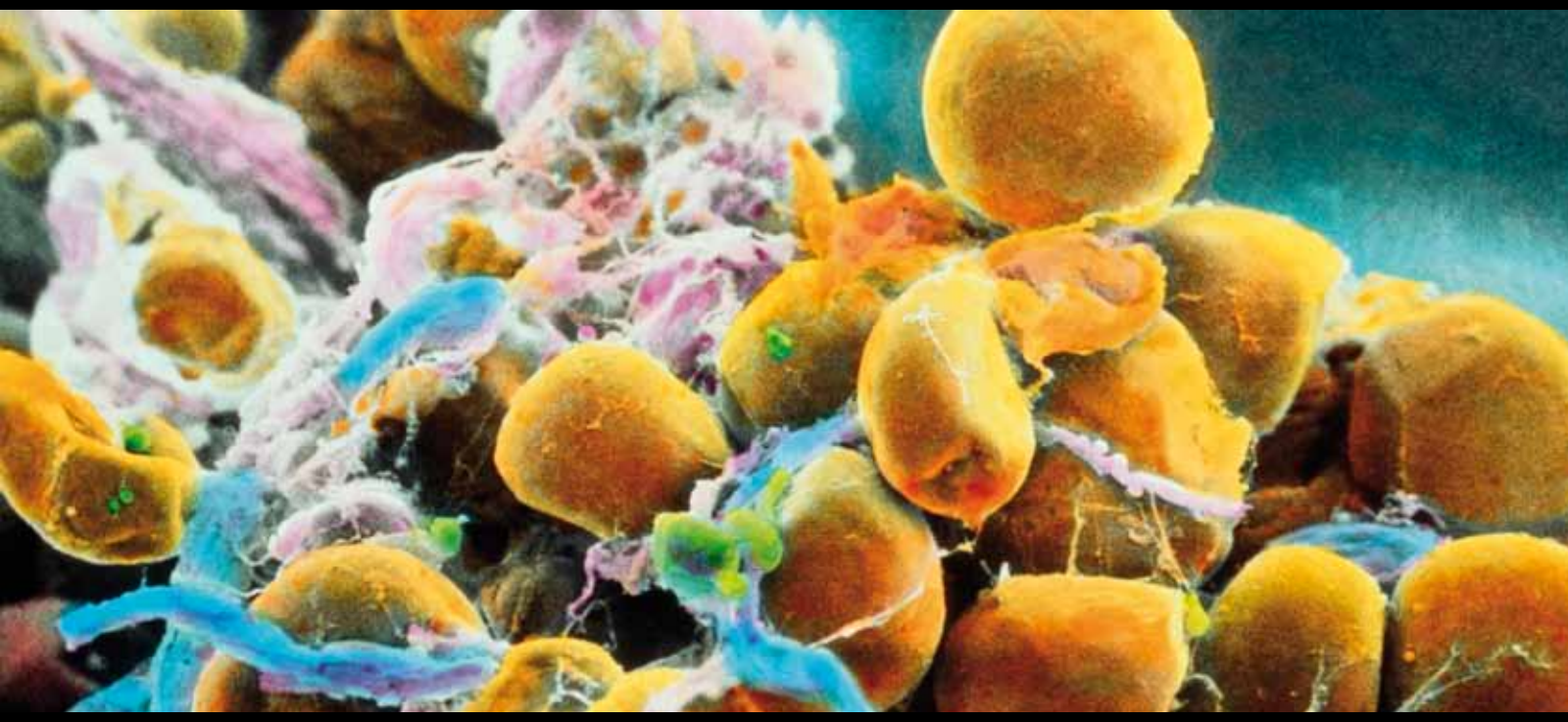
Journal of Obesity

Pharmacologic Treatment of Obesity

Guest Editors: Alfredo Halpern, Steven Heymsfield, and Luc Van Gaal



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Editorial Board

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Editorial

Pharmacologic Treatment of Obesity

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The pharmacological treatment of obesity still faces many barriers. Notably, these include the disregard of obesity as a disease and the very limited treatment options for obesity. In this context, this special issue is of special importance as it focuses on several topics, related to this challenge.

After careful screening, review, and revision, we selected 26 articles which deal with a variety of aspects related to the pharmacological therapy of obesity. Topics covered include possible etiologies of the disease (e.g., A. Niijima's rat study and B. Bjorndal et al.'s research on adipogenesis), the role of diet as an anti-inflammatory mediator (studies of B. Sears and C. Ricordi and W. R. Hamilton et al.), some new aspects of the drugs being used today, particularly orlistat (A. Hollywood and J. Ogden, J. Martin et al., and N. J. Perera et al. studies), and outlining the treatment of special conditions such as in children and adolescents (L. Iughett et al.), polycystic ovary syndrome (H. Kahal et al.), and diabetes mellitus (V. Cheng and S. R. Kashyap). Additional reports include reviews on the pharmacological treatment of obesity in the present and future (M. Glandt and I. Raz and L. L. Ioannides-Demos et al.).

To conclude, this edition of the journal describing advances in the pharmacological treatment of obesity will provide those in the field with new and useful insights on obesity pathogenesis and management.

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Luc Van Gaal

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Research Article

Hepatoportal Leptin Sensors and Their Reflex Effects on Autonomic Outflow in the Rat

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Afferent nerve signals were recorded from a peripheral cut end of the small nerve bundle of the hepatic branch of the vagus nerve in anesthetized rats. An injection of leptin (100 pg, 0.1 mL) into the portal vein facilitated the afferent activity. The response was dose dependent. Further, an intravenous (IV) injection of leptin (1 ng, 0.1 mL) facilitated the efferent nerve activity of the sympathetic nerve to the adrenal gland and suppressed that of the celiac branch of the vagus nerve. In hepatic vagotomized rats, no change in efferent activity of the adrenal sympathetic nerve nor celiac branch of the vagus nerve was observed following iv administration of leptin. These observations suggest that leptin sensors in the hepatoportal region play a role in reflex modulation of autonomic outflow in relation to metabolic functions.

1. Introduction

Leptin is a satiety hormone secreted by white adipose tissue (WAT). Both central and peripheral administration of leptin reduce food intake and body weight [1, 2]. Leptin receptors are localized to the hypothalamus [3, 4] and choroid plexus [5]. Direct application of leptin has been shown to increase the activity of glucoreceptor neurons in the ventromedial hypothalamus (VMH) and inhibit the activity of glucose-sensitive and nonglucose-sensitive neurons in the lateral hypothalamus (LHA) [6]. This finding suggests that leptin is liberated from WAT into the bloodstream, transported into the hypothalamus through the choroid plexus, and bound to receptors in the hypothalamic neurons to modulate their activities. In addition to the direct action of blood-borne leptin in the hypothalamus, the existence of afferent signaling from the leptin sensors in the WAT of the epididymis and the sensors' role in reflex modulation of sympathetic nerve activity have been reported [7, 8]. It is reasonable to assume that leptin sensors exist in the hepatoportal region because the portal vein is a main pathway for leptin secreted from the visceral WAT to arrive in the portal venous blood. Further, it has been reported that several types of chemosensors, such as glucose sensors [9], amino acid sensors [10], and interleukin-1β sensors [11], exist in the hepatoportal region and send their information through the hepatic branch of the vagus nerve to the brain. The aim of the present study was to investigate the effect of leptin on afferent signals from leptin sensors in the hepatoportal region and the sensors' reflex effect on autonomic outflow.

2. Materials and Methods

Male Wistar rats weighing 250–300 g were used. All animals were housed in a room maintained at approximately 24°C and illuminated for 12 h (07:00–19:00). Food (type MF; Oriental Yeast Co., Tokyo) and water were freely available. All procedures were performed in accordance with The Japanese Physiological Society's guidelines for animal care. Experiments were conducted after the animals had been allowed to adapt to their housing conditions for one week. Food, but not water, was removed 12 h before the experiment. The animals were anesthetized by intraperitoneal injection of 1 g/kg urethane. After the laparotomy, a catheter was inserted into the portal vein or the inferior vena cava for

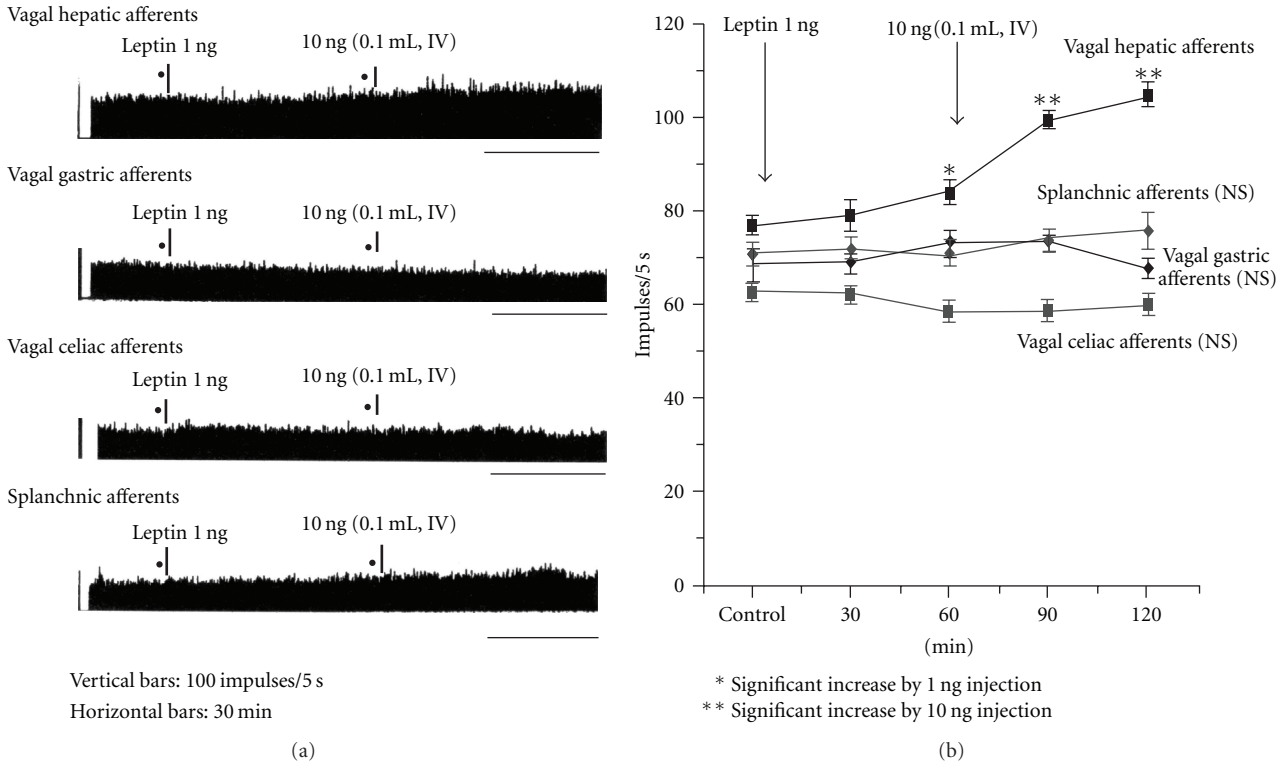

Figure 1: Effect of intravenous (IV) injection of leptin (1 ng and 10 ng, 0.1 mL) on the afferent discharge rate of the hepatic, gastric, or celiac branch of the vagus nerve, and that of the splanchnic nerve.

administration of leptin. With a dissection microscope, an isolated nerve filament from the peripheral cut end of the hepatic branch of the vagus nerve was placed on a pair of silver wire recording electrodes to record the nerve activity. Afferent signals were sometimes recorded from the gastric or celiac branches of the vagus nerve.

To record the efferent nerve activity of the sympathetic or vagal nerve branch, a nerve filament dissected from its central cut end was used. The recording electrodes were immersed in a mixture of liquid paraffin and petroleum jelly to prevent dehydration. The nerve activity was amplified in a condenser-coupled differential amplifier, monitored by an oscilloscope, and stored on magnetic tape. All nerve activity were analyzed after conversion of raw data to standard pulses by a window discriminator, which separated discharges from background noise. The discharge rate was displayed on a pen recorder by means of a rate-meter with a reset time of 5 seconds. The effects of leptin injection on nerve activity were investigated by comparing the mean number of impulses per 5 seconds over 50 seconds (i.e., the mean value of 10 successive measured samples) before and after the injection. Data were expressed as the mean ± SEM. Statistical significance was determined by analysis of variance (ANOVA) ($P < .05$). Recombinant leptin (SIGMA) was kept in a freezer (−20°C) and dissolved in physiological saline before use. The leptin solution was injected through a catheter inserted into the inferior vena cava or portal vein. The animals' body temperatures were maintained by means of heating pads.

3. Results

3.1. Effect of IV Injection of Leptin on the Visceral Afferents. Figure 1 illustrates typical examples of the effects of two successive IV injections of leptin on the afferent nerve activities of the hepatic, gastric, and celiac branches of the vagus nerve, as well as of the splanchnic nerve. All recordings were made from the nerve filament dissected from the peripheral cut end of the nerve. The second IV injection (10 ng, 0.1 mL) was made 60 minutes after the first injection (1 ng, 0.1 mL).

Afferent discharge rates of vagal hepatic afferents just before, 30 minutes after, and 60 minutes after the first injection were 77.6 ± 1.9, 79.7 ± 36.1, and 84.7 ± 2.3* impulses per 5 seconds, respectively. Those just before, 30 minutes after, and 60 minutes after the second injection were 84.7 ± 2.3, 100.2 ± 1.9*, and 105.7 ± 2.8* impulses per 5 seconds, respectively (*represents significant increase, $P < .05$). These results indicate that IV injections of leptin at doses of 1 ng and 10 ng significantly increased the afferent discharge rate, and the responses were dose related. However, no significant changes in afferent discharge rates were observed

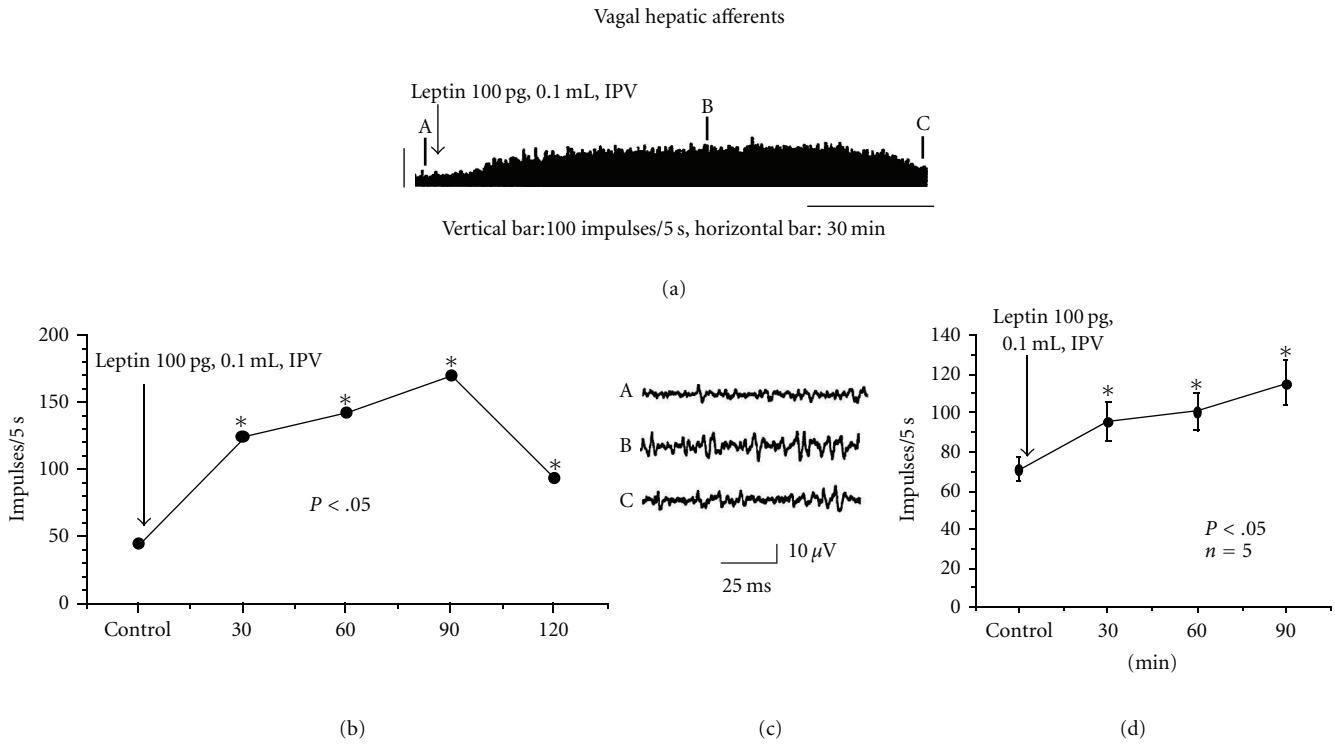

Figure 2: Effect of intraportal injection (IPV) of leptin 100 pg on afferent activity of the hepatic branch of the vagus nerve. (a) Time course of afferent activity before and after injection. Vertical bar: 100 impulses/5 seconds. The arrow shows the time of injection. (b) Afferent discharge rate just before 30, 60, 90, and 120 minutes after injection. (c) Afferent nerve activity (A) just before injection, (B) 60 minutes, and (C) 110 minutes after injection. (d) Effect of IPV injection of leptin (100 pg, 0.1 mL) on mean discharge rates of vagal hepatic afferents ($n = 5$). *Shows significant increase in discharge rates from the control value just before injection.

in the gastric or celiac branches of the vagus nerve, nor in the splanchnic nerve, after IV injections of leptin (1 ng and 10 ng). The results of these experiments clearly suggest the existence of leptin sensors at the terminals of vagal hepatic afferents.

3.2. Effect of Intraportal Injection of Leptin on Vagal Hepatic Afferents. To confirm the existence of leptin sensors in the hepatoportal region, the effect of leptin injection into the portal vein (IPV) on the afferent activity of the hepatic branch of the vagus nerve was investigated. Figure 2(a) shows an example of the effect of an IPV injection of leptin (100 pg, 0.1 mL) on the vagal hepatic afferents. As shown in the trace, an injection of leptin caused a gradual and long-lasting increase in afferent activity, which reached a peak value approximately 60 min after the injection. The discharge rates just before and 30, 60, 90, and 120 minutes after IPV injection were 65.2 ± 2.2, 113.6 ± 4.1*, 128.5 ± 2.9*, 149.8 ± 5.8*, and 83.1 ± 2.5* impulses per 5 seconds, respectively (*indicates a significant increase, $P < .05$) (Figure 2(b)). Figure 2(c) presents the afferent action potentials at times A, B, and C in Figure 2(a). Figure 2(d) presents the mean discharge rates of five different preparations before and 30, 60, and 90 minutes after IPV injection of leptin (100 pg, 0.1 mL). The mean discharge rates were 71.0 ± 5.7, 95.7 ± 10.0*, 100.7 ± 9.6*, and 115.3 ± 11.7* impulses per 5 seconds, respectively (*indicates a significant increase, $n = 5$, $P < .05$). Further, each of five preparations demonstrates a significant increase in afferent discharge rates 60 and 90 minutes after IPV injection (Table 1). It was further observed that an IPV injection of saline (0.1 mL) as a control resulted in no significant change in discharge rates; IPV injection of 10 pg leptin resulted in no significant increase. The least effective dose to increase afferent activity was 100 pg.

3.3. Reflex Effects from Hepatoportal Leptin Sensors to Autonomic Outflow. Reflex effects of IPV infusion of leptin on the efferent activity of the sympathetic branch to the adrenal medulla, pancreas, liver, epididymal WAT, interscapular brown adipose tissue (BAT), as well as that of the celiac and pancreatic branches of vagus nerve, were investigated. Figure 3 presents typical examples of the effects of IPV infusion of leptin 1 ng (0.1 mL) on sympathetic and vagal outflows. The injection activated sympathetic nerve activity and inhibited vagal nerve activity to the organs mentioned above. Figure 4 and Table 2 show the mean discharge rates of sympathetic or vagal nerve activities before and after IV injection of leptin (1 ng, 0.1 mL). These observations demonstrated that IPV or IV administration of leptin at a dose of 1 ng activates sympathetic and inhibits vagal outflow.

In subsequent experiments, the effects of IPV injection of leptin (1 ng, 0.1 mL) on the efferent nerve activity of the

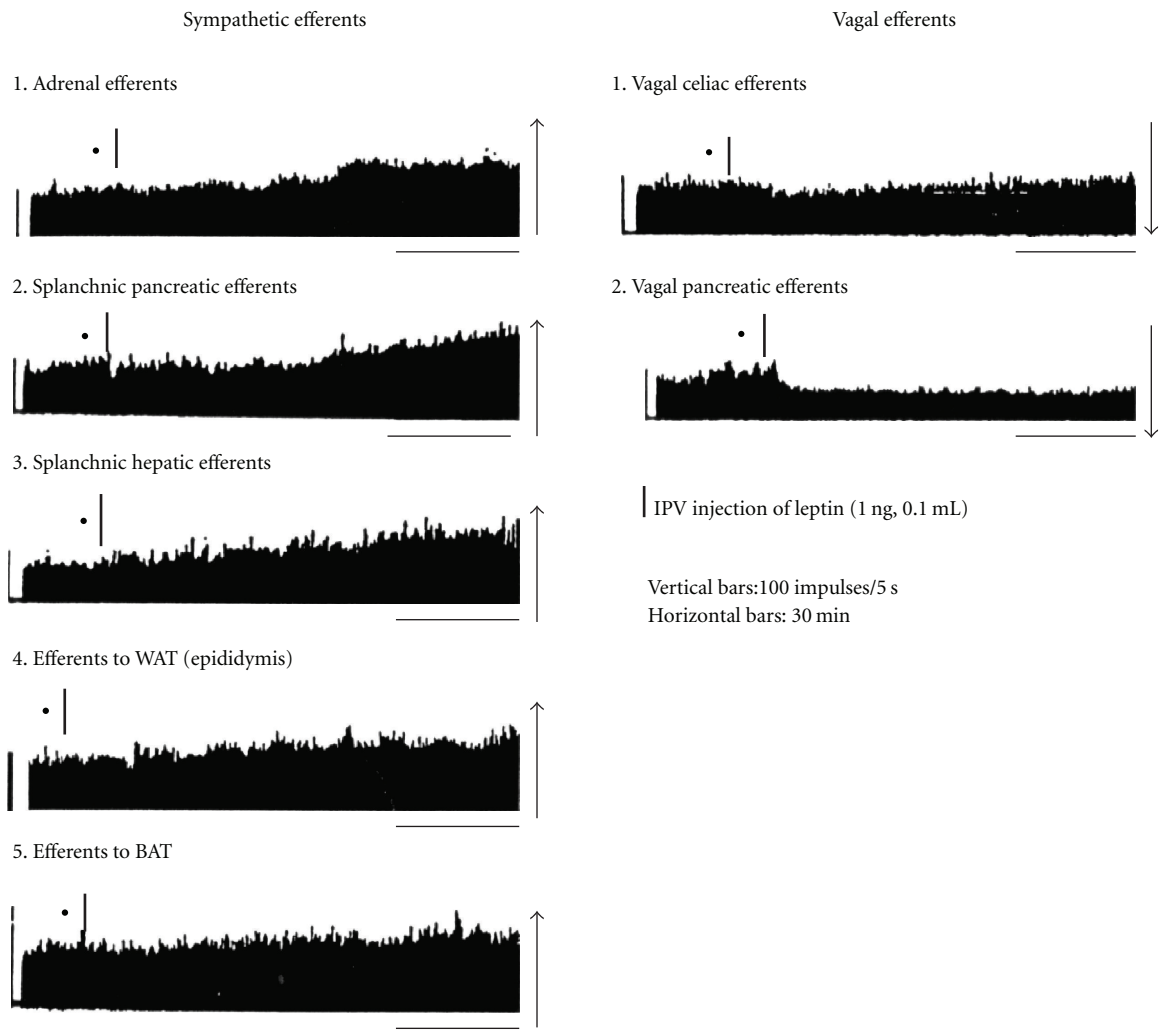

Figure 3: Reflex effects of IPV injection of leptin (1 ng, 0.1 mL) on efferent discharges of sympathetic and vagus nerve. Arrows indicate the time of injection.

Table 1: Firing rate response of vagal hepatic afferent fibers before and after intraportal injection of leptin (100 pg. 0.1 mL).

	Impulses/5 seconds (mean ± SEM)			
No.	Before	30	60	90 minutes
1	54.0 ± 3.1	61.2 ± 1.9	70.6 ± 2.8*	91.3 ± 6.3*
2	73.2 ± 2.2	89.1 ± 3.0*	107.3 ± 2.6*	122.1 ± 1.2*
3	65.2 ± 2.2	113.6 ± 4.1*	128.5 ± 2.9*	149.8 ± 5.8*
4	88.5 ± 2.4	116.9 ± 2.7*	106.1 ± 4.0*	126.2 ± 3.3*
5	74.2 ± 2.7	97.7 ± 2.6*	90.8 ± 2.2*	86.8 ± 2.9*
Mean	71.0 ± 5.7	95.7 ± 10.0*	100.7 ± 9.6*	115.3 ± 11.7* ($n = 5$, $P < .05$)

(*Significant increase, $P < .05$).

adrenal sympathetic and vagal celiac nerves were compared in normal and hepatic vagotomized rats. As shown in the upper part of the left panel of Figure 5, leptin injection (1 ng, 0.1 mL) caused a significant increase in adrenal nerve activity in normal rats; however, no significant change in nerve activity occurred following IPV injection of leptin at the same dose in hepatic vagotomized rats. The lower part of the left panel of the Figure 5 presents the effects of leptin on vagal celiac efferents. In the normal rat, leptin injection suppressed vagal nerve activity, although no significant change in nerve activity was observed following injection of leptin in hepatic vagotomized rats. The right panel of Figure 5 demonstrates that IV administration of leptin (1 ng, 0.1 mL) caused an increase in efferent activity of the sympathetic adrenal

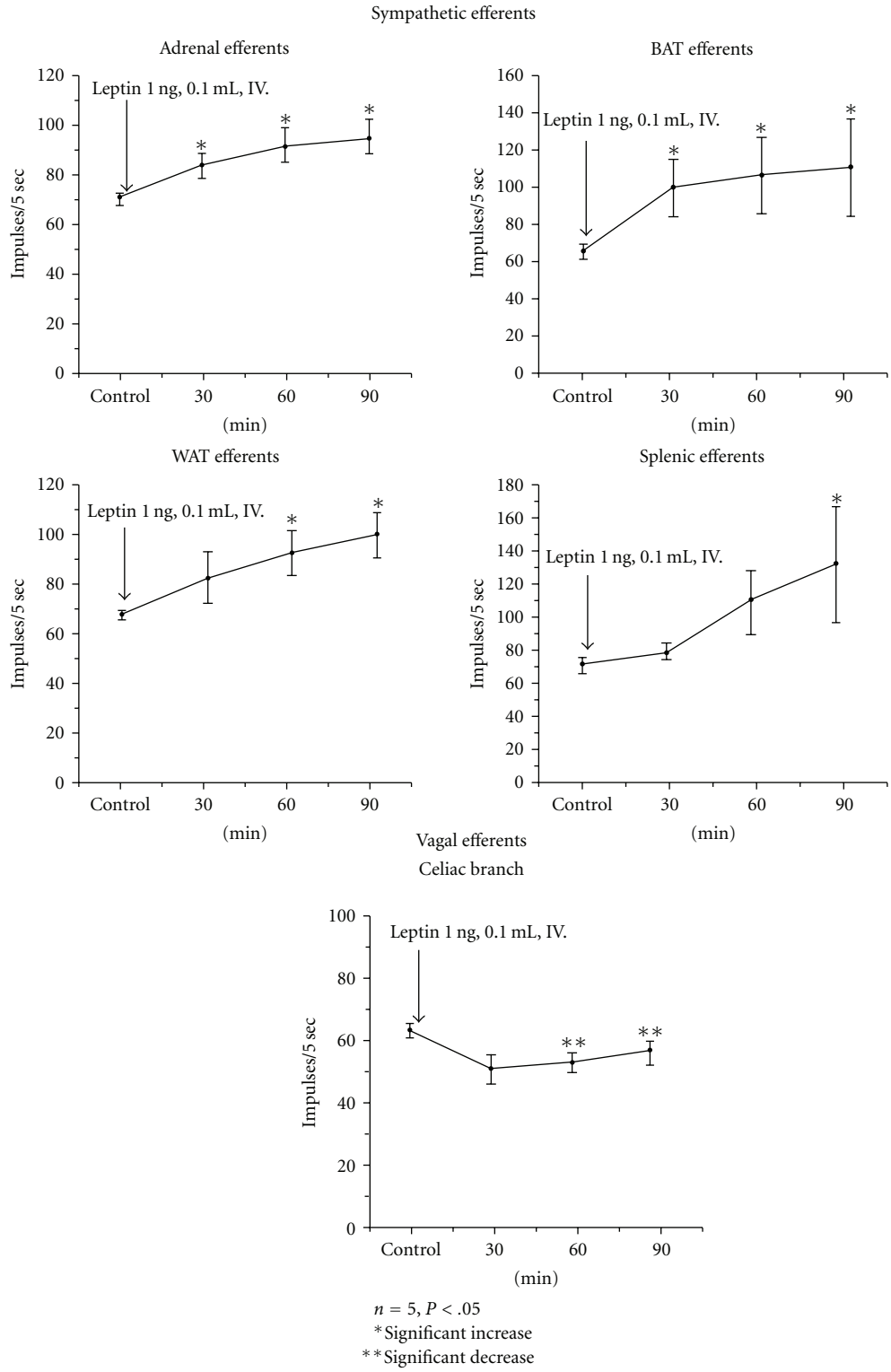

Figure 4: Reflex effects of IV injection of leptin (1 nfg, 0.1 mL) on the mean discharge rates of sympathetic and vagus nerve.

efferents; further, in bilateral or hepatic vagotomized rats, no remarkable changes in efferent activity following IV injection of leptin at doses of 1 ng, 10 ng, 100 ng, or 1 μg were observed. However, IV administration of a large amount of leptin (10 μg) resulted in a remarkable increase in efferent activity. This response might be the direct effect of blood-borne leptin on the hypothalamus. These observations indicate that IPV injection of leptin results in reflex activation of sympathetic outflow and reflex suppression of vagal outflow to visceral organs that regulate metabolic functions.

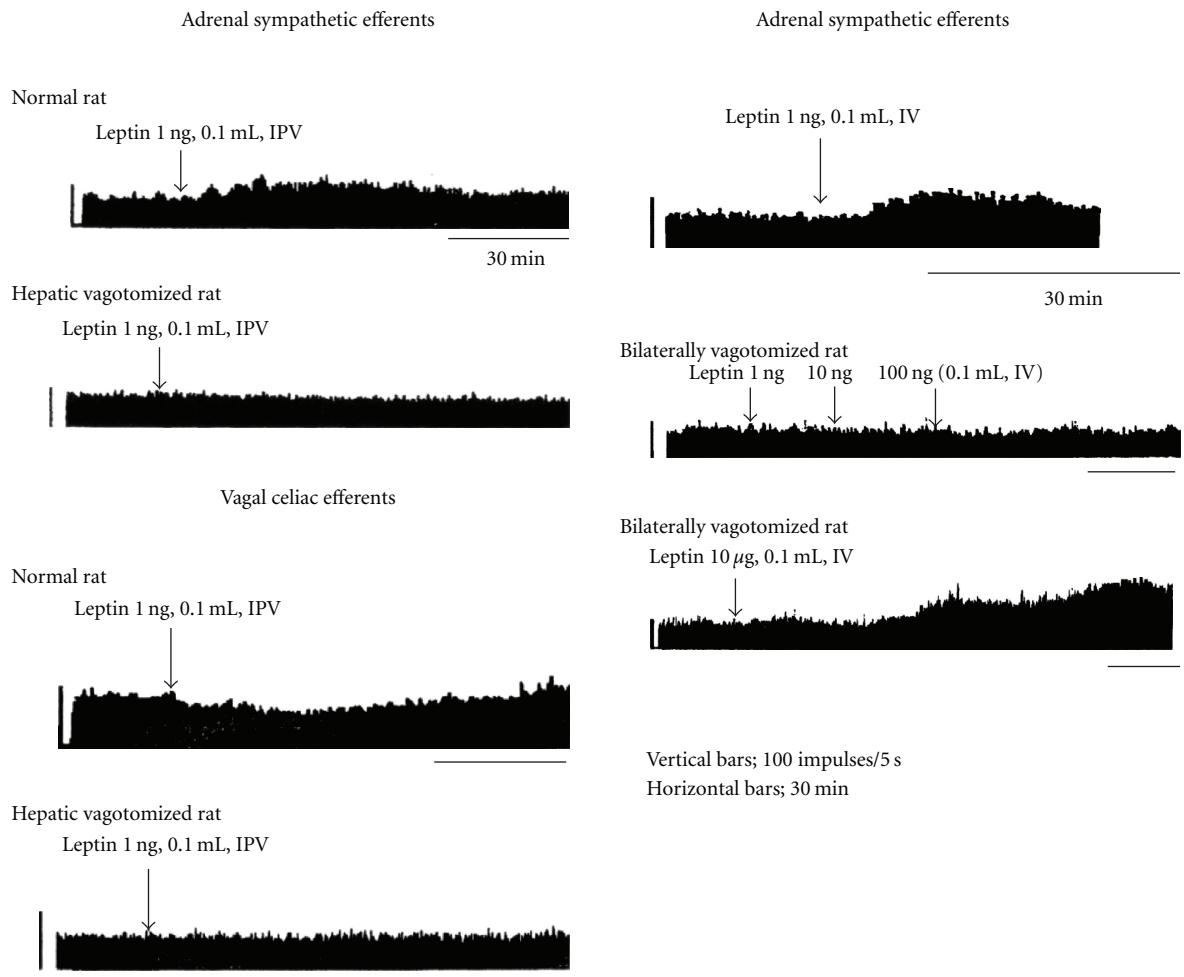

Figure 5: Effects of IPV or IV injection of leptin on the efferent activity of sympathetic nerve and vagus nerve in normal and hepatic or total vagotomized rat.

Table 2: Reflex responses of sympathetic and vagal efferents following IV injection of leptin (l ng, 0.1 mL).

	Impulses/5 seconds ± SE			
	Before injection	30 minutes	60 minutes	90 minutes
Sympathetic efferents				
Adrenal efferents ($n = 5$)	70.7 ± 2.7	83.6 ± 5.4*	92.0 ± 6.8*	95.5 ± 6.8*
WAT efferents ($n = 5$)	69.3 ± 1.8	84.8 ± 10.8	95.3 ± 9.4*	102.3 ± 9.6*
BAT efferents ($n = 5$)	65.0 ± 3.7	100.2 ± 16.0*	106.3 ± 20.6*	110.7 ± 26.2*
Splenic efferents ($n = 5$)	71.4 ± 4.9	79.3 ± 5.0	110.0 ± 19.6	132.8 ± 35.2*
Vagal efferents				
Vagal celiac efferents ($n = 5$)	63.5 ± 2.4	50.6 ± 4.7**	53.0 ± 3.2**	56.2 ± 3.9

(*Significant increase, **significant decrease, $P < .05$).

4. Discussion

It was stated in the introduction that both central and peripheral administration of leptin reduces food intake and body weight, and that the site of action of leptin is the hypothalamus [1, 2, 12]. It was also reported that leptin receptors are distributed within the hypothalamus and choroid plexus [3–5], and that leptin directly stimulates activity of VMH (satiety enter) neurons and inhibits activity of LHA (feeding center) neurons [6], and stimulates/inhibits activity of ARC (dual center) neurons [13]. Interestingly, several lines of evidence support that leptin signaling in the central nervous system regulates autonomic outflow to white adipose tissue [14], brown adipose tissue [15], muscle and

liver [16]. In relation to this, the importance of the role played by the hepatic sympathetic nerves has been reported [7].

The most widely accepted hypothesis is that blood-borne leptin is transported into the brain by the choroid plexus and acts on the leptin receptors in the hypothalamus; chemical signals may thereby be translated into neural signals.

It was recently reported that leptin sensors exist in the WAT of the epididymis and send signals through afferent nerve fibers innervating WAT to the central nervous system; those afferent signals then evoke reflex activation of sympathetic nerve activity to the visceral organs, such as the adrenal medulla, pancreas, liver, WAT, and BAT, and evoke reflex inhibition of vagal nerve activity to the pancreas and liver [13, 14].

Further, it is possible to assume that peripheral leptin sensors also exist in the hepatoportal region, are sensitive to leptin liberated from the visceral WAT into the portal venous blood, and send signals to the brain via vagal hepatic afferent fibers.

The results of the present study demonstrated that an IPV or IV injection of leptin (100 pg to 1 ng, 0.1 mL) activated afferent nerve activity of the hepatic branch of the vagus nerve; an IV or IPV injection of leptin (1 ng, 0.1 mL) caused facilitation of sympathetic nerve activity to visceral organs such as the adrenal medulla, WAT, and BAT, and inhibition of vagal celiac and pancreatic nerve activity; the same amount of leptin injection was without effect in totally vagotomized or hepatic vagotomized rats; the doses of leptin (100 pg to 10 ng) used for IV or IPV injections were in the physiological range of plasma leptin concentrations (6 ± 1 ng/mL) [6]; IV or IPV injection of physiological saline was without effect on the afferent activity of the hepatic branch of the vagus nerve as well as on the reflex change in autonomic outflow; IV or IPV injection of a larger amount of leptin (1 μg or 10 μg) increased adrenal sympathetic nerve activity in totally or hepatic vagotomized rats. Further, the author observed that injections of leptin at doses of 0.5–1.0 μg (1.5 μl) into the lateral ventricle activated efferent activity of the adrenal sympathetic nerve (unpublished data).

In addition to the blood-borne leptin pathway and afferent signaling system from leptin sensors in the WAT, the present study demonstrated a third pathway of the leptin signaling system from hepatoportal leptin sensors to the brain. Leptin secreted from the abdominal visceral white adipose tissue into the portal vein may play an important role in reflex regulation of metabolic function. The existence of three different leptin signaling systems (the blood-borne system, afferent signaling system from WAT, and that from hepatoportal leptin sensors), is likely to represent a fail-safe alarm system.

The results of the present experiments demonstrate the reflex modulation of metabolic functions, including acceleration of lipolysis in WAT, increase in catecholamine secretion from the adrenal medulla, facilitation of glycolysis in the liver, and reduction in insulin release from the pancreas. The reflex center is in the hypothalamus or brainstem, and the afferent limb is made up of afferent fibers in the hepatic branch of the vagus nerve. The exact sites of hepatoportal leptin sensors, as well as morphological and immunohistochemical properties of these sensors, remain to be studied.

5. Conclusion

The results of the present experiments indicate that afferent signals from leptin sensors in the hepatoportal region play a role in reflex regulation of metabolic functions through modulation of autonomic outflow.

Support for Work

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Review Article

Different Adipose Depots: Their Role in the Development of Metabolic Syndrome and Mitochondrial Response to Hypolipidemic Agents

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Adipose tissue metabolism is closely linked to insulin resistance, and differential fat distributions are associated with disorders like hypertension, diabetes, and cardiovascular disease. Adipose tissues vary in their impact on metabolic risk due to diverse gene expression profiles, leading to differences in lipolysis and in the production and release of adipokines and cytokines, thereby affecting the function of other tissues. In this paper, the roles of the various adipose tissues in obesity are summarized, with particular focus on mitochondrial function. In addition, we discuss how a functionally mitochondrial-targeted compound, the modified fatty acid tetradecylthioacetic acid (TTA), can influence mitochondrial function and decrease the size of specific fat depots.

1. Introduction

In a modern lifestyle people are chronically exposed to elevated amounts of lipids and nutrients, potentially leading to tissue dysfunction and disease [1]. The adipose tissue is highly involved in the development of metabolic disorders such as cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). It is the body's largest storage site for triglycerides and plays an important role as an endocrine organ in energy homeostasis [2]. The adipose tissue is divided into specific regional depots with differences in structural organization, cellular size, and biological function [3]. The distribution of fat between these depots seems to be more important than the total adipose tissue mass for the risk of developing obesity-associated diseases (Figure 1).

Normally, fat storage in the form of neutral triglycerides takes place during food intake to be replaced by fat mobilization processes during fasting or situations with elevated energy demand (Figure 2(A)). Adipocytes produce a number of endocrine hormones that contribute to the regulation of this mechanism, such as adiponectin and leptin. As adipocytes play a key metabolic role as a source of fuel, they are required to respond acutely to changes in the nutritional levels. As a result they are tightly regulated by both hormonal (e.g., insulin) and sympathetic (e.g., adrenergic) stimulation [2].

Metabolic syndrome is a group definition on several diseases connected to obesity. Subjects will display elevated levels of glucose and nonesterified fatty acids, also called free fatty acids (FFAs), and show symptoms of one or more afflictions amongst which hypertension, hypertriglyceridemia, diabetes, and obesity are the most usual. Lipotoxitcy and the accumulation of saturated fat in peripheral tissues are an important step in the development of metabolic syndrome. Fatty liver (liver steatosis) is found in a large proportion of individuals with body mass index (BMI) above 30. Liver steatosis contributes to insulin resistance and may lead to the development of nonalcoholic fatty liver diseases (NAFLDs), including nonalcoholic steatohepatitis (NASH) and cirrhosis [4].

T2DM is usually linked to obesity and lipid redistribution to nonadipose organs and tissues and is caused by a reduced sensitivity to insulin in muscle, liver, and adipose tissue [1]. During insulin resistance, an increase in insulin

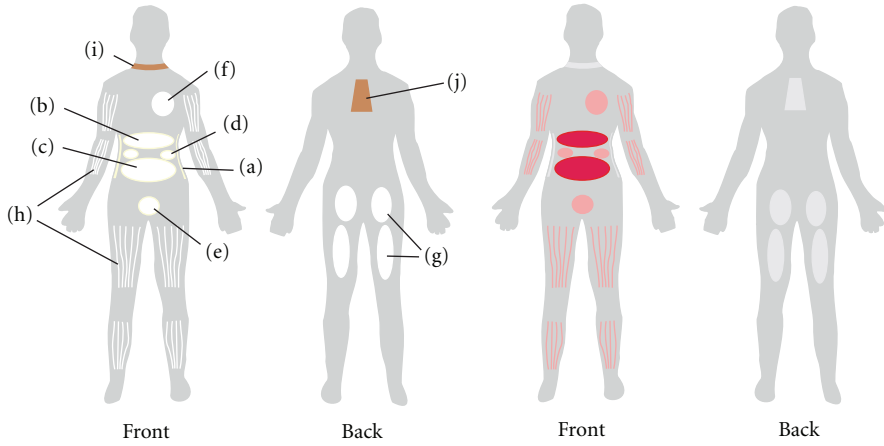

Figure 1: (A) The main white adipose tissues (WATs) are abdominal subcutaneous adipose tissue (SAT, (a)), and visceral adipose tissue (VAT). VAT surrounds the inner organs and can be divided in omental (b), mesenteric (c), retroperitoneal ((d): surrounding the kidney), gonadal ((e): attached to the uterus and ovaries in females and epididymis and testis in men), and pericardial (f). The omental depot stars near the stomach and spleen and can expand into the ventral abdomen, while the deeper mesenteric depot is attached in a web-form to the intestine. The gluteofemoral adipose tissue (g) is the SAT located to the lower-body parts and is measured by hip, thigh, and leg circumference. WAT can also be found intramuscularly (h). Brown adipose tissue is found above the clavicle ((i): supraclavicular) and in the subscapular region (j). Although the mentioned subcutaneous and visceral adipose tissues are found in humans, depots (d) and (e) are mostly studied in rodents. (B) The adipose tissue depots that have been linked to risk of developing obesity-related diseases are indicated in red. The best-documented link to risk is found for the omental and mesenteric VAT.

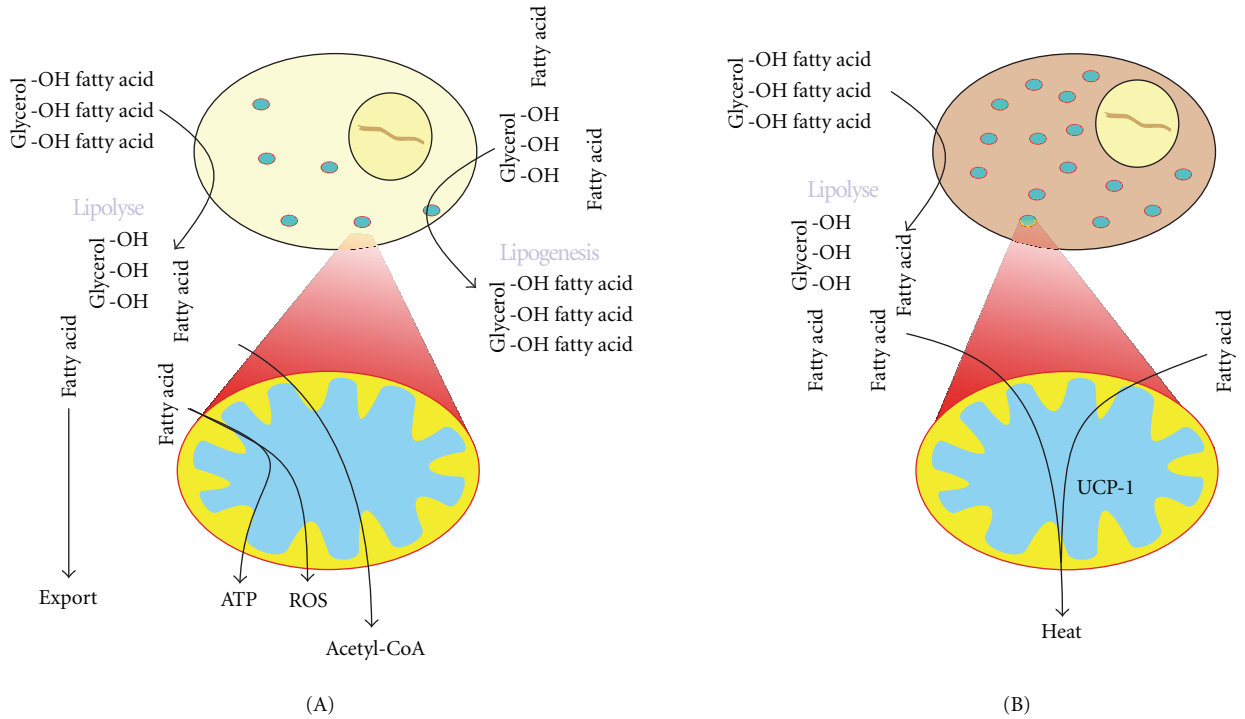

Figure 2: (A) The role of white adipose tissue (WAT) is to store excess dietary fat in the form of triglycerides (TGs) and to release free fatty acids (FFAs) in times of starvation or energy demand. In addition WAT releases several important factors that regulate energy homeostasis. Lipolysis takes place in the cytosol and is also dependent on processes in the mitochondria. The resulting FFA can be released to the blood or directly used as an energy source by β-oxidation in the mitochondria. In lipogenesis, glycerol and acyl-CoA produced in the mitochondria are used to generate TG for storage in the adipocytes. (B) In brown adipose tissue (BAT) the FFAs are β-oxidized, and the resulting acetyl-CoA enters the Krebs cycle. The reduction potential is used to form a mitochondrial proton gradient that instead of producing ATP is released by uncoupling protein 1 (UCP1), a process that generates heat.

does not lead to a corresponding increased uptake of glucose in muscle and liver. As a compensatory mechanism insulin production is increased in pancreatic beta cells. T2DM occurs when the increased insulin production is no longer sufficient, or when lipid-induced beta cell malfunction takes place, particularly in genetically predisposed individuals. It is believed that high levels of circulating FFA and lipotoxic mechanisms can cause damage on β-cell and nonislet tissues.

CVD includes hypertension, atherosclerosis, heart disease, vascular disease, and stroke. Several of the high-risk factors for CVD result directly or indirectly from obesity and a malfunctioning adipose tissue: dyslipidemia, chronic inflammation, diabetes, and hypertension. Mitochondrial dysfunction could also contribute to CVD due to their role as targets and sources of reactive oxygen species (ROS), both in the formation of vascular lesions and in their involvement in the development of insulin resistance and T2DM [5].

A well-functioning adipose tissue with capacity to neutralize and store nutritional overload is therefore necessary to protect the body from peripheral insulin resistance. Two closely linked processes in adipose tissue are important for the development of metabolic disease: adipose tissue inflammation and adipose tissue hypertrophy. Adipose tissue inflammation due to the recruitment of T-cells and macrophages has been shown to contribute to insulin resistance in obese individuals. This inflammation leads to a disturbed adipokine-balance and an uncontrolled release of free fatty acids and inflammatory cytokines [6]. As a result, lipoprotein metabolism and insulin sensitivity in other organs will be affected. During obesity, the adipocyte size increases (hypertrophy), and they are eventually unable to store excess lipids even with enhanced adipocyte proliferation (hyperplasia). This redirects fatty acids to the liver promoting dyslipidemia, characterized by elevated plasma FFA, triglycerides (TGs), and small dense low-density lipoprotein (LDL), and the reduction of high-density lipoproteins (HDLi). In addition large adipocytes are characterized by an increased lipolysis, the breakdown of stored TG by lipases. This results in FFA and glycerol, which further increases plasma FFA-levels and upregulates TG synthesis in the liver [7]. A recent publication demonstrated that enlarged adipocytes in obese men had a reduced rate of FFA-delivery to the blood stream, resulting in normal systemic FFA concentrations. However, after meals the adipose tissue storage capacity was severely impaired, thus potentially causing ectopic lipid deposition [8].

How well the adipose tissue functions as a storage organ for excess energy intake and the distribution of fat and mitochondria between the different adipose depots may determine the large differences in health observed in obese individuals. Here, we will first focus on the importance of mitochondrial function in adipose tissue and then describe the different adipose tissue depots, in rodent animal models as well as humans (Figure 1), and their impact on the development of metabolic diseases. Pharmacological treatments that specifically target mitochondria have great potential in the treatment of obesity-related disorders, and their differential effect on adipose depots will be discussed.

2. Reactive Oxygen Species during Obesity

An elevated metabolism during obesity results in increased production of reactive oxygen species (ROS). ROS damage is most likely involved in all features of metabolic syndrome. Increased ROS production is an early event in glucose intolerance, contributing to pancreatic β-cell dysfunction as well as liver steatosis. β-cells may be specially susceptible to ROS-damage due to their relatively low expression of free-radical-detoxifying enzymes compared to most other cell types [9].

There are indications that mitochondrial content and function in adipose tissue might be disrupted in metabolic disorders. It has been suggested that insulin resistance is linked to mitochondrial dysfunction [10, 11]. Furthermore, it is increasingly evident that oxidative capacity and thus mitochondria are fundamental to avoid the development of toxicity during excess energy intake. For instance, increase in mitochondrial ROS is observed in adipose tissue during obesity [12–14], and in skeletal muscle, the mitochondrial H_2O_2 emission is higher using fatty acids as substrates versus carbohydrate-based substrates [15, 16]. Two models of insulin resistance, induced by either tumor necrosis factor α (TNFα) or glucocorticoid treatment, were both shown to involve increased ROS levels [17]. Also, antioxidants attenuating mitochondrial H_2O_2 emission completely restored insulin sensitivity in skeletal muscle [16]. These findings show that mitochondria play a central role in the physiology leading to insulin resistance.

In addition, the NOX family of NADPH oxidases, located to the plasma membrane, cytosol and cytosolic membranes, is a major source of ROS generation. NOX2 is an energy transporter that catalyses the reduction of oxygen to O_2^- in an NADPH-dependent reaction. The involvement of NOX enzymes in the development of metabolic syndrome has been reviewed by Krause and coauthors [18, 19]. In low concentrations, ROS participates in the cell signaling as secondary messengers [20]. In response to insulin, NOX will release ROS that activates the distal insulin-signaling cascade, mobilizing glucose transporters to the surface of adipocytes. However in higher amounts it will have serious harmful effects on the cells, including decreased glucose uptake in adipocytes [21]. Several mouse obesity models have found an increase in oxidative stress in adipose tissue associated with overexpression of NADPH oxidase and repression of antioxidative enzymes such as catalase [13]. Also, mitochondrial proliferation is inhibited by increased ROS production [22, 23], indicating that ROS can modulate adipocyte generation.

3. Mitochondria in Adipose Tissue

Well-functioning mitochondria are essential in adipose tissue. Mature adipocytes require large amounts of ATP to maintain their activities such as lipolysis, fatty acid β-oxidation, and fatty acid synthesis. In the organism, mitochondria produce energy and are central in the fatty acid metabolism since more than 98% of fatty acids are oxidized in mitochondria [24]. Recent evidence indicates

that a reduced mitochondrial function and altered biogenesis play important roles in the etiology of obesity, insulin resistance, and T2DM [25]. An impaired metabolic flexibility is the inability of an organism to adapt fuel oxidation to fuel availability. The ability to switch from fat to carbohydrate oxidation is usually impaired in subjects prone to obesity and in subjects with a family history of diabetes [26–29]. Indeed, there is strong evidence that defects in substrate switching cluster together with disturbances in mitochondrial content and/or function and might be a manifestation of an underlying mitochondrial disorder [30]. In this respect, it has been demonstrated that insulin-resistant offspring of patients with T2DM have reduced mitochondrial function, reduced ATP synthesis and accumulation of fat in skeletal muscle, the liver, and other cells [31].

3.1. Role of White Fat Mitochondria. The major adipose depots in the body consist of white adipose tissue (WAT). Mature white adipocytes require large amounts of ATP to maintain their diverse functions, and during adipogenesis mitochondrial biogenesis is an important process [20]. One of the main tasks of adipose tissue is to generate glycerol 3-phosphate and acetyl-CoA for esterification into TGs (lipogenesis), processes localized to the mitochondrial matrix as well as the cytosol, that require an abundant mitochondrial population [32, 33]. Mitochondria in WAT are also believed to be important for the regulation of lipolysis, the process of degrading TG into FFA and glycerol (Figure 2(A)). When inhibiting the mitochondrial respiratory chain or decreasing intracellular ATP by the use of mitochondrial uncouplers, lipolysis is inhibited [34]. Inhibition of the AMP-activated protein kinase (AMPK), which is an essential molecular sensor regulated by the ATP/AMP level in the cells, will decrease lipolysis stimulated by agonists of β-adrenoreceptors [35]. The FFAs produced by lipolysis can subsequently be utilized in the adipocytes as a source of energy, through β-oxidation in the mitochondria. Thus, mitochondria in white fat cells are involved in both *de novo* lipogenesis and lipolysis [36].

Depending on the anatomical position in the body, adipose tissue shows differences in metabolic activity due to different mitochondrial density. For example, rat epididymal (VAT) adipocytes have more mitochondria than inguinal (SAT) adipocytes [37]. The higher number of mitochondria per mg tissue in VAT than SAT is also observed in obese human individuals. Although mitochondrial respiratory flux per cell and per mitochondrial content was lower in VAT compared to SAT due to smaller cells, the visceral fat was bioenergetically more active and responsive to substrates of the electron transport chain [38]. In general, some studies show that obesity and T2DM in humans reduce mitochondrial number in white adipocytes [39], while others show a connection between mitochondrial number and lipogenesis, but not body mass index (BMI) or overall insulin sensitivity [40]. The link between mitochondrial dysfunction and T2DM is indicated by the reduced oxidative phosphorylation (OXPHOS) capacity in elderly subjects [41], and in obese subjects, where a reduced FA β-oxidation in several tissues including adipocytes will increase blood FFA levels and alter glucose uptake [10, 11]. Nonalcoholic steatohepatitis, a disease caused by lipid accumulation in the liver, is associated with mitochondrial dysfunction due to increased lipid peroxidation, alterations in mitochondrial ultrastructure, depletion in mtDNA, and low OXPHOS activity. These patients also commonly display abdominal obesity, diabetes, and hypertriglyceridemia with insulin resistance [42]. In rodent genetic or high-fat fed obesity models, the expression of genes involved in mitochondrial ATP production, energy uncoupling and other genes important for mitochondrial function was downregulated compared to lean control animals [43]. This indicates a strong impairment of mitochondrial biogenesis during obesity.

3.2. Role of Brown Fat Mitochondria. Although brown adipose tissue (BAT) is mainly found in newborns, recent studies have demonstrated its presence in adults [44, 45]. BAT originates from a different cellular lineage than WAT [46], but they share many features. However, mature BAT contains a higher number of mitochondria and lower numbers of lipid droplets. During cold temperatures and activation of the sympathetic nervous system [47], brown adipocyte lipolysis occurs (Figure 2(B)). Instead of being released for subsequent oxidation in organs such as liver and skeletal muscle, the resulting FFAs are oxidized in the BAT mitochondria and used to generate heat (see review in [48]). This process involves transport of FFA complexed to fatty acid binding proteins (FABPs) into the mitochondria by the carnitine shuttle system, where they are oxidized and used to generate heat by uncoupling protein 1 (UCP1), a process called nonshivering thermogenesis. This process is important in newborn humans and in rodents, but appears to be almost lost in adult humans. Although UCP1 is the hallmark of BAT, increased UCP1 expression and energy expenditure can also be seen in WAT under some conditions [49] and may be a desirable effect during the treatment of obesity. The increased expression of the *Ucp1* gene in both BAT and WAT by a fat-specific *aP2* promoter generated mice partially resistant to age-related obesity, both genetically and high-fat diet induced [50, 51]. The effect of BAT on obesity is further discussed below.

4. Transcriptional Regulation of Lipolysis and Lipogenesis

Peroxisome proliferator-activated receptors (PPARs) are important regulators of fatty acid metabolism. They serve as lipid sensors since they are activated by metabolic derivates of fatty acids in the body. There are three PPAR superfamily members PPARα, PPARγ, and PPARδ (also called PPARβ) that act upon ligand activation by controlling networks of target genes. It exceeds the scope of this paper to describe their function in detail, but the relevant PPARs will be mentioned briefly here. For a comprehensive review on the role of PPARs in metabolic syndrome, see Guri et al. [52].

PPARα is the most important PPAR in the liver and has many important target genes involved in fatty acid β-oxidation. During fasting, lipolysis is stimulated while

lipogenesis is decreased. The increase in lipolysis provides FFAs that are used as energy source in other tissues [53]. The subsequent PPARα-regulated metabolism of fatty acids mainly takes place in the liver, through mitochondrial and peroxisomal β-oxidation, but the heart, adipose tissue, and skeletal muscle can also utilize FFA for energy.

The decrease in lipogenesis is on the other hand due to downregulation of the two main lipogenic transcription factors, PPARγ and steroyl regulatory element binding protein 1c (SREBP1c) [54, 55]. Two isoforms of PPARγ exist, of which PPARγ1 is expressed in a wide variety of tissues, while PPARγ2 is mainly expressed in adipose tissue [56]. PPARγ activation stimulates preadipocyte differentiation, promotes the storage of fatty acids in mature adipocytes [56], and can activate GLUT4, facilitating increased fatty acid synthesis from glucose [57]. SREBP1c is necessary for the insulin-stimulated increase in fatty acid synthesis [58]. Its activation has been shown to create ligands for PPARγ [59], as well as regulate PPARγ expression in cultured adipocytes [60]. Lipogenesis mainly takes place in the cytosol, but the activation of PPARγ using rosiglitazone has been shown to alter both mitochondrial density and morphology in adipocytes suggesting that PPARγ also controls mitochondrial functions [36]. In obese db/db mice, the adiponectin expression and mitochondrial content of white adipocytes are reduced, and these effects are reverted by the activation of PPARγ by rosiglitazone [61]. It is interesting to note that rosiglitazone can increase expression of mitochondrial genes/mitochondrial biogenesis in rodent obesity models [43].

5. Differential Role of Fat Depots

Although the total adipose tissue is important for the development of insulin resistance, it is believed that some fat depots are more linked to risk factors for disease than others. The main adipose depots of interest are found in the abdomen and can be divided into the SAT and VAT, and the visceral tissue can again be divided into omental and mesenteric, the latter being the more deeply buried depot surrounding the intestine (Figure 1). Some studies have also included a deep subcutaneous layer (dSAT). The distribution of SAT and VAT shows person-to-person variations and is dependent on several factors such as age, nutrition, sex, and the energy homeostasis of the individual adipose tissues [62]. Both human studies and rodent obesity models will be discussed below. Although several similarities exist, the differences in rodent and human adipose tissues entail caution when choosing which depots to study and when extrapolating information between species [63].

5.1. Visceral Fat. Particularly, visceral fat depots, including omental and mesenteric adipose tissue, represent a risk factor for the development of CVD and T2DM. Visceral adipose tissue mass correlates with development of insulin resistance, while total or subcutaneous tissue mass does not [62, 64, 65]. It has been thoroughly confirmed that the adipocytes of visceral fat tissue are more lipolytically active than subcutaneous adipocytes and thus contribute more to the plasma free fatty acid levels [62, 66]. This was found in particular in diabetic obese individuals, where it was linked to a significant upregulation of leptin and downregulation of adiponectin gene expression in mesenteric VAT compared to SAT and omental VAT [67].

The metabolic activity of a cell is dependent on its mitochondrial content, and it has been shown that, in rats, epididymal (VAT) adipocytes have more mitochondria than inguinal (SAT) adipocytes [37]. In adipose tissue from obese individuals undergoing bariatric surgery the relative OXPHOS activity was found to be higher in omental VAT than SAT [38]. Individuals with a polymorphism in the *UCP1* promotor reducing *UCP1* gene expression are prone to have a high BMI, in particular due to abdominal obesity [68]. Thus, the level of mitochondrial uncoupling and energy efficiency may have an effect on obesity in WAT as well as BAT. The higher expression level of beta-adrenergic receptors in VAT could contribute to the higher lipolytic activity [69]. In addition, insulin-stimulated glucose uptake was found to be higher in VAT compared to SAT [70]. As a result, excess visceral fat will enhance the level of free fatty acid delivered to the liver, thus increasing hepatic glucose and very low-density lipoprotein particles (VLDLs) output, and impair the hepatic insulin response.

The expression of *PPARγ* mRNA is increased in the adipose tissue of obese subjects [71], and it has been shown that while there was no difference in omental VAT and SAT, *PPARγ* was significantly higher expressed in mesenteric VAT and remarkably so in obese diabetic subjects [67]. This supports the involvement of PPARγ in mesenteric adipose tissue lipolysis. In rats, lipid synthesis is shown to be higher in internal adipose tissues compared to SAT [72]. A study comparing the gene expression pattern in rat retroperitoneal (rVAT), mesenteric VAT (mVAT), and inguinal SAT (iSAT) showed that the larger cells in rVAT expressed high amounts of the lipogenic transcription factors *Pparγ* and *Srebp1c* compared to mVAT and iSAT that showed a high expression of lipogenesis-related genes and a low expression of fatty acid oxidation-related genes [73]. They also showed that the genes involved in lipid metabolism changed more rapidly as a result of fasting in the internal rVAT depot compared to SAT [74].

5.2. Subcutaneous Fat. As SAT is less metabolically active than VAT, it may have better short-term and long-term storage capacity. Thus, this depot is important to accumulate TG in periods of excess energy intake and supply the organism with FFAs in periods of fasting, starvation, or exercise. Another suggested role of SAT is to be a buffer during intake of dietary lipids, thus protecting other tissues from lipotoxic effects [75].

In humans, SAT is anatomically divided by a stromal fascia (fascia superficialis) into superficial (sSAT) and deep subcutaneous adipose tissue (dSAT), with distinct histological features. While sSAT has not been linked to risk for T2DM, the size of dSAT depots is significantly linked to the fasting insulin level and insulin-stimulated glucose

utilization, as is total fat and VAT [76, 77]. The association between dSAT and insulin resistance is particularly seen in male obese patients [77, 78]. When studying the expression and secretion of hormones and cytokines in lean subjects, dSAT was found to be more similar to VAT than sSAT [79]. Interestingly, SAT seem to have an exclusive role in leptin secretion, since it correlates with plasma leptin levels (in contrast to plasma insulin levels which correlate with inter abdominal fat [80]). Rat studies have shown that *Pparγ2* expression is higher in male than in female SAT [81], indicating a gender-linked variation in this adipose depot.

5.3. Gluteofemoral Fat. The subcutaneous gluteofemoral fat tissue is measured by hip or thigh circumference, or leg adipose tissue mass. Accumulation of fat in this depot is believed to have a protective role against diabetes and cardiovascular disease [82]. Indeed, low amount of this tissue has been associated with an unfavorable lipid and glucose profile [83]. Likewise, an increase in gluteofemoral tissue size has been connected to increase in HDL-cholesterol and decrease in total- and LDL-cholesterol levels in several studies [83–85]. Gluteofemoral fat is also positively associated with adiponectin and leptin serum levels [86]. Tracing of specific lipid fraction in blood samples from veins draining femoral or abdominal fat showed that there was a lower metabolic flux from femoral fat [87] and that femoral fat had a preference for uptake of FFA and VLDL-TG compared to chylomicron-TG, thus accumulating recycled fat rather than dietary fat.

The gluteofemoral fat tissue may thus provide protection from ectopic deposition of excess fat. While the abdominal subcutaneous adipose tissue has a role as buffer during daily fatty acid intake [75], the gluteofemoral fat tissue may have an important role in TG storage. Since it shows less metabolic activity and is more lipolytically inert than upper-body adipose depots, it seems to be involved in the long-term sequestering of fatty acids.

5.4. Intermuscular Fat. Few studies have focused on intermuscular adipose tissue (IMAT), and little is known about its specific metabolic activities. However, due to its increased level in T2DM patients, IMAT has been suggested be a risk factor along with VAT for the development of obesity-related diseases [88]. The amount of IMAT seems to be associated with age and lack of activity [89], and increases the likelihood of metabolic abnormalities in elderly subjects with normal body weight. In one study, only one third of men and less than half of women with T2DM were obese, but in the normal weight subjects, high amounts of IMAT was associated with higher fasting insulin levels [90]. Also, IMAT correlates negatively with glucose infusion rate [91], and fasting glucose and total cholesterol level in Caucasians [92], indicating its involvement in the development of insulin resistance. The amount of IMAT seem to be hereditary since African-Americans, who are also prone to develop T2DM, have more of this fat type [93].

5.5. Epicardial Fat. Epicardial adipose tissue (EAT) is the visceral fat layer located around the heart and is believed to be important for the buffering of the coronary arteries, and in providing fatty acids as a source of energy for the cardiac muscle. The release of adiponectin and adrenomedullin could have a protective effect on the heart during metabolic or mechanical insults [94]. On the other hand, it has recently been shown that EAT will locally influence heart and vasculature through the secretion of pro-inflammatory cytokines and will contribute to coronary atherosclerosis [95–97]. Studies indicate that the amount of EAT can be related to carotid artery stiffness in obese patients with hypertension, while waist circumference shows no statistically significant link [98]. Age seem to determine the risk associated with epicardial fat, since there is no relationship between EAT thickness and insulin resistance and metabolic syndrome in obese children [99].

A study of patients undergoing coronary artery bypass surgery showed that the fat layer closest to the heart expressed 5-fold higher *UCP1* mRNA than the more distal substernal fat, while *UCP1* expression was undetectable in subcutaneous thoracic fat [100]. The *UCP1* expression was influenced by age and body mass index, but showed no relationship to epicardial fat volume, waist circumference, metabolic syndrome or T2DM. This, along with the increased expression of brown adipocyte differentiation transcription factors *PRDM16* and *PGC-1α*, indicate that epicardial fat may have a function similar to brown fat. This could possibly give an additional protection of the myocardium and coronary vessels from hypothermia, but its effect on obesity-linked disease is unknown.

5.6. Gonadal Fat. Gonadal VAT is one of the largest adipose depots in rodents and is found around the testis of males (epididymal) and around the ovaries of females (periovarian). This depot is specifically increased in rodents fed a cafeteria diet. Gonadal VAT has been shown to express more of *Pparγ* and *Srebp1c* as well as another key adipogenic transcription factor, CCAAT enhancer-binding protein alpha (*C/EBP-alpha*) compared to SAT [101]. Interestingly, PPARγ2 protein was found at a significantly higher level in female than in male rat perigonadal adipose tissue [81], indicating that sex hormones can affect the regulation of PPARγ2 in WAT. It is possible that this could contribute to the gender differences observed with different PPAR agonists.

5.7. BAT. The largest region of brown adipose tissue is found in the upper back region of rodents (interscapular BAT). In humans, small areas are found in the thorax region (supraclavicular), and in the chest and abdomen [44]. There are indications that the nonshivering heat generation is important for the development of obesity [48]. Mice lacking UCP1 demonstrates increased obesity with age when fed high-fat diets [102], and selective destruction of BAT leads to obesity and reduced energy expenditure and insulin resistance [103]. Interestingly, overnurished rat pups displayed excess weight gain, reduced thermogenic capacity, and lower levels of UCP1 as adults [104]. It seems

that reduced amount and thermogenic capacity of BAT may contribute to a life-long predisposition for obesity. Although adult humans possess only a small percentage of BAT compared to WAT, the possibility to pharmacologically increase BAT amount or activity would be a potential treatment for obesity. Recent studies have demonstrated that the forced expression of transcription factors PRDM16 and C/EBP-beta is sufficient to generate brown fat cells from myoblastic precursors [105]. The activation of PPARγ by different agonists has been shown to induce the expression of brown adipose genes in white adipocytes [106], the differentiation of brown adipose progenitor cells [107], and the proliferation of BAT in rats and monkeys [108]. However, long-term activation of the sympathetic nervous system by drugs such as ephedrine and BAT proliferation with the PPARγ agonist the thiazolidinedione darglitazone have been linked to numerous side effects [108, 109].

6. Targeting Mitochondrial Function by Bioactive Components

6.1. The Effect of Dietary Supplementation with Bioactive Lipids. Adipose tissue plays a major role in the inflammation, insulin resistance, and dyslipidemia associated with obesity. It is therefore beneficial to use compounds that therapeutically target adipose tissue to avoid high-risk complications of obesity such as T2DM and CVD. Dietary lipids may influence the energy balance and are shown to have a differential effect on different fat depots. Studies in rats have shown that fish oil or n-3 polyunsaturated fatty acids (n-3 PUFA) intervention reduces epididymal and perirenal adipose tissues although the body weight stays constant [110, 111]. It is believed that fish oil can affect adipose tissue by the activation of PPARα and γ, leading to decreased lipolysis, improved lipid storage capacity in subcutaneous adipose tissue, as well as anti-inflammatory effects possibly due to inhibitory action on NF-κB [112]. It has been shown that fish oil at 15% (w/w) level of substitution in a 35% high-fat diet prevented fat accumulation in C57BL/6 mice, preferentially in abdominal fat depots [113–115]. This dose of fish oil also induced mitochondrial biogenesis in white fat, with a stronger effect in epididymal than in subcutaneous adipose tissue [114]. AMPK could be involved in this metabolic switch that increases adipocyte fatty acid metabolism, decreases lipolysis, and upregulates mitochondrial biogenesis [116]. The anti-inflammatory effect of n-3 PUFA in mice has been associated with increased adiponectin secretion [116, 117], possibly resulting from AMPK activation. This induction of adiponectin is probably mediated by PPARγ and is higher in epididymal fat than in SAT [118].

The artificially made 3-thia sulphur fatty acid tetradecylthioacetic acid (TTA) has a targeted effect on mitochondria and exhibit high potency compared to fish oil, both with regard to increased fatty acid β-oxidation and anti-inflammatory effects [119]. TTA thus shows great promise in the treatment of metabolic syndrome (Figure 3). It is a 16-carbon saturated fatty acid where a sulfur atom is inserted between the second and the third carbon in the β-position, thus making it unavailable for fatty acid β-oxidation. The mechanism of action of TTA is based mainly on the activation of PPARs, which induce mitochondrial biogenesis and enhance fatty acid oxidation in the liver [119–121]. This has been suggested to remodel WAT tissues due to drainage of fatty acids towards the liver. TTA was shown to specifically reduce epididymal fat in young obese Zucker (fa-fa) rats and epididymal and retroperitoneal fat in male Wistar rats fed a high-fat diet for 3 weeks [122]. To further study the effect of TTA-induced adipose tissue remodelling, Wistar rats were fed a high-fat lard diet (40% energy from fat) for 7 weeks, and the distribution of fat was studied by magnetic resonance imaging (MRI). The adipose tissue mass was significantly reduced in the TTA-fed rats, particularly in the perirenal and epididymal depots [123]. Interestingly, while most of the genes involved in lipolysis, fatty acid β-oxidation, mitochondrial biosynthesis, and immune response were unchanged by TTA in all studied adipose tissues, *Ucp1* was highly increased at the mRNA level in epididymal and mesenteric depots [123]. Also, a *de novo* production of *Ucp3* in the liver was detected both at the mRNA and protein level along with high induction of genes involved in β-oxidation [123]. The possible increased energy expenditure due to uncoupling of mitochondria combined with the enhanced hepatic fatty acid β-oxidation supports the hypothesis of hepatic lipid drainage. As a result, TTA treatment will channel TGs to the liver for β-oxidation and facilitate adipose tissue lipolysis. The higher metabolic activity of the VAT tissues could explain the specific reduction of these risk-linked depots, perhaps also influenced by their increase in BAT-like features (Figure 2). Although the effect of TTA is mostly due to activation of hepatic mitochondrial genes, activation of some adipose genes involved in β-oxidation was observed. The lipogenic genes acetyl-CoA carboxylase alpha (*Acaca*) and fatty acid synthase (*Fasn*) were induced by TTA in perirenal and epididymal VAT [123]. In a directly comparable long-term rat study, the 3-n PUFA eicosapentaenoic acid (EPA) was found to increase *Acaca* and *Fasn* in VAT while *Cpt2* mRNA-level was increased in mesenteric VAT, indicating an increase in genes involved in fatty acid degradation also with marine oils [124]. *Ucp1* was on the other hand increased only by TTA. Similar results have been found in human adipocytes (not published results). TTA and EPA were able to significantly increase the gene expression of *AMPK*, *CPT2*, *UCP1*, and acyl-coA oxidase 1 (*ACOX1)*, but TTA induced a higher level of *AMPK* and *UCP1*. Importantly, AMPK is believed to function as a metabolic switch resulting in upregulation of FA oxidation in parallel with reduced maloyl-CoA level and downregulated lipogenesis and TG biosynthesis.

The mitochondrial gene pyruvate kinase (*Pklr*) was upregulated by TTA specifically in perirenal VAT [123], indicating mitochondrial biogenesis in this fat depot. This is important as the generation of new mitochondria is severely compromised in several obesity-models [36, 43]. In accordance, a coactivator central for mitochondrial biogenesis, PPAR coactivator 1α (*Pgc-1*α) was reduced in a rodent obesity model [125] and in morbidly obese humans [126].

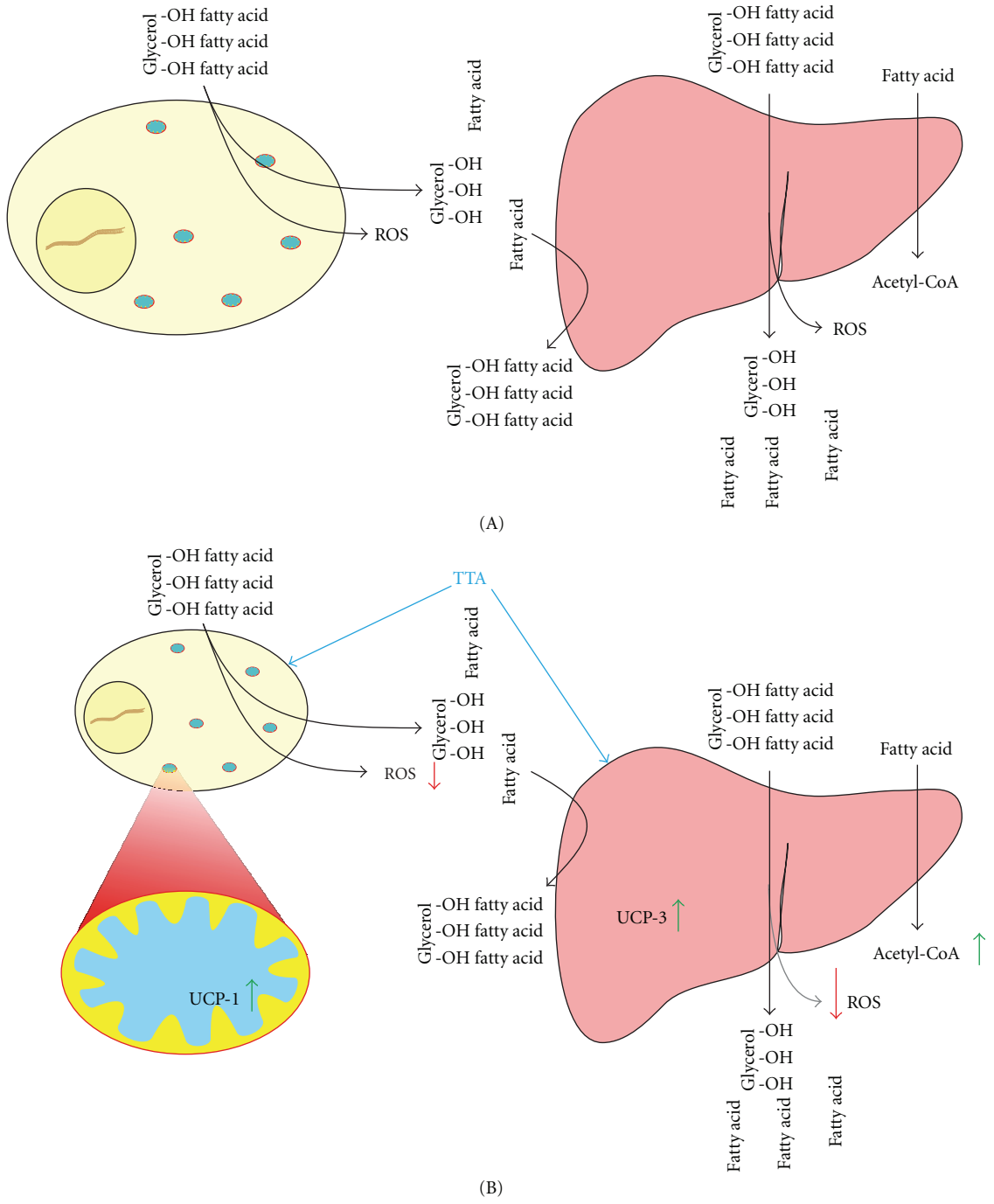

Figure 3: (A) During obesity, the adipocytes will have reduced lipid storage capacity, leading to increased lipolysis and release of free fatty acids (FFAs), inflammatory agents, and disturbed adipokine release. The FFAs will be repackaged to triglycerides (TGs) in the liver where they are released as very low-density lipoprotein particles (VLDL). Together this has secondary effects on organs such as skeletal muscle and pancreas, as well as liver, and can result in hyperlipidemia, tissue lipid deposition, mitochondrial malfunction, insulin resistance, increased insulin production, and pancreatic β-cell disruption. (B) The effect of tetradecylthioacetic acid-(TTA-) treatment on metabolic syndrome. In the liver, TTA treatment will increase the degradation of FFA, by the induction of both mitochondrial and peroxisomal genes involved in β-oxidation. The excess FFA released from adipose tissue during metabolic syndrome (hyperlipidemia) is drained from the blood, thereby reducing TG, cholesterol, and FFA levels. In addition the TTA-induced increase in uncoupling protein 3 (UCP3) could increase energy expenditure, as well as function as a protection from the excess ROS production observed with obesity and high levels of FA degradation. The effect of TTA on liver is probably due to PPARα-mediated mechanisms, while the effect on adipose tissue may arrive from PPARγ as well as PPARα activation. In adipose tissue, the main effect of TTA is an increase in the brown adipose tissue marker *Ucp*1 in visceral adipose tissue (VAT) indicating higher energy expenditure and heat production. The higher metabolic activity of VAT compared to SAT will cause it to be the major source of FFA during increased hepatic β-oxidation. Together, this may explain the specific reduction of these risk-linked adipose depots with TTA-treatment.

Although TTA reduces dyslipidemia in diabetic patients [121], a direct effect on glucose metabolism and insulin sensitivity is more disputed [121, 123, 127]. A reduced amount of risk-related adipose tissue in obese individuals would however be important to reduce the likelihood of developing disease. The efficient reduction of plasma FFA and TG by TTA, seen in both human and animal studies [128], may also prevent ectopic lipid deposition and lipotoxic effects.

TTA induces mitochondrial biogenesis in liver, but the effect on mitochondrial respiration has not been fully elucidated. Although the number of mitochondria increases in the liver during TTA-treatment, the hepatocytes are enlarged, and the respiration per mg tissue stays constant (not published results). Little work has been done to measure oxygen consumption/OXPHOS in adipose tissue, probably due to the difficulties caused by a low mitochondrial density in adipocytes compared to skeletal muscle [129]. Some studies have successfully determined mitochondrial OXPHOS in cells or organelles isolated from both brown and white adipose tissues [130], and VAT and SAT have been compared using similar techniques [38]. Information on mitochondrial OXPHOS activity will be of importance when testing for improved adipose tissue function during treatment with mitochondrial-targeted compounds.

6.2. Regulation of Gene Expression in the Treatment of Obesity-Related Diseases. PPAR is the target of many agents that restore insulin sensitivity. However, they do not necessarily decrease adipose tissue size. Thiazolidinediones (TZDs), such as pioglitazone and rosiglitazone, activate PPARγ and are associated with increased body weight due to the ability to stimulate preadipocyte differentiation [131, 132]. In particular the retroperitoneal VAT [133, 134] and intermuscular fat infiltration was increased by TZDs [134]. Studies show that the activation of PPARα by fenofibrates or oleoyletanolamide reduces adiposity and increases lipolysis [132], while a pan-PPAR ligand, LM 4156, had no effect on adipose tissue size [134]. Another study in accordance with this showed that a PPARα agonist reduced feed intake and body weight gain but had less effect on glucose intolerance (GI), a PPARγ agonist improved GI and adiponectin release but enhanced feed intake and body weight gain, while an agent with dual PPAR-activation demonstrated a combined, more beneficial effect [135]. TTA activates both PPARα and γ, the former with higher efficiency [136–138], and this may partly explain the effect on lipid degradation in the liver, as well as the reduced weight gain and hepatic induction of UCP3 primarily associated with PPARα activation [132, 139]. However, we have shown the existence of PPAR dependent and independent induction of UCP2 by TTA [140]. Therefore, mechanisms not involving PPAR-activation may also be important for the effects seen with TTA.

6.3. ROS Generation and TTA as an Antioxidant. There is mounting evidence that the increased release of H_2O_2, mainly originating from mitochondria, may be an important step in the development of insulin resistance in different tissues during high-fat intake [15, 16]. Mitochondrial damage is linked to insulin resistance and could be a secondary effect of the increase in ROS [141]. A number of studies have shown that treatment with antioxidants will both improve oxidative stress and restore insulin sensitivity [141–143]. While antioxidants studied so far have had little effect on human atherogenesis, it could be worthwhile to investigate mitochondrial-targeted antioxidants in the treatment of CVD [5].

TTA mainly affects mitochondrial functions and is a very potent antioxidant [144, 145] and anti-inflammatory agent [120, 146], both of which may be important for its reduction of dyslipedemia in diabetic patients [121]. TTA inhibits the lipoprotein oxidation in rats, indicating that its effects as an antioxidant may influence the development of atherosclerosis [145]. It also reduces the stenosis development after balloon angioplasty injury of rabbit iliac arteries [147]. Recently we have shown downregulated expression of PPAR target genes and reduced mitochondrial fatty acid oxidation in the liver of mice transgenic for hTNFα [148]. TTA enhanced the hepatic fatty acid oxidation in these animals (in preparation). Moreover, TTA reduced the hepatic gene expression of TNFα and visfatin [123].

7. Conclusions

The different adipose depots have specific roles based on their level of lipolysis and rate of TG storage. It is becoming clear that while abdominal visceral adipose tissue increases the risk of obesity-related disease, subcutaneous adipose tissue, especially located to the lower-body parts, protects from lipotoxic effects through short-term and long-term storage of TGs. In rodent studies, intake of bioactive lipids gives specific reduction of risk-related adipose tissues, and this targeted effect may be due to the higher metabolic activity of these depots. Treatments that increase energy expenditure through "mild uncoupling" of mitochondria and fatty acid β-oxidation show great promise in the treatment of obesity. The artificial fatty acid TTA is an inducer of these processes. Enhanced expression of uncoupling proteins together with an induction of hepatic β-oxidation suggests that TTA may increase energy consumption via increased uncoupling in liver and/or WAT. In addition its activity as an antioxidant and anti-inflammatory agent will have a direct positive effect on the diseases linked to obesity, such as T2DM and CVD.

Abbreviations

AMPK: Adenosine monophosphate-activated protein kinase
BAT: Brown adipose tissue
CPT: Carnitine palmitoyltransferase
CVD: Cardiovascular disease
DMT2: Diabetes mellitus type 2

dSAT: Deep subcutaneous adipose tissue
EAT: Epicardial adipose tissue
FFA: Free fatty acid
IMAT: Intermuscular adipose tissue
ME: Mesenteric adipose tissue
MRI: Magnetic resonance imaging
NOX: NADPH oxidase
OXPHOS: Oxidative phosphorylation
PPAR: Peroxisome proliferator-activated receptor
PUFA: Polyunsaturated fatty acid
ROS: Reactive oxygen species
SAT: Subcutaneous adipose tissue
sSAT: Superficial subcutaneous adipose tissue
TG: Triglyceride
TTA: Tetradecylthioacetic acid
UCP: Uncoupling protein
VAT: Visceral adipose tissue
WAT: White adipose tissue.

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Research Article

Oral Administration of Semicarbazide Limits Weight Gain together with Inhibition of Fat Deposition and of Primary Amine Oxidase Activity in Adipose Tissue

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An enzyme hitherto named semicarbazide-sensitive amine oxidase (SSAO), involved in the oxidation of primary amines, is abundantly expressed in adipocytes. Although SSAO physiological functions remain unclear, several molecules inhibiting its activity have been described to limit fat accumulation in preadipocyte cultures or to reduce body weight gain in obese rodents. Here, we studied whether oral administration of semicarbazide, a prototypical SSAO inhibitor, limits fat deposition in mice. Prolonged treatment with semicarbazide at 0.125% in drinking water limited food and water consumption, hampered weight gain, and deeply impaired fat deposition. The adiposomatic index was reduced by 31%, while body mass was reduced by 15%. Such treatment completely inhibited SSAO, but did not alter MAO activity in white adipose tissue. Consequently, the insulin-like action of the SSAO substrate benzylamine on glucose transport was abolished in adipocytes from semicarbazide-drinking mice, while their insulin sensitivity was not altered. Although semicarbazide is currently considered as a food contaminant with deleterious effects, the SSAO inhibition it induces appears as a novel concept to modulate adipose tissue development, which is promising for antiobesity drug discovery.

1. Introduction

Pharmacological agents known to inhibit a membrane enzyme involved in the oxidation of various primary amines, and hitherto named semicarbazide-sensitive amine oxidase (SSAO, E.C. 1.4.3.6, pending novel classification E.C. 1.3.4.21) have been reported to limit body weight gain in diverse animal models. Most of these observations of such "slimming effect" with the tested agents were not expected since they were evidenced in studies initially focused on vascular pharmacology. The first reported observation was made by Yu and coworkers in the obese and diabetic KKAy mice [1]. Whilst the authors aimed to demonstrate an antiatherogenic effect of (E)-2-(4-fluorophenethyl)-3-fluoroallylamine (FPFA), they observed that this compound reduced weight gain in obese KKAy mice fed an atherogenic diet. In the same report, Yu and colleagues demonstrated that FPFA was able to inhibit both SSAO and monoamine oxidases (MAO). More recently, by performing pharmacological research on arterial thickness alteration, Mercier and colleagues repeatedly administrated the reference inhibitor of SSAO, namely, semicarbazide, at 100 mg/kg bw/d to Sprague-Dawley [2], and Brown-Norway rats [3]. In both models, the authors observed a dramatically reduced body weight gain in response to the SSAO inhibitor, which also induced a decrease in the pressure resistance of arteries, as initially expected.

We have also reported that aminoguanidine, which inhibits SSAO [4] together with nitric oxide synthases (NOS) and diamine oxidase (DAO) [5], was able to limit WAT extension without notably altering calorie intake and body weight gain in obese Zucker rats [6]. Then, we observed that the combined inhibition of SSAO and MAO, obtained by repeated injections of semicarbazide plus pargyline, or

by daily i.p. administration of phenelzine (an antidepressant which inhibits both MAO and SSAO), produced concomitant limitation of body weight gain in the obese Zucker rat [7, 8]. Therefore, at least four distinct pharmacological agents tested *in vivo* to inhibit SSAO were able to alter energy balance and to lower body weight gain in rodents.

SSAO is historically known for its presence in vessels [9]: in endothelial cells, where it is known as SSAO/VAP-1 owing to its vascular adhesion properties [10] and in smooth muscle cells, where it is involved together with another copper-containing amine oxidase, the lysyl oxidase, in extracellular matrix maturation [2, 11]. In fact, SSAO is also highly expressed in white adipose tissue (WAT) [12]. Tissue-distribution studies have recently evidenced that the SSAO amount in adipocytes is extremely elevated, regarding gene expression [13], protein abundance [14], or activity level [6], including in man [15]. With an approach aiming at unravelling the role for such SSAO abundance at the surface of fat cells [15], we observed *in vitro* that exogenous amines exert insulin mimicry when added to adipocyte preparations. Actually, at submillimolar concentrations, benzylamine elicits, in a SSAO-dependent manner, an activation of glucose transport and an inhibition of lipolysis in isolated fat cells, from human [15] or rodent origin [16]. In addition, benzylamine [17], methylamine [18], or other SSAO substrates [19], activate adipocyte differentiation in several preadipocyte lineages and therefore partly reproduce the adipogenic action of insulin. Lastly, *in vitro* experiments showed that the hydrazine derivative phenelzine (which inhibits SSAO) alters the adipocyte differentiation of cultured human and mouse preadipocytes [20]. We therefore hypothesized that endogenous or dietary amines may reproduce *in vivo* such anabolic insulin-like effects, and if the amines can reach WAT, any sustained pharmacological inhibition of their oxidation could hamper fat deposition. In this context, it appeared essential to verify whether inhibition of fat mass extension was an important issue occurring during the body weight gain reduction induced by the above-mentioned agents, all of them sharing the property to inhibit SSAO.

This prompted us to further test whether the *per os* administration of the prototypical SSAO inhibitor, semicarbazide, was limiting fat deposition. The following results show that, when given in the drinking water, semicarbazide not only inhibited the SSAO activity in WAT, but also limited food consumption, and to a larger extent, hampered fat accretion in both visceral and subcutaneous fat depots. Moreover, the prevention of body weight gain observed during oral administration of semicarbazide was not accompanied by any worsening of the plasma levels of metabolites or oxidative stress markers. Finally, since semicarbazide oral toxicity has been recently suspected [21–23], the need for further investigations of the putative antiobesity effects of other SSAO inhibitors is discussed.

2. Materials and Methods

2.1. Animals and Treatments. FVB/n male mice (Charles River, l'Arbresle, France) were separated in two groups of 8 mice with equivalent body weight at the age of 5 weeks. They were housed at 2 animals per cage with unlimited access to standard rodent chow (Global rodent diet, Harlan, France) and water (control), or to a semicarbazide solution (semicarbazide-drinking). Semicarbazide hydrochloride (Sigma-Aldrich, Saint Quentin Fallavier, France) was dissolved in drinking water and given as a 0.125% solution that was changed weekly. Body mass, food, and water consumption were checked weekly, and at the end of an 8-week treatment period, the mice were sacrificed after overnight fasting. Ten other 10-wk-old FVB/n mice grown under standard conditions were used for tissue distribution study of SSAO activity and preliminary lipolytic studies. Housing conditions and experimental procedures followed in the IFR 150 animal unit were in accordance with the European Union regulations on the use of animals for scientific research.

2.2. Tissue Sampling and In Vitro Functional Assays on Adipocytes. Once obtained, plasma was immediately frozen at −80°C, and circulating metabolites (glucose, insulin, triglycerides, fatty acids, etc.) were determined using a Cobas-Mira + multi-analyser, according to the manufacturer' instructions (Roche, Neuilly, France). Once weighed, the perirenal, retroperitoneal, and epididymal white adipose tissues were pooled (and named as visceral WAT), immediately digested to obtain adipocyte preparations for glucose uptake assays and lipolysis measurements, as previously described [8], or for cell size determination under microscope using Lucia G software (Nikon). Portion of the tissues were also frozen for further determinations of DNA and protein contents and for amine oxidase assays. DNA content was assessed in WAT after proteinase K digestion, chloroform/ethanol extraction, and 260/280 nm spectrophotometric readings. Protein content was determined using DC Protein Assay kit (BioRad, Hercules, CA).

2.3. In Vitro 2-Deoxyglucose Uptake Assay. Freshly isolated adipocytes were diluted in around 10-fold their volume of Krebs-Ringer containing 15 mM sodium bicarbonate, 10 mM HEPES, 2 mM pyruvate, and 3.5% serum bovine albumin. Then 400 μL of cell suspension was distributed into plastic incubation vials and incubated 45 min at 37°C with the tested agents, just before 10 min exposure to 0.1 mM [^{3}H]-2-deoxyglucose (2-DG). Separation between extracellular and internalized hexose was performed on 200 μL aliquots by centrifugation through dinonyl-phthalate layer which allowed to separate buoyant intact fat cells from medium, as previously described [6]. 2-DG uptake was expressed as fold increase over basal uptake or even as percentage of maximal response to insulin, with basal uptake set at 1 or at 0%, respectively [8].

2.4. Amine Oxidase Activity. Oxidative deamination was measured by extracting the oxidation products of 0.1 mM [^{14}C]-benzylamine (from Amersham Biosciences, Buckinghamshire, UK) or 0.5 mM [^{14}C]-tyramine (from Sigma-Aldrich, St Quentin Fallavier, France) after incubation for 30 min in 200 μL of 200 mM phosphate buffer, pH 7.5, in the

presence of protease inhibitors and approx. 100 μg protein of homogenates prepared just before assays from thawed tissues, as previously described [24]. 15-min preincubation with 1 mM semicarbazide or 0.5 mM pargyline was used to selectively inhibit SSAO or MAO activity, respectively, as previously reported [25].

2.5. Gene Expression Analysis. Real-time quantitative RT-PCR was performed using oligonucleotide primers specific for the indicated genes, designed with Primer Express software (Perkin-Elmer Life Sciences, Courtaboeuf, F), and the sequences of which are reported in [25], for *MAO-A*, *MAO-B*, *AOC2,* and *AOC3*, or in [26] for adipokines, or either given as supplemental data to [27]. Total RNAs were extracted from mature adipocytes, using RNeasy minikit, then reverse-transcribed using random hexamers and Superscript II reverse transcriptase (Invitrogen, Cergy-Pontoise, F). Reactions without reverse transcriptase (RT-) were performed in parallel to estimate DNA contamination. Real-time RT-PCR was performed using the primers in the presence of 6.25 ng cDNA and SYBR green Universal PCR Mix (Eurogentec, Angers, F). Fluorescence was analysed in an ABI PRISM 7500 Sequence Detection device (Taqman, Applied Biosystems, Foster City, CA). Analysis of 18 S RNA was performed in parallel using the Ribosomal RNA Control Taqman Assay Kit (Applied Biosystems) to normalize gene expression as already reported [25, 26]. For each gene, results were expressed as arbitrary units: $2^{(\text{Ct18S-Ctgene})} \times (1 - 1/(2^{\text{Ct RT-Ctgene}})) \times 10^6$, where Ct corresponds to the number of cycles reaching fluorescence threshold.

2.6. Statistical Analysis. Data are given as means ± standard error of the mean (SEM). Comparisons between semicarbazide-treated and control groups were determined using an unpaired Student's *t*-test.

3. Results

3.1. Tissue Distribution of SSAO Activity in Mice. Table 1 shows that the SSAO activity was higher in adipose depots than in any other studied organ. As previously reported [28], the oxidation of the prototypic SSAO substrate benzylamine was inhibited by semicarbazide in all the homogenates. However, the visceral WAT of FVB/n mouse was richer in SSAO activity than the subcutaneous WAT and than the interscapular brown fat. Aorta, duodenum, and kidney exhibited a lower capacity to oxidize benzylamine than the adipose depots.

3.2. Changes in Body Weight and Adiposity in Semicarbazide-Drinking Mice. FVB/n mice exhibits a rapid growth after weaning, which accounts for a week increase of about 10% of their body mass. We have therefore tested whether semicarbazide oral treatment could hamper adipose tissue development during such period of intense anabolism and substantial fattening. To this aim, semicarbazide was administered at 125 mg/100 mL in the drinking water of 5-week-old mice. Since changes in body mass and in water consumption occurred between the beginning and the end of the 8-week treatment, the ingestion of such dose of semicarbazide was calculated to reach a mean daily intake comprised between 123 and 150 mg/kg/day (equivalent to 1100–1350 μmol/kg/d). This range is similar to the dose used in toxicology studies showing no signs of any distress [29] and is lower than the oral LD_{50} stated in mouse (225 mg/kg, available at http://www.carl-roth.de/jsp/en-de/sdpdf/4681e.pdf). The semicarbazide oral administration clearly limited the growth of young mice. An immediate decline was detected in the growth curve after the first week of treatment, while retardation persisted throughout the treatment (Figure 1). At the end of the treatment, the semicarbazide-drinking mice weighed 15% less than their control, since body weights were 24.7 ± 0.6 and 29.1 ± 0.6 g, respectively ($n = 8$, $P < .001$). The subsequent lowered body weight gain found after 8 weeks of semicarbazide ingestion (2.4 ± 0.5 g) represented only one third of that observed in the control group (6.9 ± 0.7 g, $n = 8$, $P < .001$). It was concomitant with a significant reduction in the cumulative water and food consumption, observed during the same period (equivalent to a 29% and 12% reduction, resp., not shown).

A clear reduction of adiposity was found in the semicarbazide-drinking mice, at least when comparing the mass of the epididymal, perirenal, retroperitoneal, and inguinal fat pads (Figure 2). Since the body weight was lower in the semicarbazide-treated mice, it was more convenient to compare the adiposomatic index between the two groups, that is, the percentage of the sum of dissected fat depots relative to body weight [8]. This index fell from 5.8 ± 0.3% to 4.0 ± 0.3% ($n = 8$, $P < .001$), corresponding to a 31% reduction. The mass of the interscapular brown fat pad was also reduced by approximately 20% in semicarbazide-drinking mice: 82 ± 4 versus 103 ± 6 mg in control ($P < .01$).

Two approaches were performed to further examine the limitation of WAT development. First, the determination of adipocyte diameter distribution clearly showed a fall in the proportion of the larger adipocytes, together with an

Table 1: Tissue distribution of semicarbazide-sensitive amine oxidase activity in control mice.

Tissue	Semicarbazide-inhibited oxidation of 0.1 mM benzylamine (nmoles/mg protein/min)
Visceral WAT	4.402 ± 0.368 (8)
Subcutaneous WAT	2.250 ± 0.144 (4)
Interscapular brown fat	0.860 ± 0.027 (4)
Aorta	0.506 ± 0.064 (10)
Duodenum	0.201 ± 0.018 (4)
Kidney	0.018 ± 0.005 (10)

Homogenates were preincubated for 15 min without or with 1 mM semicarbazide before a 30-minute incubation in the presence of 0.1 mM [^{14}C]-benzylamine. The semicarbazide-inhibited oxidation is representative of the SSAO activity. Mean ± SEM of the number of determinations indicated in parentheses. Analysis of variance showed that the factor tissue was highly influencing SSAO activity ($P < 10^{-18}$).

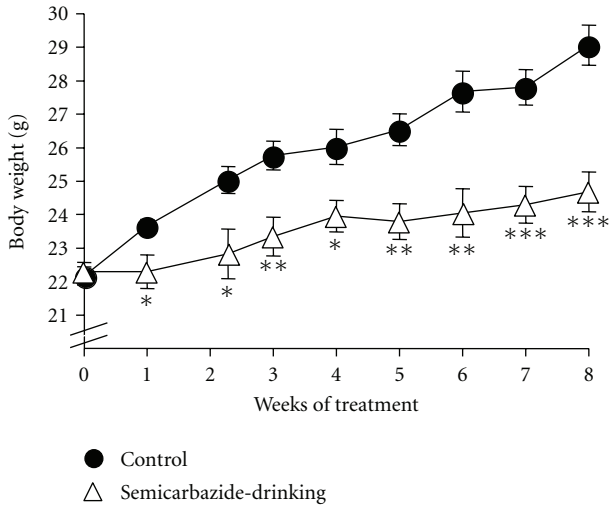

Figure 1: Body weight changes in mice treated or not with orally administered semicarbazide. Two groups of age- and weight-matched mice were constituted after weaning and before oral administration of semicarbazide (week 0), given at 125 mg/100 mL in drinking solution (semidrinking, open triangles), while control (black symbols) had free access to tap water. Mean ± SEM of 8 determinations. Different from corresponding control at $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

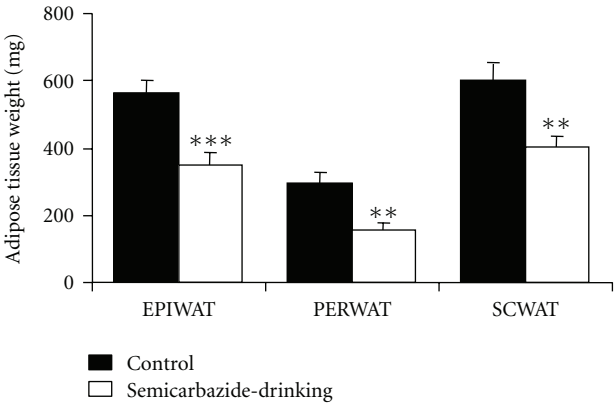

Figure 2: Effect of chronic semicarbazide ingestion on adipose tissue mass in male mice. Mean ± SEM of 8 determinations of epididymal (EPIWAT), perirenal + retroperitoneal (PERWAT), or inguinal (SCWAT) fad pad weights. Different from corresponding control (black columns) at $^{**}P < .01$, $^{***}P < .001$.

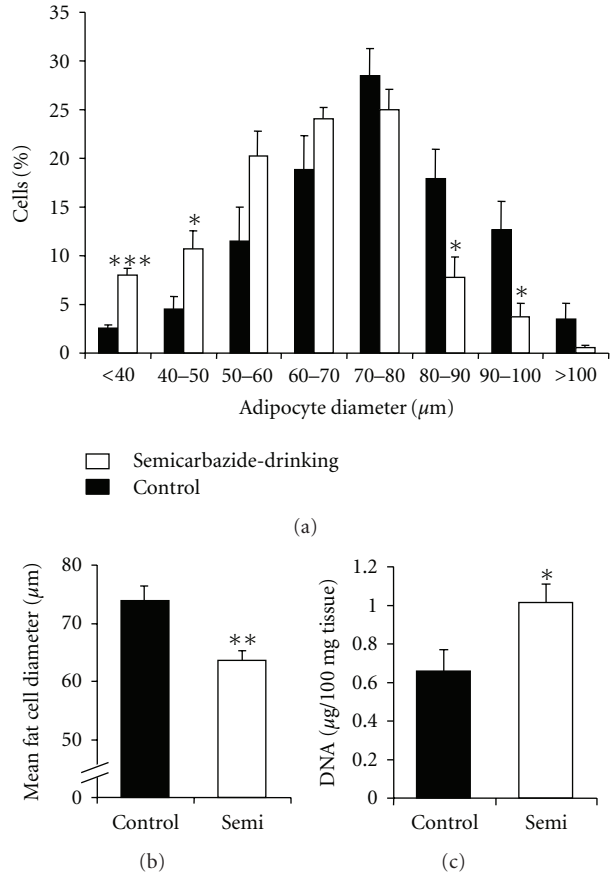

Figure 3: Effect of chronic semicarbazide ingestion on the cellularity of visceral adipose tissues. Adipocytes isolated from visceral adipose tissues were subjected to size analysis and counted according to the indicated distribution of cell diameter classes (a). Mean fat cell diameter was calculated (b) while DNA was extracted from pieces of visceral WAT and determined as described in Section 2 (c). Mean ± SEM of six mice per group. Different from control at $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

increase in the proportion of small fat cells (Figure 3(a)). This resulted in a significant decrease of the mean fat cell size (Figure 3(b)), reflecting an impaired lipid accumulation inside the fat cells. Second, the DNA content per 100 mg tissue was greater in WAT from semicarbazide-drinking mice than in control, indicating that more numerous and smaller cells were present per unit of tissue mass in the treated than in the control animals, the fat depots of which contained larger, heavier, lipid-laden adipocytes (Figure 3(c)). These two approaches, together with the lack of increased adipogenic markers (see below), indicated that the WAT of semicarbazide-drinking mice partially escaped to the age-dependent lipid accumulation and contained adipocytes having a reduced cell size/mass when compared with age-matched control mice.

Moreover, the maximal lipolytic activity of the adipocytes was elevated in the semicarbazide-treated group: in fat cells isolated from epididymal plus retroperitoneal and perirenal fat pads (pooled as visceral WAT, to obtain sufficient amount of biological material for the various determinations), the glycerol released in response to 10 μM isoprenaline was 3.14 ± 0.72 versus 1.90 ± 0.20 μmol/100 mg lipids/90 min in control ($n = 4$, $P < .05$). Such observation supported that these small adipocytes were more metabolically active than larger fat cells. Therefore, chronic semicarbazide ingestion clearly appeared to prevent adipocyte hypertrophy and to facilitate lipid mobilization.

3.3. Benzylamine-Dependent Effects in Adipocytes. Of worth was to investigate whether the insulin-like effects of amines

were abolished in fat cells after SSAO blockade. The glucose uptake was measured *ex vivo* in adipocytes isolated from visceral WAT. The submaximal stimulation of deoxyglucose uptake by 10 nM insulin reached 2.9 ± 0.3-fold increase over basal in control adipocytes, while it was equivalent to a 4.5 ± 0.5-fold increase in semicarbazide-drinking mice ($n = 8$, $P < .01$). Again, the smaller fat cell size of semicarbazide-treated mice likely accounted for such enhanced insulin responsiveness. On the opposite, the insulin-like effect of benzylamine was dramatically impaired in the semicarbazide-treated mice, especially when the SSAO substrate was tested at 0.1 mM in the presence of 0.1 mM vanadium (Figure 4). The combination of vanadium and benzylamine produced more than 80% of the maximal insulin effect in control cells, as previously reported [16], whereas this effect did not exceed 25% after semicarbazide treatment. Similarly, the weak effect of benzylamine alone on glucose uptake also exhibited a tendency to be reduced in semicarbazide-treated mice (not shown).

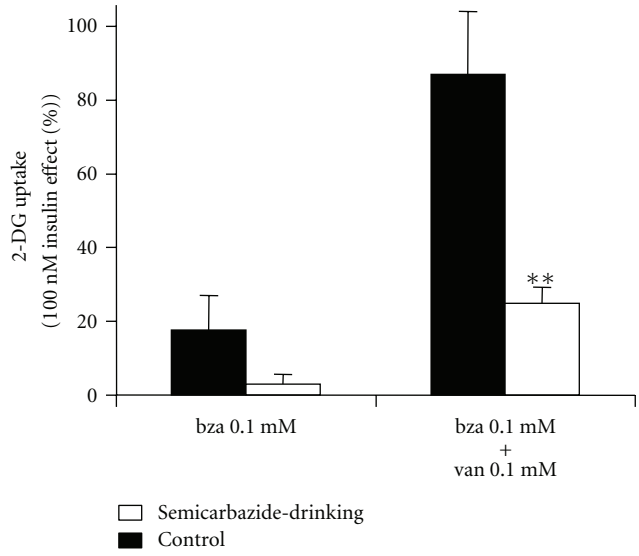

Figure 4: Influence of 8-week oral administration of semicarbazide on benzylamine-induced glucose transport in mouse adipocytes. Visceral adipocytes were incubated with 0.1 mM benzylamine alone (bza) or combined with 0.1 mM vanadate (bza + van) for 2-DG uptake assay. Data are expressed as percentage of response to 100 nM insulin, which maximally stimulated basal uptake by 3.3 ± 0.4-fold in control (black columns) and by 4.8 ± 0.8-fold in semicarbazide-drinking mice (open columns). Mean ± SEM of 8 determinations. Different from control at $^{**}P < .01$.

3.4. Amine Oxidase Activities in Adipose Tissue. It was then verified whether SSAO activity was really blocked in WAT after semicarbazide treatment. As shown in Figure 5(a), the oxidation of benzylamine was totally abolished in the subcutaneous WAT of semicarbazide-drinking mice. This was in agreement with the fact that, in control WAT, benzylamine oxidation was exclusively SSAO-dependent. On the contrary, tyramine was oxidized by SSAO and MAO as well. Its oxidation was lower in the semicarbazide-treated mice than in control. The remaining tyramine oxidation found after semicarbazide treatment was explained by an unaltered MAO-dependent oxidation together with an almost total disappearance of the SSAO component (Figure 5(b)). A similar obliteration of SSAO activity was found in visceral WAT after semicarbazide treatment: the oxidation of 0.1 mM benzylamine fell from 2.74 ± 0.28 to 0.06 ± 0.02 nmol/mg protein/min ($n = 6$, $P < .001$).

The total blockade of SSAO activity obtained after semicarbazide treatment was not accompanied by any alteration of MAO activity. This was supported by the lack of change in the expression of genes encoding for the monoamine oxidases A and B in adipocytes (Table 2). Similarly, there was no change for AOC2 gene, which is poorly expressed in WAT. More surprising was the decline in AOC3 expression, the gene encoding for SSAO, traducing that prolonged semicarbazide treatment did not only inhibit the SSAO enzymatic activity but also reduced its expression in WAT.

3.5. Semicarbazide-Induced Changes in Gene Expression in Adipocytes. To further analyse the changes occurring in adipose tissue after semicarbazide treatment, the expression of several genes was quantified in adipocytes from visceral WAT. As expected, the leptin expression was strongly inhibited in adipocytes from the smaller fat pads found in the semicarbazide-treated group (Table 2). However, no noticeable change in the expression of other adipokines was found, irrespective of their detrimental (TNFα, IL-6, resistin) or beneficial (adiponectin) effect on insulin resistance, with the exception of apelin, the mRNA abundance of which exhibited a 50% reduction that failed to reach statistical significance ($P < .1$). Regarding the genes involved in energy supply to adipocytes, there was no clear change in LPL or FAS expression (Table 2). The expression of the ubiquitous glucose transporter GLUT1 was reduced, but not that of the insulin-sensitive GLUT4. Catalase expression remained unaffected (not shown). Lastly, the expression of the adipogenic markers aP2 and PPARγ_2 genes was unchanged in the semicarbazide-treated group (they were equivalent to 77% and 93% of the levels found in control, resp.) while a tendency to decrease was found for the macrophage marker F4/80 (not shown). Taken together, these last observations argued that the increased DNA content per mass unit of WAT was not due to an increased adipogenesis or to an infiltration of immune cells but was rather the consequence of the reduced size of the adipocytes: being of smaller mass, they were in higher number per 100 mg tissue than in control, the WAT of which was constituted with larger fat cells.

3.6. Absence of Abnormalities in the Circulating Metabolic Parameters of Semicarbazide-Drinking Mice. Regarding the adverse effects that might be provoked by semicarbazide prolonged ingestion, no change was found at the plasma level for the circulating values of glucose, insulin, triglycerides, free fatty acids, or cholesterol (not shown). Similarly, the plasma markers of lipid peroxidation did not differ between control and semicarbazide-drinking mice (malonyldialdehyde equivalents were 23.9 ± 2.0 and 22.5 ± 3.2, resp., $n = 8$).

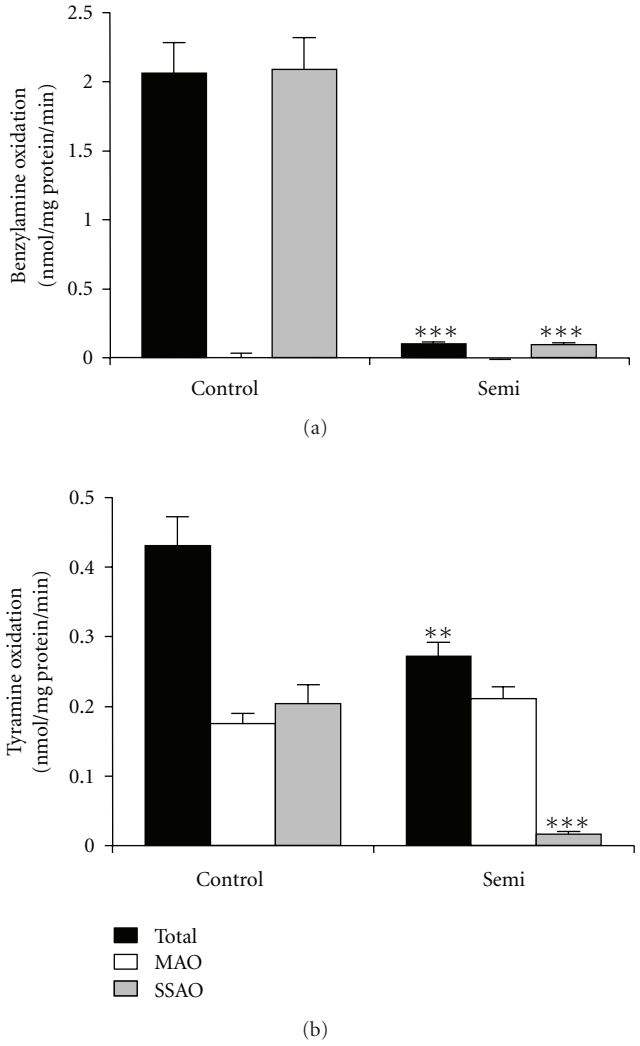

Figure 5: Influence of 8-week oral treatment with semicarbazide on the benzylamine (a) and tyramine (b) oxidation in subcutaneous WAT. Homogenates were preincubated for 15 minutes without (total oxidation, black columns), with 0.5 mM pargyline (in order to determine pargyline-sensitive component as MAO, open columns), or with 1 mM semicarbazide (to determine the semicarbazide-sensitive component as SSAO, shaded columns). Then, they were incubated for 30 minutes in the presence of 0.1 mM benzylamine (a) or 0.5 mM tyramine (b). Mean ± SEM of 6 determinations. Different from corresponding control at $^{**}P < .01$, $^{***}P < .001$.

Table 2: Gene expression in visceral adipocytes from semicarbazide-drinking or control mice.

Gene	Control	Semicarbazide-treated
Amine oxidases:		
MAO-A	66.2 ± 22.3	35.9 ± 11.7
MAO-B	154 ± 21	133 ± 31
AOC2	2.0 ± 0.4	1.5 ± 0.2
AOC3	4750 ± 555	2420 ± 275**
Adipokines:		
Adiponectin	22568 ± 1942	19068 ± 2195
Resistin	3837 ± 300	4143 ± 374
leptin	2068 ± 365	853 ± 80**
IL-6	792 ± 230	718 ± 198
TNF-alpha	16.9 ± 2.6	23.5 ± 8.1
Apelin	26.0 ± 9.3	12.5 ± 4.3
Fuel metabolism:		
GLUT1	187 ± 28	103 ± 18*
GLUT4	440 ± 38	429 ± 51
LPL	47742 ± 3860	39274 ± 3152

mRNA abundance of the indicated genes was determined by rt-PCR and expressed using the arbitrary units detailed in Section 2. Mean ± SEM of 8 determinations. Different from control at $^{*}P < .05$; $^{**}P < .01$.

4. Discussion

The present study highlights decrease in both body weight gain and fattening as marked consequences of semicarbazide subchronic ingestion. The total SSAO blockade found in WAT, together with the decreased lipogenic effect of benzylamine, which can be considered as one representative of the dietary amines reaching visceral fat during the digestion process, led us to propose that pharmacological inhibition of SSAO can be considered as a way to mitigate obesity. Since SSAO is largely expressed in fat depots, a peripheral mode of action can be envisaged, but other central mechanisms, participating to the observed 12% reduction of calorie intake cannot be excluded.

The clear-cut blockade of benzylamine oxidation found in the WAT of treated mice agrees with the fact that benzylamine oxidation is almost exclusively catalyzed by SSAO activity in this tissue. As a consequence, the insulin-like action of the SSAO substrate benzylamine on glucose transport was abolished in adipocytes from semicarbazide-treated mice. This was concomitant with substantial reductions in the fat mass and in the mean fat cell size. Whether the decrease in SSAO expression was a consequence of fat cell size reduction appears unlikely since the shrinking of lipid stores observed during prolonged fasting is not accompanied by a reduction of SSAO activity in adipose depots [28]. On the opposite, agents known to inhibit SSAO have been observed to limit lipogenesis *in vitro* [20]. On the basis of these previous observations, it can be proposed that inhibition of lipogenic effects of endogenous/dietary SSAO substrates participates to the limitation of fat deposition. The reduced number of large adipocytes together with the increased DNA content per WAT mass and the unchanged adipocyte differentiation markers (aP2, PPARγ_2), indicated that the differentiated adipocytes already present at the beginning of treatment, at the age of 5 weeks, were less capable to accumulate lipids in semicarbazide-drinking mice than in the control group. The resulting fat cells of moderate size were present in a larger number per mass unit and consequently gave higher DNA richness per mass unit of WAT, higher lipolytic capacity, and higher insulin responsiveness. In this view, the inhibition of peripheral SSAO, which is abundant in fat cells [15, 25], but which is not expressed in neurones, may represent a fascinating novel

approach to modulate WAT development. Such paradigm is relevant only whether endogenous or dietary amines spontaneously exert insulin-like effects, which is far from being demonstrated. Indeed, it is widely accepted that numerous amines are spontaneously present in foods [30, 31] or may be generated during digestion process by the intestinal flora [32]: for instance, tyramine is present at around 45 mg/kg in rodent chow [33] and benzylamine reaches around 3–10 mg/kg in edible vegetables [34]. However, it has never been demonstrated (or ruled out) whether such dietary amines might exert *in vivo* the lipogenic effects they exhibit *in vitro* [19], and so facilitate fat accretion once ingested.

Moreover, SSAO is not only expressed in fat cells but also in vessels, in endothelial cells, where it is involved in leukocyte extravasation, and in smooth muscle cells, where it is involved in glucose transport [11] and in collagen-elastin maturation [2, 3]. The tendency to decrease the F4/80 macrophage marker level found in mice after semicarbazide exposure is probably related to the anti-inflammatory properties of SSAO/VAP-1 blocking agents [35]. Whether the improved *in vitro* responsiveness to insulin in the adipocytes from semicarbazide-drinking mice is a consequence of such reduced low-grade inflammation or is related to the smaller size of fat cells remains to be determined. In the treated adipocytes, there was no change in the expression of TNFα and IL-6, two cytokines involved in insulin resistance, together with a decrease of the proinflammatory adipokine leptin and a tendency to lower the expression of apelin, another adipokine recently shown to facilitate glucose utilization [36]. Such absence of enhanced WAT inflammation was associated with an absence of oxidative stress (evidenced by unchanged lipid peroxidation markers or catalase content). This context may explain the increased *ex vivo* insulin responsiveness of adipocytes, which was not sufficient to fill the fat cells of semicarbazide-drinking mice via sustained lipogenic pathways, since it was occurring together with a reduced calorie intake. Of interest is to test whether the insulin-sensitizing action of SSAO blockade also occurs acutely in obese insulin-resistant models, and improve the insulin-dependent and independent antilipolytic responses, since adipose tissue lipolysis regulation has been proposed to be instrumental in the treatment of obesity and metabolic syndrome [37].

The major concern is that semicarbazide itself cannot currently be proposed as antiobesity drug since this derivative has been described to exert deleterious toxic effects. In 2003, the European Food Safety Authority has banned the use of azodicarbonamide as a blowing agent for plastics used in food processing or packaging, or as a flour treatment, since it can be transformed into semicarbazide, which is suspected to be a food contaminant exerting toxicological effects on consumers. However, this hydrazine derivative is not only a food contaminant, the origin of which is limited to the transformation of known chemical substances used (or even prohibited) in food processing [23], but can also naturally occur in certain foods [38, 39]. As a consequence, the DNA damaging effects of ingested semicarbazide have been intensively studied to bring health risk assessments. But these recent toxicological studies inconsistently demonstrated the genotoxic properties of semicarbazide, since they raised variations according to the model and the protocol used. Briefly, semicarbazide has very poor genotoxic effects on human lymphocytes when tested *in vitro* [23] and is not genotoxic in mice when administered *in vivo* at 120 mg/kg b.w. [29]. However semicarbazide exhibits carcinogenic potential—with marginal statistical relevance—when orally given to rats at 150 mg/kg [23]. Of note, other aspects of semicarbazide toxicity have also been stated very recently and have produced more consensual results. Indeed, a toxicological study showed a dramatic decrease in body weight with orally given semicarbazide as mixed in the food at doses up to 1000 ppm, giving a daily intake of 65 to 70 mg/kg in male and female rats [22]. The decrease in adiposity was not assessed in this study. However, the relative weights of the brain, heart, and kidneys were unchanged or even increased, depending on the sex, while body weights were clearly lower in the semicarbazide-treated rats. This may indicate that while semicarbazide ingestion limits WAT expansion, as assessed in our conditions, other organs are preserved during the body weight loss. Unfortunately, exposure to semicarbazide was accompanied by serious adverse effects such as deformation of bones and lesions of articular cartilages [22]. In the aorta, alteration of connective tissues was suspected to increase fragility of arterial walls. Nonetheless, no inflammatory disease was found. In view of these toxicological effects of chronic exposure to semicarbazide, the no observed adverse effect level (NOAEL) was estimated by the authors to be lower than 18–21 mg/kg/day in rat. Accordingly, the NOAEL in juvenile rats on another strain has recently been proposed to be lower than 40 mg/kg for semicarbazide oral administration [21]. In this study describing the toxicity of semicarbazide on biochemical and behavioural parameters, the authors observed a clear-cut inhibition of body weight gain and defects in cartilage mineralization [21]. During the completion of the present work, the same italian group reported that semicarbazide behaves as an endocrine disrupter, being able to reduce estrogen levels or to alter testosterone catabolism [40]. Anyhow, semicarbazide inhibited body weight gain and food consumption in postweaning male and female rats when orally given from the dose of 40 to 140 mg/kg/d period.

Thus, semicarbazide administration is univocally recognized to reduce body weight gain in diverse obese or nonobese models [2, 3, 7, 8, 21] and regarding this aspect our findings are confirmatory. Our detailed analysis of adiposity of juvenile mice, together with the high expression of SSAO in adipocytes, brings novel insight, focusing the attention on WAT as a major target of semicarbazide. However, this hydrazine derivative cannot be safely used as a "slimming" agent for toxic issues in bones, joints, and vessels. Although other SSAO inhibitors have also been reported to limit body weight gain, several semicarbazide effects other than inhibiting SSAO will be discussed here in an attempt to detect whether SSAO blockade is necessary and/or sufficient to limit fat deposition.

Another well-recognized effect of semicarbazide is to inhibit lysyl oxidase, a copper-containing amine oxidase

involved in the stabilization of extracellular matrix by cross-linking of proteins such as collagen and elastin [2, 3], therefore explaining part of the semicarbazide toxic effects on bones and joints. However, the lysyl oxidase inhibitor β-aminopropionitrile, reported to decrease insoluble elastin and collagen content and to impair collagen cross-linking does not decrease body weight gain, excepted when combined with semicarbazide [3].

Semicarbazide has been reported to inhibit glutamic acid decarboxylase, a γ-amino butyric acid-synthesizing enzyme [41, 42], and its administration is therefore believed to impair γ-amino butyric acid (GABA) formation and to lower GABA brain levels. This has been proven to induce "freezing" behaviour in rodents after central administration [43]. However, it is striking to note that relatively high doses of gamma-vinyl GABA, an irreversible inhibitor of the enzyme GABA-transaminase, which is involved GABA catabolic pathway, have been recently reported to produce significant weight loss in rats [44]. Likewise, another inhibitor of the GABA degradation exhibits anorectic potency in obese rats [45], while it has been proposed that a rat strain resistant to high-fat diet feeding reduces its food intake via an elevation of brain GABA levels [46]. In fact, it is difficult to understand how the chronic *in vivo* administration of semicarbazide, supposed to inhibit GABA synthesis, alters the GABA-ergic systems in a manner that results in reduced food intake, as it is the case with inhibitors of GABA degradation. To pour more complexity, it has been recently reported that several semicarbazide derivatives elevate GABA levels in the midbrain and exert anticonvulsant activity [47]. It could be therefore of interest to determine the relative proportion of the central GABA-ergic alterations and of the peripheral SSAO blockade in the "slimming" action of semicarbazide before establishing whether more selective SSAO blockers, devoid of interaction with the GABA-ergic system, also elicit antiobesity effects. Otherwise, the current number of SSAO-interacting agents that have been also reported to reduce weight gain (FPFA [1], aminoguanidine [6], phenelzine [8]) suggests a nonnegligible influence of the component related to SSAO inhibition in the observed effect of semicarbazide.

Semicarbazide has also been described to inhibit sphingosine-1-phosphate (S1P) lyase [48], but it is not clear whether such nonspecific inhibition may have some bearing on our observations. As in the case of GABA-ergic system, it must be quoted that novel semicarbazide derivatives act differently from semicarbazide itself, since instead of inhibiting a S1P-degrading enzyme, they are selective inhibitors of a subset of the G protein-coupled S1P receptors (S1P2) [49]. This emphasizes that semicarbazide is a molecule with multiple aspects, each one being selectively developed by the association of a functional and selective moiety to the semicarbazide turntable structure. Such drug design is out of the scope of the present study, while SSAO inhibition, obtained by the various above-mentioned pharmacological agents was always found to be concomitant with a decreased body weight gain. The need for searching novel, selective SSAO inhibitors is not only a concern for our proposed antiobesity pharmacology, but also relies with the general biology of the enzyme, which will soon change of denomination, from SSAO to primary amine oxidase, a fact indicating that its definition based on its sensitivity to semicarbazide will become historical once the many other inhibitors under development will be fully characterized.

5. Conclusions

Taken together, our results demonstrate that oral administration of the prototypical SSAO inhibitor semicarbazide limits weight gain and fat deposition in mouse. In spite of the toxicity of the high doses of this agent, the use of other specific SSAO inhibitors might reveal more beneficial for the pharmacologic treatment of obesity.

Conflict of Interests

The authors have no conflict of interests to declare.

Acknowledgments

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Review Article

Present and Future: Pharmacologic Treatment of Obesity

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Obesity now presents one of the biggest health problems of our times. Diet and exercise are best for both prevention and treatment; unfortunately, both require much discipline and are difficult to maintain. Medications offer a possible adjunct, but their effect is modest, they are limited by side effects, and the weight loss lasts only as long as the drug is being taken, since as soon as treatment is stopped, the weight is regained. Sibutramine, a sympathomimetic medication which was available for long-term treatment, is the most recent of the drugs to be withdrawn from the market due to side effects; in this case it was an increased risk of cardiovascular events. This paper reviews those medications which are available for treatment of obesity, including many of those recently taken off the market. It also discusses some of the newer treatments that are currently being investigated.

1. Introduction

Obesity has become one of the biggest health problems of our times. The World Health Organization's (WHO) latest projections indicated that globally in 2005 approximately 1.6 billion adults were overweight and at least 400 millions were obese. The WHO further projects that by 2015 approximately 2.3 billion adults will be overweight and more than 700 million will be obese. At least 20 million children younger than 5 years old were overweight in 2005 [1].

Although obesity has been labeled a disease for over 200 years [2], only recently has it been recognized as a condition that warrants medical attention. This is especially true given the mounting data on the correlation between obesity and type 2 diabetes, hypertension, dyslipidemia, cardiovascular disease, stroke, and depression. Obesity is associated with osteoarthritis, obstructive sleep apnea, increased risk of cancer, and nonalcoholic fatty liver disease. It is related to a variety of other complications, such as gastrointestinal reflux disease, gout, headache, cellulitis, chronic renal failure, hypogonadism, and erectile dysfunction, among others. Moreover, obesity is associated with a reduced quality of life and social stigmatization [3, 4]. Unfortunately, doctors remain reluctant to bring up the topic of obesity when seeing patients in their clinic. Less than 50% of obese patients are even advised to lose weight [5].

This low involvement on the part of the doctor may be due, in part, to the fact that at this time there is still no magic bullet for the treatment of obesity, and diet and exercise continue to be the cornerstone of treatment. Given the frustrations doctors and patients face in achieving results by diet and exercise, drug therapy offers an attractive option, even if the results are modest. Unfortunately, despite the frantic search at the moment for pharmacotherapy, few options currently exist. In the last few years many drugs that have been effective weight loss medications have had to be withdrawn from the market secondary to unacceptable side effects. Fen-Phen, which was marketed as a combination of two drugs fenfluramine and phentermine, was removed from the market in September 1997, after it was reported that it could cause valvular heart disease and pulmonary hypertension [6]. In 2000, phenylpropanolamine, an over-the-counter weight loss drug, was found to be an independent risk factor for hemorrhagic stroke in women [7]. The first selective CB1 receptor blocker, Rimonabant, was never approved in the USA, but was available in 56 countries from July 2006 to January 2009, when it was withdrawn from the European market due to the fact that it caused increased depression

and suicidal ideation [8]. More recently, in October 2010, sibutramine, a centrally acting serotonin-norepinephrine reuptake inhibitor structurally related to amphetamines, was withdrawn from the market because of its association with increased cardiovascular events and strokes [9].

This paper reviews those medications which are available for treatment of obesity, including many of those recently taken off the market. It will also discuss some of the newer treatments that are currently being investigated. While bariatric surgery has been increasing in popularity, and certainly offers a powerful tool with which to treat obesity, it is beyond the scope of this paper.

2. Indications for Medical Therapy

Obesity is defined as a body mass index (BMI; calculated as weight in kilograms divided by the square of height in meters) of 30 or more. Clinical practice guidelines for the pharmacologic and surgical management of obesity in primary care have been issued by the American College of Physicians (ACP) [10]. These guidelines are based upon the results of two meta-analyses [11, 12] and the existing guidelines from the United States Preventive Services Task Force.

The guidelines include five recommendations. (1) Counsel all obese patients (BMI $\geq 30\,kg/m^2$) on diet, lifestyle, and goals for weight loss. (2) Pharmacologic therapy may be offered to those who have failed to achieve weight loss goals through diet and exercise alone. (3) Pharmacologic options include sibutramine, orlistat, phentermine, diethylpropion, and two drugs that are not approved for obesity treatment, fluoxetine and bupropion. (4) Bariatric surgery should be considered for patients with BMI $\geq 40\,kg/m^2$ who have failed diet and exercise (with or without drug therapy) and who have obesity-related comorbidities (hypertension, impaired glucose tolerance, diabetes mellitus, dyslipidemia, sleep apnea). (5) Bariatric surgery should be performed at high-volume centers with experienced surgeons. The NIH also suggests that weight loss drugs approved by the Food and Drug Administration (FDA) for long-term use can be used or for patients with a BMI of ≥ 27 accompanied by risk factors or diseases. These risk factors are hypertension, dyslipidemia, coronary heart disease, type 2 diabetes, and sleep apnea [13]. These recommendations may possibly change soon, in light of the new findings linking sibutramine to an increased rate of cardiac events [9]. The NIH also suggests that adults with a BMI > 35 who have serious comorbidities such as diabetes, sleep apnea, obesity-related cardiomyopathy, or severe joint disease may also be candidates for bariatric surgery [14].

3. Goals of Therapy

Perhaps the most important aspect of starting pharmacological treatment for obesity is to set realistic goals. Given the difficulties of dieting, it is nearly impossible to reach the patients expectations. Numerous studies have shown that obese individuals want to lose the equivalent of 25–35% of their initial weight and expect to do so in approximately 1 year of treatment [15]. Dieters maintain these expectations even when repeatedly informed that they are likely to lose only 5–15% of initial weight, which is the size of the losses typically induced by current behavioral and pharmacological interventions [16]. These data illustrate the dramatic disparity between patients' expectations and professional recommendations and the need to help patients accept more modest weight loss outcomes. A realistic treatment goal is usually the loss of 5–10% of initial body weight over a 6- to 12-month period, followed by long-term maintenance of reduced weight. The NIH guidelines recommend that weight loss should exceed 2 kg during the first month of drug therapy (1 pound per week), fall more than 5% below baseline by three to six months, and remain at this level to be considered effective [17]. If this is not achieved, then the dose should be adjusted or the medication discontinued.

Although weight loss is an important treatment outcome, a major goal of obesity management should be to improve cardiovascular and metabolic risk factors in order to reduce obesity-related morbidity and mortality. Modest weight loss as low as 5% of initial body weight can lead to favorable improvements in blood pressure, serum lipid concentrations, increased insulin sensitivity, and improved glucose levels [18–21]. Patients who have impaired glucose tolerance, type 2 diabetes, or hypertension are the ones who benefit the most as far as improvement in cardiovascular risk factors [22]. The ongoing Look AHEAD study, initiated in 1999, is a 12-year prospective trial designed to determine whether a 7% weight loss will reduce the occurrence of myocardial infarction and stroke in overweight patients who already have type 2 diabetes [23].

When starting pharmacological therapy it is important that the patient understands that once the maximal therapeutic effect is achieved, weight loss plateaus and that when drug therapy is discontinued, the weight is regained [17]. Because the body reduces its energy expenditure as it loses weight, more and more effort must be made to maintain the weight loss [24]. Weight regain results from complex interactions between multiple factors including physiologic, environmental, and psychological factors. Physiologic factors include reduced metabolic rate, both resting and nonresting [25, 26], and increased adipose tissue lipoprotein lipase activity [27]. These counterregulatory mechanisms protect against starvation by causing an increase in appetite and a decrease in energy expenditure, making it very difficult to keep off the weight that was lost.

Patients must also be made aware of the fact that the data on the use of these medications is limited, given that the longest trials for sibutramine have a duration of only two years, and for orlistat a maximum of four years. The trials for the remaining medications are even shorter. Therefore, if there is a good response to the medication and the patient wishes to continue, the decision should be a shared discussion between the physician and patient.

4. Review of Medications

4.1. Orlistat-Lipase Inhibitor. Orlistat (Xenical) was approved in 1998 for use for more than 12 weeks, which is considered a long-term use. It reduces dietary fat absorption

by 30% by inhibiting pancreatic and gastric lipase [28]. Orlistat is available in 120 mg capsules. The recommended dose is 120 mg three times daily. A lower dose (60 mg), which is not as effective, is available over-the-counter in some countries, including the United States.

Three randomized, double-blind, placebo-controlled trials of orlistat lasting two years have been published. These showed that orlistat 120 mg (three times/day), taken with an appropriate diet, resulted in clinically significant weight loss and reduced weight regain when compared to placebo. The orlistat group, on average, lost 10.2% versus placebo which lost 6.1% of body weight ($P < .001$) from randomization to the end of the first year. During the crossover portion of the study in the second year, patients who continued with orlistat regained, on average, half as much weight as those patients switched to placebo ($P < .001$) [28–30].

In a longer four-year study involving 3305 obese patients, 21% of whom had impaired glucose tolerance, orlistat led to weight loss during the first year of more than 11% below baseline in the orlistat-treated group versus 6% in the placebo-treated group [31]. Over the remaining years of the trial, both groups regained weight, but overall, the orlistat-treated patients were 6.9% below baseline, compared with 4.1% for those receiving placebo.

In a meta-analysis of 22 trials that included patients with and without diabetes and reported data with 12-month outcomes, the mean difference in weight loss due to orlistat was −2.89 kg (−3.51 to −2.27 kg) [11]. With an average placebo effect of −5.5 kg, this translates into an expected loss of −8.83 kg on average for patients who adhered to the treatment for this long. Almost two-thirds of the subjects completed the first year of treatment. Weight loss at one year varied from 5.5 to 6.6% of initial body weight in the placebo groups and 8.5 to 10.2% in the orlistat groups. Also in diabetic patients orlistat resulted in significantly more weight loss and a decrease in hemoglobin A1c at one year than placebo [32–34]. In all the studies, the lowest body weight was achieved during the first year.

The moderate effect on body weight is sufficient to improve several metabolic parameters. In a multicenter trial, as an example, serum total and LDL cholesterol concentrations both decreased by 4–11% in subjects treated with a weight-maintaining diet plus 30 to 360 mg of orlistat per day for eight weeks [35]. Blood pressure was reduced by 1.8 mmHg systolic and 1.6 mmHg diastolic. A retrospective analysis showed reductions in triglyceride and cholesterol blood levels, improved oral glucose tolerance, and a fall in systolic and diastolic blood pressure [30].

The major side effects of orlistat therapy are gastrointestinal, including intestinal cramps, flatus, fecal incontinence, oily spotting, and flatus with discharge [34]. These occur in 15–30% of the patients and tend to occur early and to subside as patients learn how to avoid high fat diets and stick to the recommended intake of no more than 30% fat. Levels of fat-soluble vitamins (A, D, E) and beta-carotene are reportedly lowered by orlistat therapy, with vitamin D the most frequently affected; therefore it seems prudent to also give a multivitamin supplement when prescribing orlistat [34].

4.2. Sibutramine-Sympathomimetic Drug, Formerly Approved for Long-Term Use. Sibutramine (Meridia, Reductil) was first developed as an antidepressant, and although it was ineffective for this, it was found to reduce body weight and appetite. It is a centrally acting noradrenaline and serotonin reuptake inhibitor that enhances weight loss by increasing satiety. It may also have a thermogenic effect in human beings, but this remains controversial. It is given as a 10 mg dose once daily, usually in the morning, and could be increased to 15 mg daily after 4 weeks if needed. It was available from 1997 until October 2010. We will discuss the side effects that led to its withdrawal, but first we will review its efficacy.

More than 10 prospective, randomized, controlled trials of sibutramine lasting 6–24 months have supported its efficacy [11]. An analysis of three trials of at least 1-yr duration shows that patients on sibutramine lost 4.3 kg or 4.6% more weight than those taking placebo; 34% more patients achieved at least 5% weight loss, and 15% more patients achieved at least 10% weight loss in the sibutramine arm compared with placebo [36]. The weight loss seems to be dose dependent [37], and the extent of initial weight loss in subjects treated with sibutramine predicts the long-term response. In two different weight maintenance trials comparing sibutramine to placebo, more weight loss was seen in the drug-treated patients (mean 5.2 kg) while the placebo group gained weight (mean 0.5 kg) [38, 39].

There are benefits to using sibutramine intermittently, as was seen in a randomized, placebo-controlled trial lasting 52 weeks [39]. The patients received either continuous treatment with sibutramine at 15 mg/day for 1 year or sibutramine with two 6-week periods when the drug was withdrawn. During the periods when the drug was replaced by placebo, there was a small regain in weight that was again lost when the drug was resumed. At the end of the trial, the continuous-therapy and intermittent-therapy groups had lost the same amount of weight.

Another study highlights how much intense lifestyle modification contributes to the success of medical treatment. In a 1-year randomized trial, 224 obese adults received sibutramine alone, sibutramine plus brief individualized lifestyle modification (8–10 visits of 10–15 min each), group lifestyle modification alone (30 sessions), or sibutramine plus 30 sessions of group lifestyle modification [40]. All subjects were prescribed a calorie-restricted diet of 1200 to 1500 kcal per day and the same exercise regimen. At one year, subjects in the combined therapy group lost a mean of 12.1 ± 9.8 kg, while those receiving sibutramine alone, lifestyle modification alone, or sibutramine plus brief lifestyle intervention counseling lost 5.0 ± 7.4 kg, 6.7 ± 7.9 kg, and 7.5 ± 8.0 kg, respectively.

A number of studies have demonstrated that obese patients with diabetes also seemed to benefit from sibutramine therapy [41–44]. As an example, in a six-month trial in patients with diabetes, those treated with sibutramine lost weight and had a decline in hemoglobin A1C values, but no change in blood pressure [42]. In a meta-analysis of eight randomized trials in 1093 obese patients with type 2 diabetes, significant decreases were noted in body weight,

waist circumference, fasting blood glucose, and HbA1C in the sibutramine group compared to placebo. Other treatment benefits included decreased serum triglycerides and increased HDL concentrations [44].

In all these trials weight loss is associated with improved lipid profiles [41, 42, 44]; however, the data for blood pressure has been conflicting. In many trials patients treated with sibutramine had significant, albeit small, changes in blood pressure [45, 46], on average by 1 to 3 mmHg. This includes patients with hypertension controlled with other agents, such as calcium-channel blockers with or without concomitant thiazide treatment [47]. However, a large surveillance study of 6360 patients suggested a significant decrease in blood pressure with sibutramine in obese hypertensive patients [48].

The different trials also show that a small, but significant increase in pulse rate is seen with sibutramine use. In a systematic review of 29 trials, the mean increase in heart rate with sibutramine was 3.76 beats/min (95% confidence interval, 2.70–4.82 beats/min) [47]. None of the antihypertensive drugs, including beta-blockers, appear to prevent the rise in pulse rate with sibutramine.

Uncertainties about the cardiovascular safety of sibutramine led to the initiation of the randomized, double-blind, placebo-controlled Sibutramine Cardiovascular Outcomes Trial (SCOUTs), which is the longest and largest study with sibutramine [49]. This recently published study enrolled nearly 10,000 patients who were followed for a mean of 3.4 years. It was designed to determine the impact of weight loss with sibutramine on cardiovascular problems in a population already at high risk for cardiovascular disease, with 60% of the patients having a history of cardiovascular disease and diabetes. The primary endpoint was the time from randomization to the first occurrence of a primary outcome event, which included nonfatal myocardial infarction, nonfatal stroke, resuscitation after cardiac arrest, or cardiovascular death. All patients were first in a 6-week lead-in period with sibutramine, in which the mean weight loss was 2.6 kg. The subjects were then randomized to sibutramine or placebo. The subjects in the sibutramine group then achieved a further weight loss of 1.7 kg, while the placebo group gained weight, an average of 0.7 kg by month 12. The mean blood pressure decreased in both groups, with greater reductions in the placebo group than in the sibutramine group (mean difference, 1.2/1.4 mmHg). The trial showed that the risk of a primary outcome event was increased by 16% in the sibutramine group as compared with the placebo group (hazard ratio, 1.16; 95% confidence interval (CI), 1.03 to 1.31; $P = .02$), with overall incidences of 11.4% and 10.0%, in the two groups, respectively. The rates of cardiovascular death and death from any cause were not increased. Due to these findings, where the weight loss did not seem to outweigh the cardiovascular risks, the European Medicines Agency recommended the suspension of marketing authorizations for sibutramine across the EU. Months later, due to pressures exerted by the FDA, Abbot Laboratories announced that it too would withdraw sibutramine from the US market [50].

4.3. Benzphetamine, Diethylpropion, Phendimetrazine, and Phentermine-Sympathomimetic Drugs Approved for Short-Term Treatment. Benzphetamine (Didrex), Diethylpropion (Tenuate), Phendimetrazine (Bontril), Phentermine (Adipex-P), and Mazindol (Mazanor, Sanorex) are approved for use for a period of up to 12 weeks. They are all amphetamine derivatives that lead to weight loss by suppressing appetite. Like amphetamines, they are centrally acting medications that increase blood pressure and pulse rate. Of these, phentermine is the most frequently prescribed weight loss medication [51]. Phentermine comes in 15 mg, 30 mg, and 37.5 mg doses and should be given once daily. Tablets may be divided in half, and dose may be given in 2 divided doses, while avoiding late evening administration.

Phentermine administered continuously in a 36-week double-blind trial in 108 women led to 12.2 kg loss, versus placebo which was 4.8 kg [52]. Diethylpropion has been around since the 1950s, but up until very recently it had rarely been studied beyond 20 weeks. However, a recent randomized double-blind placebo-controlled study lasting for one year showed that diethylpropion was safe. In the study, 69 obese healthy adults received a hypocaloric diet and were randomized to diethylpropion 50 mg BID or placebo for 6 months. After this period, all participants received diethylpropion in an open-label extension for an additional 6 months. After 6 months, the diethylpropion group lost an average of 9.8% (s.d. 6.9%) of initial body weight versus 3.2% (3.7%) in the placebo group ($P < .0001$). From baseline to month 12, the mean weight loss produced by diethylpropion was 10.6% (8.3%). Participants in the placebo group who were switched to diethylpropion after 6 months lost an average of 7.0% (7.7%) of initial body weight. The difference between groups at month 12 was not significant ($P = .07$). There was no difference between the two groups with respect to cardiovascular and psychiatric side effects [53].

Phentermine and diethylpropion have been shown to reduce weight significantly when used intermittently. In a way, this means that they can be used as chronic medications, as long as they are used in alternating months, and therefore still remaining within the short-term limitations [52]. Phentermine and diethylpropion are classified by the U.S. Drug Enforcement Agency as schedule IV drugs, meaning that they have a very low potential for drug abuse. Benzphetamine and phendimetrazine are schedule III drugs, meaning that they have a slightly higher potential for abuse; however, this potential appears to be very low.

5. Diabetes Medications That Lead to Weight Loss

The increased weight associated with many commonly prescribed antidiabetic medications such as insulin, sulfonylureas, and thiazolidinediones leads many doctors and patients to resist intensifying therapy as needed [54]. Fortunately not all medications cause weight gain, and recently new medications have been introduced that actually cause weight loss.

5.1. Metformin. Metformin (Glucophage) was first investigated for its effects on weight loss in trials as early as 1969 [55], but though the results were promising, metformin was put aside when the following year a trial comparing metformin with fenfluramine showed that fenfluramine was much more effective. Since then, only 9 trials have evaluated weight loss as a primary endpoint of metformin treatment. All of these studies, sometimes combined with orlistat or sibutramine, showed significant weight reduction, but they were short-term studies, consisting of no more than 50 patients each [56–59].

Metformin comes in doses of 500 mg, 850 mg, or 1000 mg. Generally, clinically significant responses are not seen at doses <1500 mg daily; however, a lower recommended starting dose and gradual increased dosage are recommended to minimize gastrointestinal symptoms. Extended release formulations are available.

There are few large randomized controlled trials evaluating the effect of metformin on weight as a secondary outcome. The largest one is the Diabetes Prevention Program [60]. This was a large-scale trial involving 3234 patients with impaired glucose tolerance, which primarily evaluated the incidence of diabetes at the end of the treatment period among patients treated with either metformin, standard lifestyle intervention (placebo group), or intensive lifestyle modification. After an average of 2.8 years of follow-up, the metformin-treated group lost 2.5% of their body weight over placebo ($P < .001$).

The BIGPRO (Biguanides and Prevention of the Risks in Obesity) trial evaluated the relationship between insulin resistance and cardiovascular disease by giving metformin to insulin-resistant patients [61]. The preliminary stage was reported in the BIGPRO1 trial, which randomized 324 patients to receive metformin 850 mg daily or placebo. The metformin-treated patients had a nonsignificant decrease in weight after 1 year (−2 kg in metformin versus −0.8 kg in placebo) ($P < .06$ versus placebo). Significant differences were observed for systolic blood pressure, which decreased considerably more with metformin versus placebo ($P < .003$), and for fasting plasma glucose and total and LDL cholesterol, which decreased slightly with metformin, while increasing with placebo ($P < .04$; $P < .02$ for LDL cholesterol). There was also a (nonsignificant) trend towards a greater reduction of diastolic blood pressure in the metformin group ($P < .09$), whereas weight, BMI, waist-to-hip ratio, 2 hPG, fasting and 2-h insulin, HDL cholesterol, triglycerides and fibrinolytic markers did not differ between the two treatment groups [62].

The most common side effects of metformin are gastrointestinal, including a metallic taste in the mouth, mild anorexia, nausea, abdominal discomfort, and soft bowel movements, or diarrhea. Lactic acidosis is an uncommon side effect; however, it remains a concern because of the high case fatality rate. Thus, metformin should not be administered to individuals with predisposing factors for developing lactic acidosis, such as those with impaired renal function.

5.2. GLP-1 Agonists: Exenatide and Liraglutide. GLP-1 is a hormone secreted by L cells in the terminal ileum after food intake. It decreases blood glucose by inhibiting glucagon secretion and stimulating insulin secretion. GLP-1 also delays gastric emptying, reduces caloric intake, and promotes satiety. Exenatide (Byetta) is a GLP-1 receptor agonists that shares 53% homology to GLP-1 and has a much longer half life because it is resistant to dipeptidyl peptidase-4- (DPP-4-) mediated degradation. It is administered as a subcutaneous injection twice daily. It is approved to treat type 2 diabetes and produces similar effects to GLP-1, reducing fasting and postprandial glucose levels, decreasing HbA1c, slowing gastric emptying, and decreasing food intake by about 19%. In diabetic patients it has been shown to cause weight loss by an average of 1.6 kg without any change in lifestyle, diet, or exercise [63].

Exenatide, available in prefilled syringes that hold a month's supply of either 5 or 10 mcg doses, is administered subcutaneously twice daily immediately before or within one hour of morning and evening meals [64]. Exenatide should not be used in patients with severe renal impairment (creatinine clearance <30 mL/min) or end-stage kidney disease.

A study that followed 314 overweight patients with type 2 diabetes, the first 30 weeks of which were double blind, followed by an open-label observation to week 82, showed progressive weight reduction in 80% of patients, with an average reduction of 4.4% body weight [65]. Recently another study was published that assessed the effects of exenatide on body weight and glucose tolerance in 152 nondiabetic obese subjects with normal or impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) [66]. They were randomized to receive exenatide or placebo, along with lifestyle intervention, for 24 weeks. The exenatide-treated subjects lost 5.1 ± 0.5 kg from baseline versus 1.6 ± 0.5 kg with placebo ($P < .001$), with a placebo-subtracted difference in percent weight reduction of $-3.3\% \pm 0.5\%$ ($P < .001$). IGT or IFG normalized in 77% and 56% of exenatide and placebo subjects, respectively. Exenatide offers a good alternative for weight loss, particularly in nondiabetic obese subjects with glucose intolerance.

A once a week version of exenatide is currently being tested, so far, only in diabetic patients. The DURATION-1 study evaluated the safety and efficacy of treatment with the exenatide once weekly (2 mg) compared to exenatide twice daily in 295 patients with type 2 diabetes. It appears that once a week exenatide will be just as effective as twice daily injections, since at 52 weeks the A1c changes were similar and the weight loss was similar −4.1 kg (−5.3 to −2.9 kg) and −4.5 kg (−5.7 to −3.3 kg) in the two groups [67].

The most common side effects of exenatide are predominantly gastrointestinal. Nausea is a common adverse effect, but it is generally mild to moderate in intensity and wanes with duration of therapy [68]. Nausea can be reduced with dose titration. There have been 36 postmarketing reports of acute pancreatitis in patients taking exenatide [69]. In some cases, necrotizing or hemorrhagic pancreatitis was reported, and fatalities occurred. There is insufficient data to determine the overall frequency of pancreatitis in exenatide

users, but overall it seems similar to the background rate in patients with diabetes mellitus.

Liraglutide (Victoza) is another long-acting GLP-1 analog with a 97% structural homology to human GLP-1. It is available for use in the United States, Europe, and Japan for the treatment of type 2 diabetes. The drug is administered subcutaneously once daily. In diabetes trials, liraglutide was associated with a significant reduction in weight (2.0 to 2.5 kg) when compared with placebo or glimepiride [70].

Like exenatide, liraglutide is only available as an injection, in prefilled pens. The initial dose is 0.6 mg once daily for one week to reduce gastrointestinal side effects [71]. After one week, the dose should be increased to 1.2 mg once daily for one week. If blood glucoses remain above the goal range, the dose can be increased to 1.8 mg once daily.

Weight loss has also been studied in patients without diabetes who received liraglutide. In the 20-week study, 564 obese individuals were randomized to one of four liraglutide doses (1.2 mg, 1.8 mg, 2.4 mg, or 3.0 mg) or to placebo administered once a day subcutaneously, or orlistat 120 mg three times a day orally [72]. All individuals had a 500 kcal per day energy-deficit diet and increased their physical activity throughout the trial. Treatment with liraglutide led to a sustained, dose-dependent weight loss that was significantly greater than that with placebo (all doses) and significant for orlistat at the doses of liraglutide of 2.4 mg and 3.0 mg. Mean weight loss with liraglutide ranged from 4.8 to 7.2 kg, depending on the dose. The weight loss was accompanied by reductions in waist circumference, systolic and diastolic blood pressure, and frequency of both metabolic syndrome and prediabetes. Liraglutide was generally well tolerated. However, as in other trials, nausea and vomiting were more frequent with liraglutide than with the other treatments, although these events were mostly of mild or moderate intensity and transient, tending to occur in the first four weeks.

5.3. Pramlintide. Pramlintide (Symlin) is an analogue of amylin, a small peptide hormone that is released into the bloodstream by the β-cells of the pancreas along with insulin in response to nutrient stimuli. Amylin has been shown to slow gastric emptying, reduce postprandial rises in blood glucose concentrations, and improve hemoglobin A1C concentrations in both type 1 and type 2 diabetes patients. It must be given by subcutaneous injection, at a maximum dose of 120 micrograms with each meal.

Pramlintide is associated with modest weight loss in patients with type 1 diabetes. In one such trial, where 651 patients with type 1 diabetes were randomly assigned to placebo or subcutaneous pramlintide in addition to their insulin, weight decreased 0.4 kg in the pramlintide group and increased by 0.8 kg in the placebo group [73].

A few trials evaluating the effects of pramlintide on weight in adults with type 2 diabetes have been done. One large double-blind, placebo-controlled trial lasting 52 weeks found that patients treated with pramlintide 30 μg, 75 μg, and 150 μg 3 times daily experienced significant weight loss compared to placebo at weeks 13, 26, and 52. Patients were included in this trial if they were on stable doses of metformin and/or a sulfonylurea, which is noteworthy since metformin is associated with weight loss and sulfonylureas are associated with weight gain [74]. Another double-blind, placebo-controlled trial confirmed significant weight loss at 52 weeks [75]. In this trial, only the highest dose of 120 μg BID treatment group had a sustained weight loss to week 52 ($P < .05$ versus placebo), whereas in the 90 μg BID treatment group, the placebo-corrected treatment difference was no longer significant.

Pramlintide has also been evaluated for the treatment of obesity in adults without diabetes. In two studies in obese subjects pramlintide significantly reduced 24-hr caloric intake by ~500–750 kcal when compared to placebo and also reduced caloric intake by 20% at a highly palatable fast-food challenge [76]. In a 4-month, randomized, double-blind, placebo-controlled, nonforced dose-escalation study in which 88% of subjects escalated to the maximum dose (240 μg t.i.d.), pramlintide induced a placebo-corrected reduction in weight of 3.7% ($P < .001$), with 31% of pramlintide-treated subjects achieving ≥5% weight loss (versus 2% for placebo; $P < .001$) [77].

A more comprehensive study assessing the long-term weight loss efficacy and safety of pramlintide at different dosing regimens was carried out in 411 obese subjects [78]. These were randomized to receive pramlintide in one of 7 arms: 120 μg b.i.d. and t.i.d, 240 μg b.i.d. and t.i.d, and 360 μg b.i.d. and t.i.d. or placebo. All subjects received a lifestyle intervention program. At 4 months, 77% opted to continue preexisting treatment during an 8-month single-blind. At month 4, mean weight loss from baseline in the pramlintide arms ranged from 3.8 to 6.1 kg compared with 2.8 kg in the placebo arm. By month 12, the initial weight loss was regained in the placebo group but was maintained in all but the 120-μg b.i.d. group. Nausea, the most common adverse event with pramlintide in the 4-month study (9–29% pramlintide versus 2% placebo), was generally mild to moderate and occurred in <10% of subjects during the extension.

6. Antiseizure Medications That Lead to Weight Loss

6.1. Topiramate. Topiramate (Topamax) is an anticonvulsant agent approved in the mid-1990s for the treatment of refractory seizures in conjunction with other anticonvulsant agents. While it was being studied, it was discovered that the agent mitigated the weight gain often observed with antidepressant treatment. A 6-month dose-ranging study showed that it does so in a dose-dependent manner; the mean percent weight loss was −2.6% in placebo-treated subjects versus −6.3% in subjects treated with 192 mg/d topiramate for 24 wk [79]. In a meta-analysis of six trials which followed patients for an average of six-months, the weight loss was 6.51% (4.77 to 8.25%) with a placebo effect of about 2.0% [11].

Significant side effects of topiramate included fatigue, paresthesias, difficulty concentrating, and changes in taste. In addition, patients using topiramate are at risk for secondary acute angle glaucoma during the first month of therapy.

Topiramate is also associated with decreases in serum bicarbonate and development of metabolic acidosis [80], markedly lower urinary citrate excretion, and increased urinary pH. These changes increase the propensity of topiramate to form calcium phosphate stones [81]. The high frequency of side effects has led to the termination of phase III trials, while an extended release formulation is being developed by the manufacturer.

6.2. Zonisamide. Zonisamide (Zonegran) is another antiepileptic drug approved by the FDA in 2000 for the treatment of partial seizures in adults with epilepsy. Whereas the anticonvulsant activity of zonisamide is believed to be related to its sodium and calcium channel blocking activity [82], the drug is also known to exert dose-dependent biphasic dopaminergic [83] and serotonergic [84] activity. Weight loss was noted in clinical trials for the treatment of epilepsy [85], hence prompting a trial for obesity.

Only one randomized controlled trial has been done thus far, in which 60 obese subjects on a calorie-restricted diet were randomly assigned to zonisamide (100 mg/d increasing to 400 to 600 mg/d) or placebo for 16 weeks [86]. Mean weight loss in the zonisamide group was 6.6% compared to 1% in the placebo group. In 37 subjects who elected to continue in the trial for an additional 16 weeks, mean weight loss was 9.6 and 1.6% in the zonisamide and placebo groups, respectively ($P < .001$). The most common side effects of zonisamide are cognitive effects and fatigue and a small, but significant, increase in serum creatinine. Zonisamide is not FDA-approved for the treatment of obesity.

7. Antidepressant Medications That Lead to Weight Loss

7.1. Bupropion. Bupropion is a norepinephrine and dopamine reuptake inhibitor that is approved for treating depression and smoking cessation. Bupropion seems to have a weight-neutral effect for most depressed individuals of normal weight. However, controlled trials with depressed individuals suggest that bupropion SR may be associated with weight loss in overweight or obese subjects [87, 88]. In addition, weight gain after treatment for smoking cessation was less in bupropion SR-treated subjects than in placebo-treated subjects [89].

In a 6-month, randomized, double-blind, placebo-controlled trial 327 obese patients were treated with SR bupropion (300 or 400 mg/d) versus placebo [90]. Initial body percent weight lost for subjects completing 24 weeks was 5.0% for placebo, 7.2% for bupropion SR 300, and 10.1% for bupropion 400 mg/d. Compared with placebo, net weight losses were 2.2% ($P = .0468$) and 5.1% ($P < .0001$) for bupropion SR 300 and 400 mg/d, respectively. Also, there was a 6-month extension where the weight loss was largely maintained. The most common side effects are dry mouth, insomnia, headaches, and nausea. However, other serious adverse events, such as seizures, occur in people who already have a low threshold for seizures. The data is too scant to recommend bupropion as a weight loss drug at this time.

7.2. Fluoxetine. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) approved for the treatment of depression, may also lead to weight loss. A clinical trial of fluoxetine for obesity included a total of 458 subjects who were randomly assigned to fluoxetine (60 mg/d) or placebo. Fluoxetine therapy (60 mg/day) resulted in statistically significantly ($P \leq .05$) greater mean weight loss than placebo at week 28. Although some patients continued to lose weight throughout the 52-week therapy period, maximum mean weight loss occurred at week 20. There was no treatment difference at 52 weeks, meaning that it would not be serving as a long-term medication [91]. In a meta-analysis, trials of fluoxetine as an antiobesity drug at a dose of 60 mg/d, which is three times the usual dose for treatment of depression, produced variable weight change ranging from 14.5 kg lost to 0.40 kg gained [11]. In summary, it can be noted that on average most of the existing medications lead to about a 5% weight loss, with the exception of the sympathomimetic drugs approved for short term use (see Table 1 in supplementary material available online at doi:10.1155/2011/636181).

8. New Drugs in Development

8.1. Leptin. The protein hormone leptin is produced by adipose tissue and appears to signal adiposity and modulate ingestive behavior [92, 93]. In mice and rare humans with leptin deficiency, administration of physiological doses of leptin decreases food intake and causes weight loss [94]. However, obese adults are found to have high levels of leptin [95], so perhaps there is a state of leptin resistance. If that resistance is overcome with even higher levels of leptin, then perhaps it could lead to weight loss.

A randomized, double-blind, placebo-controlled, multicenter, escalating dose cohort trial was conducted to determine the relationship between increasing doses of exogenous leptin administration and weight loss. In one part of the study 47 obese people on a calorie restricted diet were given either placebo or recombinant leptin at escalating doses 0.01, 0.03, 0.10, or 0.30 mg/kg. By week 24 there was a dose-dependent decrease in body weight, ranging from −1.3 kg in the placebo group to −7.1 kg in the 0.30 mg/kg group ($P = .01$). No clinically significant adverse effects were observed on major organ systems, but it was associated with an unacceptable incidence of injection site reactions [96].

A possible use for leptin may be as an adjunctive therapy for the maintenance of weight loss. The reductions in energy expenditure and thyroid hormone concentrations that occur with weight loss may be due to a decline in leptin concentrations and may also hamper the ability to maintain weight loss. These metabolic and hormonal changes are reversed to the preweight-reduced state after administrations of low replacement dose leptin. This was seen in a small proof-of-concept study carried out in 10 inpatient subjects where leptin therapy prevented weight gain after significant weight loss, by preventing the weight loss-associated decrease in energy expenditure [97].

8.2. Peptide YY. The gut hormone peptide YY (PYY) is synthesized and cosecreted with GLP-1 by the L cells in the

small intestine. It suppresses appetite and decreases food intake [98]. This was illustrated in a trial of obese and lean adults receiving short-term intravenous infusion of PYY administration. Appetite and caloric intake decreased by approximately 30% in both groups when the subjects were offered a buffet meal, and 24 hour caloric intake was reduced. Obese subjects had significantly lower fasting PYY levels and levels correlated inversely with body mass index. These observations suggest that PYY has an important regulatory role in feeding behavior, potentially offering a target for drug development [99].

In contrast, in a 12-week trial, 133 obese patients were randomly assigned to diet and exercise and either intranasal PYY (200 or 600 mcg three times daily before meals) or placebo [100]. The group receiving 600 microg t.i.d. could not be assessed because 59% of the patients discontinued due to nausea and vomiting. There was not statistically significant difference between placebo and the 200 mcg group.

8.3. Oxyntomodulin. Oxyntomodulin is a peptide produced in L cells of the GI track from the proglucagon gene product. It is released into the blood in response to food ingestion and in proportion to meal calorie content [101]. Oxyntomodulin has been reported to reduce food intake by 19.3% during an intravenous infusion administered to normal-weight humans, an effect that continues for >12 h after infusion [102].

In a randomized, double blind study oxyntomodulin or saline was self-administered for 4 weeks, three times daily before each meal. The volunteers were asked to maintain their regular diet and level of physical exercise during the study period. Body weight was reduced by 2.3 ± 0.4 kg in the treatment group over the study period compared with 0.5 ± 0.5 kg in the control group ($P = .0106$) [103].

8.4. Melanocortin-4 Receptor Agonists. The hypothalamic melanocortin system appears to enhance energy expenditure and suppress appetite. In a study in adults with normal weight, intranasal administration of the MSH/ACTH4–10 core fragment of pro-opiomelanocortin for 6 weeks resulted in a significant reduction of body weight by 1.7 kg [104]. However, a study of 23 overweight men who were given the same compound for 12 weeks did not show any significant decrease in body weight or body fat when compared to placebo [105].

8.5. Lorcaserin. Stimulation of specific central serotonin receptors represents an effective pharmacological mechanism to suppress appetite. Serotonin and agonists that activate serotonin 2C (5-HT2C) receptors promote feelings of satiety, thereby reducing food intake [106, 107]. Lorcaserin is a selective 5-hydroxytryptamine (serotonin) (5-HT) 2C agonist currently in phase 3 clinical trials for treating obesity. In a 12-week placebo-controlled randomized study of 333 obese individuals taking 10 mg once a day, 15 mg once a day, or 10 mg twice a day, lorcaserin was shown to be associated with dose-dependent weight loss without instructed lifestyle modification; 1.8, 2.6, and 3.6 kg of weight loss, respectively, were observed over the 12-week period compared with 0.3 kg with placebo [108]. Lorcaserin-treated subjects had reduced plasma cholesterol, waist circumference, fasting glucose, and serum uric acid. Lorcaserin has 7.5-fold selectivity for 5-HT2C receptors over 5-HT2A receptors and 11.6-fold selectivity for 5-HT2C receptors over 5-HT2B receptors [109]. In clinical trials, 5-HT2A receptor activation, which may cause neuropsychiatric effects, has not appeared to be significantly activated by the dosages used.

8.6. Tesofensine. Tesofensine is a sympathomimetic in the family of sibutramine initially developed for the treatment of Parkinson's disease. Although its efficacy was limited for this application, study subjects were noted to experience significant weight loss by appetite suppression [110].

In a recent multidose dose-ranging trial, 203 obese patients were randomly assigned to differing doses of tesofensine (0.25, 0.5, and 1.0 mg) or placebo once daily [111]. After 24 weeks, mean weight reduction was greater in the tesofensine groups (-6.7, -11.3, -12.8 kg, for the three doses, resp.) compared with placebo (-2.2 kg). Common adverse events included dry mouth, nausea, abdominal pain, and diarrhea. Heart rate was significantly elevated in all tesofensine groups (5 to 8 beats per min) ($P = .0001$). The 0.25 dose and the 0.5 mg dose did not show any changes in blood pressure as compared to placebo; however, the highest dose of tesofensine of 1.0 mg daily was associated with a significant increase in systolic and diastolic blood pressure (mean increase 6.8/5.8 mmHg). Insomnia, dry mouth, constipation, diarrhea, and dizziness were also more common. The highest dose of tesofensine showed the highest frequency of mood change. These mild increases in heart rate or blood pressure may end up having clinical significance, as was recently seen in sibutramine.

9. Combination Treatments

None of the single-agent drugs that have been approved or appear close to approval has consistently been able to achieve a weight loss of more than approximately 10% of body weight. The combination of phentermine and fenfluramine, which was taken off the market in 1997, was able to achieve a loss of approximately 15% of body weight [112]. Recognizing the complex pathophysiology of obesity, recent efforts have focused on combination therapies. Using more than one drug to treat obesity is similar to treating hypertension, where often several drugs are needed to reach the target goals.

Surprisingly, the combination of orlistat and sibutramine yielded disappointing results. In one trial of 34 obese women who had already lost an average of 11% of their initial weight with sibutramine during the prior one year of treatment, the addition of orlistat to the sibutramine for an additional four months did not induce any further weight loss when compared to sibutramine plus placebo [113]. In a different open-label, randomized, 12-week trial, 86 overweight patients received either orlistat 120 mg three times a day, sibutramine 10 mg/d, the combination of orlistat-sibutramine, or diet [114]. After 12 weeks, sibutramine produced more weight

loss than orlistat alone. The addition of orlistat to sibutramine did not significantly enhance weight loss, similar to the results of the first trial.

A number of novel combination obesity treatments are being investigated in clinical trials. These include phentermine plus topiramate (Qnexa), bupropion plus naltrexone (Contrave), zonisamide plus long-acting bupropion, topiramate and phentermine, and pramlintide plus leptin.

Three phase 3 trials evaluating phentermine plus topiramate (Qnexa) in over 4,500 patients as a treatment for obesity were recently completed under a Special Protocol Assessment by the U.S. FDA. Qnexa is currently under review by the FDA for the treatment of obesity and is not yet approved. EQUATE, the first of the three phase 3 studies, evaluated 756 obese patients over 28 weeks at 32 sites. EQUIP and CONQUER were 56-week studies; EQUIP evaluated 1,267 morbidly obese patients with or without comorbidities, and CONQUER evaluated 2,487 overweight and obese patients with at least two comorbid conditions [115].

The combination of bupropion plus naltrexone (Contrave) is also showing promising effects. Naltrexone, which is used in the treatment of alcoholism, is not associated with weight loss, and bupropron is associated with modest weight loss, but the combination clearly leads to greater effect. The drug was tested in a 56-week clinical trial that enrolled nearly 700 obese people. All study participants enrolled in an intensive weight loss program that included counseling, diet, and exercise. Two-thirds of the study participants also took Contrave. Participants stayed in the program for 56 weeks. By the end, the placebo group, on a weight loss program, lost over 5% of their body weight—11 to 16 pounds, while the Contrave group lost 9.3% of their body weight—20 to 25 pounds. And more than 29% of them lost more than 15% of their body weight. The study had a drop-out rate of over 40%, therefore diluting the effect [115]. On March 31st 2010, Orexigen submitted a New Drug Application (NDA) to the U.S. Food and Drug Administration (FDA) for Contrave. Similar effects were seen with the combination of the long-acting anticonvulsant zonisamide with long-acting bupropion, marketed under Empatic [115].

With the combination of the anticonvulsant topiramate and phentermine in doses one quarter to one-half of those typically used with both agents, weight loss again was seen, with acceptable although definite side effects, including paresthesia in 16–23% versus 3% of those on placebo and dry mouth in 13 and 19% versus none on placebo. An increase in depression and other psychiatric issues were not reported, although there was insomnia in 10–12% versus 6% [116].

In a 24-week study, less than 3% weight loss was seen with pramlintide alone, but 11% weight loss was seen when it was combined with either phentermine or sibutramine. All patients also received lifestyle intervention. Elevations from baseline in heart rate and diastolic blood pressure were demonstrated in both the pramlintide-sibutramine and pramlintide-phentermine group. These results support the potential of pramlintide-containing combination treatments for obesity, but the blood pressure and heart rate changes with sibutramine and phentermine remain a concern [117].

The combinations of pramlintide plus leptin are also being studied. Pramlintide, in a dose 360 micrograms twice daily dose, which is much higher than the 15–120 microgram three times daily usually used in diabetes and metreleptin 5 mg twice daily showed that the agents alone result in a 7% weight loss, but the combination of the two results in a 13% weight loss in completers at 20 weeks [118]. Although the rate of weight loss in the pramlintide-metreleptin arm decreased over time, a plateau was not reached by the end of the study. The most common side effects seen with pramlintide-metreleptin combination treatment were injection site adverse events and nausea, which were mostly mild to moderate and transient in nature.

10. Conclusion

Obesity now presents one of the biggest health problems of our times. Diet and exercise are best for both prevention and treatment; unfortunately, both require much discipline and are difficult to maintain. Medications offer a possible adjunct, but their effect is modest, they are limited by side effects, and the weight loss lasts only as long as the drug is being taken, since as soon as treatment is stopped, the weight is regained. Sibutramine, a sympathomimetic medication which was available for long-term treatment, is the most recent of the drugs to be withdrawn from the market due to side effects; in this case it was an increased risk of cardiovascular events. A number of other drugs remain available, but learning more about the combination of existing medications and new drug development will hopefully provide better treatment for this very difficult challenge.

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Review Article

Weight Gain, Obesity, and Psychotropic Prescribing

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A majority of psychiatric medications are known to generate weight gain and ultimately obesity in some patients. There is much speculation about the prevalence of weight gain and the degree of weight gain during acute and longitudinal treatment with these agents. There is newer literature looking at the etiology of this weight gain and the potential treatments being used to alleviate this side effect. The authors undertook a comprehensive literature review in order to present epidemiology, etiology, and treatment options of weight gain associated with antipsychotics, mood stabilizers, and antidepressants.

1. Introduction

Weight gain is a major health problem in the United States and is a common adverse effect associated with many psychiatric drugs used to treat depression, anxiety, bipolar disorder, and schizophrenia. Clearly the new, second generation antipsychotics have gathered much press and literature about thier profound effects on weight gain and the development of metabolic disorders. Class action law suits have been filed and settled against drug manufacturers, and the FDA has issued cautions about these serious side effects with this class of medications. Psychiatric prescribers are well aware of this side effect profile of antipsychotics but also note weight gain to be associated with a majority of other commonly used psychiatric medications.

Induction of weight gain and obesity often contributes towards psychotropic medication nonadherence. This can lead to relapse and hospitalization. Weight gain clearly contributes to medical comorbidity [1, 2]. Currently prescribed psychotropics (antipsychotics, antidepressants, and mood stabilizers) may cause 2–17 kg of weight gain over the course of clinical treatment [3–6]. Unfortunately, there are only a very few psychotropics associated with weight loss [7–9]. This paper will briefly review the epidemiology, possible etiology, and available treatment options for psychotropic-induced weight changes. In a novel format, the authors will present brief clinical cases to help convey information about typical patient scenarios and management strategies.

2. Weight Gain due to Psychotropics

Case 1. HA is a 22-year-old female suffering from depressive disorder and social anxiety disorder. She had failed to respond to an initial SSRI antidepressant (paroxetine) and was subsequently placed on an SNRI. She had a partial response to this agent and developed a minimal 1-2 kg weight gain. An approved second generation antipsychotic (aripiprazole) was added for her resistant symptoms and after 6 weeks of treatment she experienced a 5 kg weight gain.

The above case is a typical and common example of iatrogenically induced weight gain. With the introduction of the newer, atypical second generation antipsychotics (SGAs), the potential to cause remarkable weight gain has been recognized. Nearly every antipsychotic has been reported to cause weight gain. Although comparison is limited by the different designs and recruitment procedures of reviewed studies [10], a MEDLINE search from 1966

to 2009 showed that the amount of body weight gain was highest in patients treated with olanzapine (average body weight gain 2.3 kg/month), quetiapine (1.8 kg/month), and clozapine (1.7 kg/month). Treatment with risperidone showed moderate changes in body weight (average body weight gain 1.0 kg/month), where ziprasidone seemed to induce only slight body weight changes (0.8 kg/month). Asenapine causes up to 0.9 kg weight gain in the first three weeks of treatment [11] and its FDA Package Insert discusses a 52-week regulatory trial causing negligible weight gain over time, suggesting it may also be less metabolically problematic [12]. Nineteen percent of patients treated with asenapine have weight gain as compared to 31% who were treated with olanzapine [13]. The other recently FDA approved second generation antipsychotic is iloperidone. It has shown mild to moderate weight gain (1.5–2.1 kg). This appears to be more than that produced by ziprasidone, but more similar to that seen with risperidone [14]. The above two antipsychotic medications may not have enough postmarketing data available as of now to clearly delineate weight gain potential. Paliperidone was approved a few years back, and both short- and long-term (52 weeks) studies have shown no significant metabolic side effects including weight gain [15, 16]. The weight gain associated with paliperidone could, in theory, be similar to risperidone as it is an active risperidone metabolite, unless the parent drug contains the weight-inducing components which are now omitted from the paliperidone pharmacodynamic profile after liver first-pass metabolism.

A common comparative finding in the literature suggests that risperdone causes weight gain between 0.3–2.6 kg while ziprasidone often causes weight loss [17–26]. Olanzapine may be the most significant second generation agent causing weight gain from 4.2–7.4 kg, and even up to an average gain of 12 kg in 45–90% of patients [27–32]. Quetiapine (regular release and extended release) also has been implicated in causing a more remarkable weight gain of 4.1 to 5.6 kg [33, 34]. Clozapine may ultimately be the most likely agent to cause weight gain of all psychotropics producing increases of 2.4 to 31.3 kg, which is often a 10 percent gain over baseline [35–39]. Finally, a short-term study using aripiprazole showed a limited, 0.5–0.9 kg weight gain as compared to placebo [40].

Case 2. SK is a 43-year-old female with depressive disorder and posttraumatic stress disorder. She failed to respond to an approved SSRI (sertraline) but experienced minimal weight gain. She was placed next on a combination of an SNRI (venlafaxineER) and a dopamine-norepinephrine reupake inhibitor (bupropionSR) also without symptomatic improvement. She did not gain weight. Given her treatment resistant symptoms, she was placed on tricyclic antidepressants (imipramine, nortriptyline) with a minimum of weight gain but experienced cardiac side effects. Finally, she was placed on an MAOi (tranylcypromine) with partial symptom improvement, but also a 15–20 kg weight gain. She developed social anxiety and agoraphobia, almost refusing to go out of her house due to the excessive weight gain.

The prevalence of weight gain due to antidepressants such as imipramine (causing a 3-4 kg weight gain) is more chronic and more common, yet underrecognized as depression and anxiety are more prevalent diagnostically and epidemiologically as compared to psychotic disorders [41].

Tricyclic antidepressants (TCAs) and perhaps monoamine oxidase inhibitors (MAOIs) may be more likely to cause weight gain than the selective serotonin reuptake inhibitors (SSRIs), while mirtazapine may be placed between the SSRIs and the TCAs in terms of relative risk of weight gain [42, 43]. Bupropion may be the only modern agent that lowers weight [5]. There is a 1–3 kg average weight gain on antidepressants in 10–20% of the population treated with them (likely a mixture of serotonergic facilitation (excess synaptic serotonin, may downregulate and desensitize serotonin-2c receptors causing increased appetite) and anticholinergic properties). This is further complicated by the fact that antipsychotic medication use is escalating after FDA approval for bipolar and major depressive disorders, which may most likely increase the incidence of metabolic effects. MAOI's use results in moderate weight gain [6]. Trazodone studies show 0.5 to 1.1 kg weight gain over time [8, 44], while five percent of baseline weight loss or 3–4.4 kg of weight loss has been reported with bupropion [45–47]. Although initial studies showed weight loss with fluoxetine, long-term followup reveals that its weight reducing effect is associated with a gain in body weight developing over time [48]. Acute losses of 0.35 kg may be noted, but an average of 2–2.5 kg of weight may be gained [49]. Citalopram may cause 1 to 1.5 kg weight gain over a year's time [50, 51]. Fluvoxamine [5, 52] has two studies showing no weight gain and no weight loss. Sertraline studies suggest more weight gain than control groups [53]. Paroxetine is the most likely of the SSRIs to cause weight gain. Tohen et al. showed that paroxetine promoted more weight gain when compared to fluoxetine and sertraline [53]. These findings suggest a lesser iatrogenic weight gain side effect profile compared to the SGAs mentioned above.

Mirtazapine has consistently shown greater weight gain when compared to SSRIs (fluoxetine, paroxetine, and citalopram) [54–56]. In clinical practice it seems to react like an SGA in this regard. However, aripiprazole which has recently been approved for adjunctive treatment of major depressive disorder, differs as it tends to have a lower risk of association with metabolic abnormalities [57]. In some patients though, the use of aripirpazole as an adjunct may contribute to a higher likelihood of weight gain [58]. As far as the newer SNRI duloxetine is concerned, in a recent 8 month double blind randomized prospective trial with subjects initially on duloxetine, escitalopram or placebo, the mean change in weight was significantly higher for escitalopram compared with duloxetine (+0.61 kg, duloxetine; +1.83 kg, escitalopram; $P < .05$). However, the incidence of treatment-emergent abnormal weight gain (FDA definition suggests greater than or equal to a 7% increase in weight from baseline) was similar between drugs and was significantly greater for both duloxetine and escitalopram compared with placebo. Mood stabilizing agents are used for bipolar disorder and schizoaffective disorder. As with the antipsychotics and antidepressants, weight gain is a common side effect.

Seventy-one percent of divalproex treated patients may gain more than 4 kg [6]. Nine tenths of a kilogram to fourteen kilogram of divalproex-induced weight gain has been reported in other studies [59, 60] with an incidence of 8–59%. Lamotrigine has a more favorable, weight neutral profile and may cause weight loss (of 2 kg) or a gain (up to 0.6 kg) [61]. Carbamazapine may show a weight change up to 15 kg based on limited studies [62, 63]. Lithium reviews suggest that weight gain is also a common side effect. Twenty percent of patients on lithium gained 10 kg or more in one study and a 6.3 kg weight gain in a second [64].

3. Cause of Weight Gain

This section will attempt to briefly review the possible causes of iatrogenic weight gain. Gothelf et al. [65] studied weight gain mechanisms and energy balance in olanzapine-treated adolescent male patients and found that an increase in body mass index was due to an increase in calorie intake, but also revealed effects on the resting energy expenditure or slowing of metabolism. Psychotropics have broad pharmacodynamic profiles, and it is likely that multiple neurotransmitters, receptors, and neurocircuits are responsible for drug-induced weight gain. Olanzapine has activity at several different receptor sites [66]. Others, like clozapine, may act at even different receptors (i.e., δ-opiod receptors) [67]. In rats, activating 5-HT_{2C} receptors decreases eating behavior and mice lacking 5-HT_{2C} receptors become obese [68]. Many of the antipsychotics (olanzapine, quetiapine, and clozapine) and some antidepressants (mirtazapine) have this 5-HT_{2C} blocking property.

Beta-3 adrenergic receptors found in adipose tissue play an active role in weight control by converting fat into energy especially in response to norepinepherine [69]. Clinically there are no data to suggest effects on weight gain with alpha-adrenoceptor antagonists but it is known that psychotropics with higher affinities (i.e., TCAs) for these receptors are associated with weight gain, whereas those with lower affinities (e.g., SSRI) are not [70]. Psychotropics with greater ability to block H-1 receptors often show greater weight gain potential [71], possibly through deactivating brain satiety centers. Another mechanism may be related to blockage of anticholinergic sites, which is associated with appetite stimulation.

4. Treatment Weight Gain

Potential weight loss strategies are outlined in Table 1. Early intervention is the key to preventing significant drug-related weight gain. Patients should be educated about weight gain as a potential adverse effect before they begin treatment and their weight should be monitored routinely as a standard of care, as long as they continue taking drugs that may increase weight. Informed consent about this risk should be the standard of care. Routine blood and vital sign monitoring for detection of increases in blood sugars, serum lipids, blood pressure, and abdominal girth would be helpful and clinically warranted [72].

Metabolic syndrome is now a well-documented effect of second-generation antipsychotic use**.** Ideally, a diet and exercise plan should be initiated to prevent or treat weight gain before medically significant weight gain occurs [73]. A successful weight loss program is one which can produce a loss of 0.5 to 1 lb of the patient's initial body weight per week, a rate of loss considered safe and acceptable [74]. Diet and exercise produces maximal benefit, but requires considerable commitment and motivation on the part of the patient. This is often difficult or impossible in the mentally ill.

Case 1: Part 2. HA had been given informed consent prior to starting her serotonergic antidepressants about the potential for weight gain and was advised to consider prophylactically increasing her current low level exercise regimen, to watch caloric intake, and to weigh herself with directions to notify the clinician if she experienced a consistent gain of 2.5 or more kilograms. After mild initial weight gain, dieting by portion control methods was discussed. After a more remarkable weight gain was noted with the second generation antipsychotic, she was offered off-label use of chromium picolinate, or metformin, or approved use of orlistat. The patient felt chromium picolinate had the most favorable risk profile and tried 1000 mcg daily without weight improvement. At a subsequent visit she was placed on metformin. (These interventions are discussed later in this paper).

Case 3. AB is a 30-year-old female with depression, anxiety, and substance use disorder. Despite gaining sobriety, she still suffered depressive symptoms and failed to respond to initial SSRI (fluoxetine) and SNRI (duloxetine) therapy. She was placed on the second generation antipsychotic, quetiapine, with moderate symptom reduction but began to gain weight (3–5 kg) and asked for other treatment options. She was cross-titrated onto aripiprazole with an acceptable weight loss as a result and continued symptom reduction.

Besides diet and exercise, formal behavior modification techniques involve changing eating habits and reinforcing desired weight controlling behavior. It is the gradual but consistent change in behavior that leads to healthier eating habits. Simple use of portion control behaviors can teach patients to eat less at every meal without the complexity of counting fat versus carbohydrate calories, and does not require the willpower to follow a bland low-salt, low-fat, low-sugar diet [75]. Behavior modification alone can generate a weight loss of 0.5 kg to 0.7 kg per week [76]. Through formal, manualized cognitive-behavioral therapy patients can achieve satisfaction with body image and acceptance of modest weight loss. In one study, the effects of cognitive-behavioral therapy on weight gain due to psychotropics was studied in 6 schizophrenia patients (mean age 37.3 years). The mean BMI (kg/m^2) decreased from 29.6 kg to 25.1 kg in the posttreatment group [77]. Cognitive therapy has been helpful in reducing weight for children and adolescents [78]. Furthermore, the addition of cognitive therapy to a diet-controlled method produces better results [79].

Generally, drug therapy with specific metabolic informed consent, active monitoring of weight and early intervention,

or even prophlyaxis with diet and exercise are the first treatment options. If this fails, or the patient is too ill to comply, then clinical practice suggests that antiobesity drugs may be appropriate. Risks and benefits should be evaluated for each antiobesity agent. Sometimes, prior to trying an antiobesity medication, one may choose to switch the current psychotropic medication to one with the same indication but less weight gain potential as noted in the case above. The risks and benefits of changing an effective medication should be adequately considered before making changes.

Chromium compounds were utilized in a case above and have been used over the counter to facilitate weight loss, although the evidence for its efficacy is lacking so far [80, 81]. However, chromium picolinate has reasonable data in regards to improving insulin sensitivity in diabetics and has been shown to help curb carbohydrate cravings in depressed patients despite continued depressive symptoms when dosed 600 mcg/d [82, 83].

Drugs that reduce caloric intake or suppress hunger, are commonly known as anorectic agents or appetite suppressants. They act centrally by decreasing appetite or increasing satiety. Sympathomimetic agents include phendimetrazine, phentermine, mazindol, diethylpropion (many are controlled substances), amphetamine and related compounds, and phenylpropanolamine. The amphetamine products are used on-label for the treatment of sleep apnea, narcolepsy, and attention deficit/hyperactivity disorders. When these conditions are comorbid with other primary psychiatric disorders a weight loss advantage is often clinically noted. Of note, however, the serotonergic agents, fenfluramine and dexfenfluramine were withdrawn from the US market in September 1997 over concerns about valvular heart disease [84].

The three most currently prescribed drugs that are FDA approved to treat obesity are phentermine, sibutramine, and orlistat. Drugs approved for treating obesity usually result in an additional weight loss of approximately 2–5 kg over placebo. At least four other types of single-agent weight loss drugs are in possible late stage development: (1) selective central cannabinoid-1 receptor blockers, (2) selective central 5-hydroxytryptamine 2C serotonin receptor agonists, (3) an intestinal lipase blocker, and (4) central-acting incretin mimetic drugs [85]. Furthermore, other agents under development that may produce beneficial changes in appetite expression in the obese include glucagon-like peptide-1 analogs such as liraglutide, an amylin analog davalintide, the 5-$HT_{(2C)}$ receptor agonist lorcaserin, the monoamine re-uptake inhibitor tesofensine, and a number of combination therapies such as pramlintide and metreleptin, bupropion and naltrexone, phentermine and topiramate, and bupropion and zonisamide [86]. For example, lorcaserin is a selective agonist of the 5-HT_{2C} serotonin receptor. Its shared mechanism of action with fenfluramine suggests that a lorcaserin-phentermine combination therapy may be particularly effective for treatment of obesity. The combination of naltrexone and bupropion has recently been examined in a large phase 3 trial for the treatment of obesity. This study demonstrated that one year of treatment with this two-drug combination produced an approximate 4% weight loss beyond that seen with placebo therapy, similar to that seen with other pharmacologic therapies. Because of the distinct mechanism of action of these two medications, naltrexone-bupropion may prove to be an attractive option for patients who are resistant to other agents. Several clinicians have noted that the combination of phentermine and topiramate can generate substantial weight loss in at least a subset of patients who exhibit little weight loss when treated with phentermine alone. These observations led to the development of fixed dose combinations of phentermine and topiramate. A large, phase 3 clinical trial has shown that one year of treatment with this combination led to weight loss of up to 9% beyond that seen with placebo therapy. There have been no head-to-head comparisons, however, and further studies will be needed to determine the relative effectiveness of these various treatments. It is noteworthy that the doses of each agent used in the phentermine topiramate combination studied were lower than the typical doses used for monotherapy with each drug [87]. In one study, following a 1-week placebo lead-in, 244 obese or overweight, nondiabetic subjects received placebo subcutaneously (sc) t.i.d., pramlintide sc (120 μg t.i.d.), pramlintide sc (120 μg t.i.d.) + oral sibutramine (10 mg q.a.m.), or pramlintide sc (120 μg t.i.d.) + oral phentermine (37.5 mg q.a.m.) for 24 weeks. Weight loss achieved at week 24 with either combination treatment was greater than with pramlintide alone or placebo [88]. As weight gain is often substantial with psychotropics, combined antiobesity therapy in clinical practice is frequently needed. Psychiatrists are often clinically savvy using rational polypharmacy to achieve remission of the psychiatric disorder at hand, and perhaps may consider polypharmacy in severe cases of psychotropic drug-induced obesity. Although risk benefits ratio for the use of single or multiple antiobesity agents needs to be determined on a case by case basis before the initiation of these therapies. Sometimes clinicians can "chase their tails" by adding anti-side-effect medications to a patient's regimen. Sometimes the anti-side-effect medications have side effects themselves that must be treated, and so on. Clinicians must make critical, Hippocratic decisions when it comes to this complex clinical polypharmacy decision.

Single sympathomimetic amphetamine agents, because of their high potential for abuse, cardiac, and psychiatric side effects (anxiety induction, insomnia), are generally not often recommended for treating obesity [84]. Sibutramine is however relatively safer, as it is a mixed serotonergic and noradrenergic reuptake inhibitor. It helps patients achieve a 10% to 15% loss of body weight [89–92]. The safety and effectiveness beyond 1 year of use have not been determined. The mechanism by which sibutramine acts is increased satiety. It decreases the levels of triglycerides, total cholesterol, and LDL cholesterol, while also increasing the levels of HDL cholesterol (seen in people who lose >5% of body weight). Sibutramine can increase blood pressure and heart rate. Other common adverse effects of sibutramine are dry mouth, anorexia, insomnia, irritability, and constipation. These studies were conducted in obese individuals who were not taking psychotropics, so the outcome may not be generalizable to the mentally ill. This agent when combined

Table 1: Weight gain treatment options.

Mode or medication	Weight loss produced	Duration of treatment
Behaviour modification	0.5–0.7 kg per week	—
Cognitive-behavioral therapy	4.5 drop in BMI	—
Naltreoxone and buropion	4%	—
Phentermine and topiramate	9%	1 year
Sibutramine	10–15%	<1 year
Orlistat	10.2%, 8.8%	1 year
Orlistat	>10%	2 years
Orlistat	34.6%	?
Amantadine	3.5 kg	3–6 months
Nizatidine	Gained 3 kg less than patients who did not take Nizatidine	16 weeks
Naltexone	5 kg	8 weeks
Topiramate augmentation	10–15 lbs	—
Metformin	15/19 patients lost weight	12 weeks

with other antidepressants may lead to serotonin syndrome and is often avoided.

Case 2: Part 2. SK had only a partial improvement on the MAOI treatment, and subsequently, it was stopped but her weight gain was not alleviated. She was ultimately treated with a complex polypharmacy antidepressant regimen with near remission of her symptoms. Similar to Case 1, she was given metformin *and* orlistat with gradual, but not complete weight loss. To gain final remission of depression symptoms she was placed on a stimulant (methylphenidate) medication which was titrated to a moderate dose. Her depression resolved and she continued to lose weight to her baseline level.

Orlistat**,** a fat or lipase blocker, has safety and efficacy data for use, up to two years. Orlistat may be a better option than sibutramine for patients already taking other drugs because it does not act systemically, so there is less risk of interaction with centrally acting medications. Specifically, it inhibits gastric and pancreatic lipases by binding covalently to the serine residue at the active site of these enzymes [89]. This allows fat not to be absorbed by the GI system when taken with meals [90–92]. It decreases triglycerides, total cholesterol, and low-density lipoprotein cholesterol while increasing high-density lipoprotein cholesterol [93]. The drug also improves glycemic control [94]. The most common adverse effects are gastrointestinal, including increased defecation, soft stools, anal leakage, fatty or oily stools, and vitamin A and E deficiencies [95, 96]. Patients take orlistat 120 mg three times daily and must take a multivitamin to avoid deficiencies and eat a low-fat diet.

Two large placebo-controlled trials [97, 98] document the efficacy of orlistat use for up to two years. After one year, the orlistat group lost 10.2% of body weight in one study and 8.8% in the second study. After 2 years, twice as many patients taking orlistat maintained a weight loss of more than 10%. Patients must take other medications one hour before or after orlistat to avoid change in absorption [99]. One study in the mentally ill reported that 13 consecutive patients with psychotropic-induced weight gain lost 34.6% of side effect weight gained [100]. Nine of the 13 subjects suffered from major depressive disorder and were taking serotonergic antidepressants. Patients were deemed obese with a body mass index (BMI) of >30 kg/m^2. The average weight gain from psychotropics prior to orlistat initiation was 16.4 kg. The average weight loss within this relatively short time period was 5.6 kg or 34.6% of the weight gained as a result of psychotropic drug use.

Amantadine was studied [101] in twelve patients who had already gained a mean of 7.3 kg during olanzapine treatment. Subjects were started on amantadine at 300 mg per day. Results of the study showed an average weight loss of 3.5 kg over 3–6 months. No adverse effects were reported. Implementation of nizatidine (histamine-2 receptor antagonist) was studied in a 16-week, randomized, double-blind, placebo-controlled study in schizophrenia patients. Dosed at 300 mg twice per day, it allowed patients to gain an average of 2.5 kg compared with the 5.5 kg gained by patients treated without nizatidine [102]. Naltrexone, an opioid antagonist, at a dosage of 50 mg/day, has been shown to decrease weight by reversing the observed hunger and craving for sweet, fatty foods caused by tricyclic antidepressants and lithium. Subjects reported decreased enjoyment ratings of food and also diminished subjective feelings of hunger. No adverse effects of opioid antagonism were seen regarding depressive symptoms. In another study, naltrexone was coadministered with antidepressants to eight female patients who had already gained more than 6 kg. After eight weeks, weight gain was reversed in five patients, stopped in two patients, and attenuated in another. However, weight increased by 1.5 ± 2.7 kg within 14 weeks after the drug was stopped. Of note, the mean weight loss was small compared to previous drug-induced weight gain.

Preliminary findings suggest that topiramate may serve as a dual purpose agent in the treatment of obese patients with affective disorders. In one case report [103], topiramate was administered to a 29-year-old male schizophrenic patient who had gained weight due to clozapine. Results showed

a sustained weight loss for the first time with an improvement of psychotic symptoms. Additionally, topiramate add-on studies for bipolar disorder have shown 33%–55% of patients losing weight (10–15 lbs) [104, 105]. Side effects of fatigue, cognitive dulling, ataxia, glaucoma, oligohydrosis, and acidosis are reported at doses of 100–400 mg/day. A recent review of studies using metformin and topiramate has shown more efficacy and fewer side effects with metformin [106].

Metformin holds promise as a treatment for weight gain in pediatric patients taking psychotropic medications. In a 12-week open label study [107] conducted on 19 patients (aged 10–18 years) who had gained over 10% of their baseline weight while on antipsychotics, 500 mg three times a day of metformin was given for 12 weeks in addition to psychotropics. The results of the study showed 15 patients lost weight, 3 gained weight, and one remained unchanged in weight. Sporadic diarrhea was noted in some patients that resolved with time**.** The results of the safety tests for lactic acidosis were unremarkable. A recent controlled study by the same group confirmed this open label finding [108].

5. Conclusions

This paper has reviewed the risks of and possible causes of psychotropic-induced obesity and concluded with some potential treatment options designed to help manage this side effect, while ideally maintaining patients on effective, psychotropic regimens. There are no FDA approved agents for reversing or preventing this iatrogenic weight gain, and all options reported above are therefore considered off-label at this time. Many studies are uncontrolled and of small scale, limiting our conclusions in the mentally ill population. A combination of diet, exercise, and medications would be the ideal approach for combating the weight gain seen in the mentally ill population, but we often find these patients unable to comply with rigorous diet and exercise regimens due to their psychiatric symptoms. Other strategies which may be useful, but need further randomized placebo-controlled studies include switching the psychotropic medication to one less likely to cause metabolic changes as well as addition of medications such as topiramate and metformin to help reduce the change which has occurred. It is important to note that, although the use of psychotropic medication has contributed to the increased incidence of metabolic effects in the mentally ill population, certain studies have shown that, at baseline, patients with severe and persistent illness have a higher prevalence of metabolic disorders. Another concern is the high likelihood that metabolic disorders are untreated in patients with schizophrenia, with particularly high rates of nontreatment for hypertension and dyslipidemia. There is a need for increased attention to basic monitoring and treatment of metabolic risk factors in this vulnerable and often underserved psychiatric population [109].

The authors would welcome future psychotropics devoid of weight gain potential and strongly suggest future, larger controlled studies focusing on weight reduction in this population using pharmacologic, nonpharmacologic, and combined strategies.

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Research Article

Serum Neopterin Is Not Increased in Obese Juveniles

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Objective. Cardiovascular disease is associated with inflammation and immune activation, concentrations of immune activation markers like neopterin predict outcome in adults. *Methods*. Serum neopterin concentrations and early metabolic and pre-atherosclerotic symptoms were analyzed in 295 obese juveniles and 101 normal weight controls of similar age. Additionally, the influence of a 12 months weight reduction program on neopterin levels was investigated in 31 obese juveniles. *Results*. Intima-media thickness of common carotid arteries (IMT) and the concentrations of C-reactive protein (CRP) were increased in the obese juveniles ($P < .001$). Also triglycerides, oxidized LDL, fasted insulin levels, HOMA-index, leptin, liver transaminases and uric acid were increased compared to the controls. However, serum neopterin was decreased in the obese versus non-obese juveniles ($P < .03$). The intervention consisting of regular sports, nutritional devices, and a psychologic attendance led after 12 months to an increase of neopterin concentration ($P < .05$; paired test). *Conclusions*. Neopterin concentrations in juvenile obesity behaved considerably different from what was demonstrated in adults, levels did not correlate with metabolic and pre-atherosclerotic symptoms found in early phases although early vascular burden and chronic low grade inflammation was indicated by increased IMT and CRP. Neopterin concentrations increased after a 12 months intervention program.

1. Introduction

Nowadays, the role of inflammation and immune activation in atherogenesis is well established [1], and the activation of macrophages appears to be of critical relevance within this process [1, 2]. Cardiovascular disease (CVD) is also associated with increased neopterin concentrations [3–10], and predictive information is provided by neopterin in patients at risk of myocardial infarction [11–16]. A recent study in 2312 patients undergoing angiography demonstrated that neopterin is an independent predictor of total and cardiovascular mortality [17]. Likewise, in patients with diabetes, both neopterin and C-reactive protein (CRP) were independent predictors of fatal ischemic heart disease [18].

Neopterin is produced by human monocyte-derived macrophages and dendritic cells preferentially upon stimulation with Th1-type cytokine interferon-γ (IFN-γ) [19, 20]. Thus, neopterin concentrations reflect activation of the Th1-type immune response [21]. In humans, increased neopterin concentrations are detected in patients with virus infections including human immunodeficiency virus, infections by intracellular bacteria and parasites, autoimmune diseases, malignant tumors, and during rejection episodes in allograft recipients [21–24].

In foregoing studies, we detected an increased carotid intima-media thickness (IMT) indicating preatherosclerosis paralleled by a subclinical inflammation in obese juveniles aged around 13 years [25]. Additionally, we showed an influence of subcutaneous adipose tissue topography on total adiponectin and adiponectin subfraction serum levels in obese juveniles and provided the first evidence that incipient atherosclerosis yet found in adolescents by an increased IMT is associated with a disturbed oligomerisation from low-molecular to high-molecular-weight adiponectin [26, 27].

In a group of otherwise healthy adults, a positive correlation was observed between neopterin concentrations and body mass index [28]. From the data obtained in adults, it appears that neopterin serum levels are associated with chronic subclinical inflammation and preatherosclerotic symptoms in early phases of obesity in a particular manner. In this study, we analysed serum concentrations of neopterin with carotid IMT, and cardiovascular risk factors in a cohort of 295 obese juveniles and adolescents compared to 101 normal weighted, healthy controls of the same age and gender distribution. Further, we investigated the influence of a weight reduction program performed for 12 months in a subgroup of 40 obese juveniles on neopterin serum concentrations.

2. Materials and Methods

2.1. Patients. Study participants were from the STYrian Juvenile Obesity Study (STYJOBS), which is designed to investigate early stages of atherosclerosis and metabolic disorders in obese juveniles. STYJOBS is registered at http://clinicaltrials.gov/ (Identifier NCT00482924), where detailed information of the study is available. The inclusion criterium for the obese probands was body mass index (BMI) > 97th percentile if under 18 years of age, BMI > 30 kg/m^2 if over 18 years of age. Exclusion criteria were endocrine diseases (e.g., hypothyroidism), infectious, inflammatory, or any other chronic diseases. Controls were healthy juvenile volunteers recruited from the Department of Pediatric Surgery, where they underwent minor elective surgery (e.g., herniotomia). Fasted blood samples were collected prior to anaesthesia and surgery. All controls had to be normal weight, free of infectious, and endocrine or any other diseases. 295 juveniles (mean age 12.9 ± 3.3 SD years) and 101 normal weight healthy controls of similar age and gender distribution were investigated.

Out of this cohort, 40 juveniles with BMI > 97th percentile (male 17, female 23, mean age 13.6 + 2.3 years (SD)) underwent a 12-month weight reduction program during the year 2006. The intervention schedule encompassed regular sports (2 times per week, one hour, 12 months), nutritional devices (1x/week, 1st three months, 1x/2 weeks, 2nd three months, 1x/4 weeks within the remaining 6 months), and a psychologic attendance. Thirtyone obese juveniles (male 13, female 18) who fully completed this program were included in the analysis of the neopterin kinetics during intervention.

The study was approved by the Ethical Committee of the Medical University of Graz. Blood collection and ultrasonography were performed after written informed consent was given by the probands if aged over 18 years, or by their parents, if they were under 18 years old. At the time of blood collection, the probands were fasting. Venous puncture was performed in a standard procedure (cubital vein approach with butterfly); blood samples were immediately centrifuged at 3500 rpm at ambient temperature and stored at −80°C until analysis.

2.2. Carotid Artery Ultrasound. The ultrasound protocol involved scanning of the bulbous near the common carotid artery on both sides with a 12-to-5-MHz broad-band linear transducer on an HDI 5000 (ATL, Bothell, Washington, DC, USA). All scans were performed by the same investigator. Longitudinal images directed through the center of the artery were taken at each vessel site. Measurements were made from stored digital images by an experienced reader. The carotid IMT was assessed at the far wall as the distance between the interface of the lumen and intima, and the interface between the media and adventitia. The maximal IMT was recorded at each of the vessel segments and averaged for the left and right sides. The lumen diameter was calculated as the interadventitial diameter minus twice the maximum far wall IMT. All diameters were measured during diastole to avoid image blurring due to systolic arterial wall motion and to minimize the influence of blood pressure [29].

2.3. Lipometry. Measurements of total percentual body fat were performed as described elsewhere by means of a lipometer, a patented optical device (EU Pat.Nr. 0516251) [30].

2.4. Laboratory Analysis. Neopterin concentrations were determined by enzyme-linked immunosorbant assay (ELISA) (BRAHMS Diagnostica, Hennigsdorf, Germany). Total adiponectin, leptin, and resistin were determined by ELISA (Biovendor Laboratory Medicine Inc., Brno, Czech Republic) according to manufacturer's instructions. Intra- and interassay coefficients of variation for all ELISAs in our study were below 10 percent. Cholesterol and triglycerides were measured by means of ECLIA (Electro Chemi Luminiscence Assay) on an Elecsys 2010 analyser (Roche Diagnostics Mannheim, Germany), and lipoproteins were separated by a combined ultracentrifugation-precipitation method (β-quantification). Blood lipids inclusive of fatty acids were analysed as outlined elsewhere [31]. Oxidized low-dense lipoprotein (oxLDL) was measured by a commercially available ELISA (Mercodia oxidized LDL Competitive ELISA, 754 50 Uppsala, Sweden). Concentrations of CRP were analysed with a highly sensitive particle-enhanced immunoturbidimetric assay (Tina-quant C-reactive protein latex ultrasensitive assay, Roche diagnostics). Plasma insulin was measured by ELISA (Mercodia, Uppsala, Sweden) and plasma glucose was measured by the glucose hexokinase method on a Hitachi 917 chemical analyser. HOMA-IR (homeostatic model assessment-insulin resistance) was calculated as the product of the fasting plasma insulin value (in μU/ml) and the fasting plasma glucose value (mm/L), divided by 22.5 [27]. Liver transaminases AST/GOT, ALT/GPT, γ-GT, creatinine, and uric acid were measured by routine laboratory methods on a Hitachi 917 chemical analyser.

2.5. Statistic Analysis. Statistical analysis was performed by SPSS version 14. Kolmogorov-Smirnov test was used to examine for normal distribution. Means were compared by a two-tailed unpaired sample t-test or by Mann-Whitney

Test, depending on the distribution of the data. A value of $P < .05$ was considered statistically significant. Least squares regression analysis was performed to test the correlation between variables according to Pearson. Significance of correlation was determined by univariate ANOVA and subsequent multiple comparison analysis. Bonferroni correction was used for multiple comparisons after ANOVA. To exclude a dependence of the resulting correlations from other variables, the correlations were adjusted by partial correlations. Stepwise multiple regression analysis was applied to investigate correlations between neopterin as dependent variable and different metabolic and vascular measures as independent variables.

3. Results

The clinical and laboratory characteristics of the study probands are summarized in Table 1. Obese juveniles had a significantly increased IMT ($P < .001$) and elevated CRP, ($P < .001$, see also Figure 1) indicating early stages of atherosclerosis and chronic low-grade inflammation (Table 1). Oxidized LDL, fasted insulin, HOMA-index, resistin, and systolic-, diastolic-blood pressure were significantly increased, and HDL cholesterol significantly decreased in the obese cohort (Table 1). Compared to the controls, obese juveniles had significantly lower neopterin serum concentrations (Table 1 and Figure 1). Neopterin concentrations did not correlate with percentual body fat or BMI-SDS values in the whole study cohort and were independent from any other variable investigated in this study and listed in Table 1 (data not shown).

After the 12-month weight reduction program consisting of regular sports, nutritional devices, and a psychologic attendance, the total percentual total body fat, oxLDL, and resistin serum concentrations decreased significantly whereas the HDL-cholesterol levels were significantly increased (Table 2). CRP concentrations increased upon intervention as compared with baseline, but the difference was not significant. Fasted blood glucose, insulin levels, IMT of common carotid arteries, and systolic and diastolic blood pressure did not differ significantly from baseline (details not shown). After the 12-month intervention program, neopterin concentrations increased and the mean value became higher than baseline (Table 2). This increase of neopterin concentrations did not correlate with the decrease of the BMI-SDS.

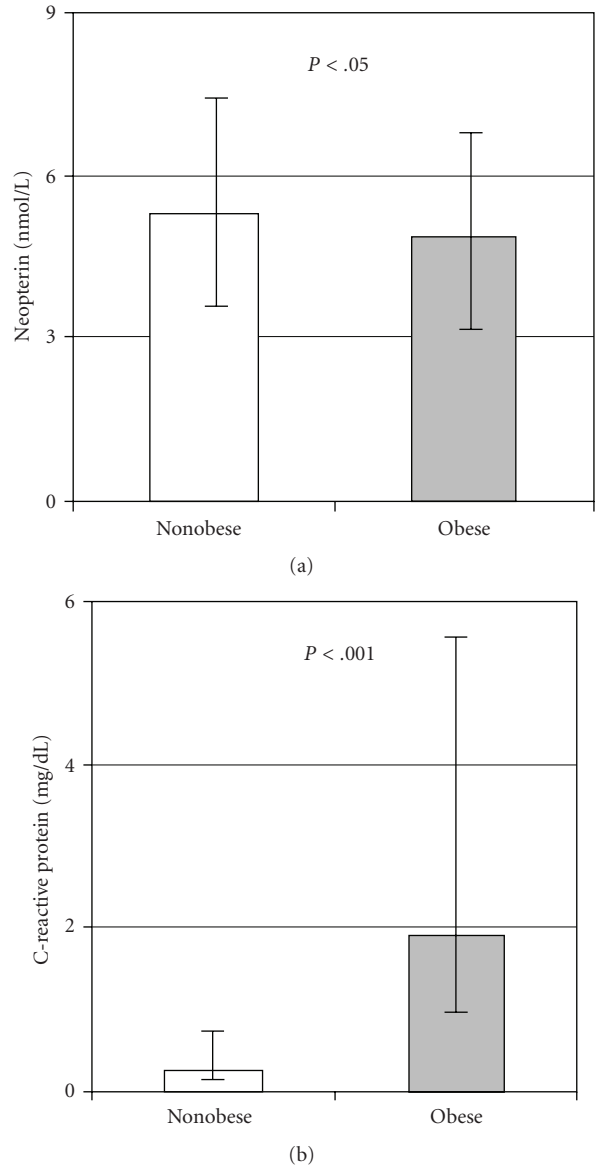

Figure 1: Serum concentrations of neopterin and C-reactive protein in obese and nonobese adolescents. Neopterin concentrations are given as mean values (boxes) and S.E.M. (bars) and because of nonnormal distribution C-reactive protein is given as medians (boxes) and 25–75th percentiles (bars).

4. Discussion

Our study shows that serum neopterin concentrations are not increased in obese adolescents compared to nonobese controls, rather a trend to subnormal levels was observed. Although neopterin concentrations were lowered only by approximately 10% compared to the nonobese controls, the difference was significant. This result is astonishing compared to results obtained in several studies performed earlier by various research groups in adults: adults with high risk for atherosclerosis presented with slightly but significantly increased serum neopterin concentrations compared to low-risk persons [11–18, 32]. Myocardial infarction (MI) is usually accompanied by a transient increase of neopterin [33]. Although only moderately elevated compared to other clinical conditions like acute virus infections or cancer, neopterin levels showed a predictive potential for future clinical events in symptomatic and stable CVD patients [12, 15, 17, 18, 34]. This predictive value of neopterin was found to be independent from other biomarkers such as CRP [16–18, 32]. The ability to enhance oxidative stress and to induce proinflammatory cytokine cascades [33, 35] via induction of nuclear factor κB suggests neopterin

Table 1: Characteristics of the study subjects.

Variable	Controls ($n = 101$)	Obese adolescents ($n = 295$)
Age (Years)	12.9 ± 2.7 (4–18)	12.4 ± 2.9 (4–18)
Body Length (m)	1.56 ± 0.14	1.56 ± 0.14
Body Weight (kg)	47.2 ± 13.4	74.5 ± 24.2**
BMI (kg/m^2)	18.7 (16.9–20.4)	28.9 (26.2–32.4)$^{+++}$
BMI-SDS	0.21 (−0.7–0.8)	5.4 (4.0–7.2)$^{+++}$
Carotid IMT (cm)	0.052 ± 0.009	0.070 ± 0.09***
Neopterin (nmol/L)	5.3 ± 1.9	4.9 ± 1.8*
C-Reactive protein (mg/dl)	0.26 (0.17–0.84)	1.9 (0.9–3.8)$^{+++}$
HDL-cholesterol (mg/dl)	48.4 ± 12.0	41.9 ± 11.2***
Oxidized LDL (mg/dl)	35.1 ± 15.3	44.3 ± 16.9***
Insulin (uE/ml)	7.9 (5.1–12.7)	15.4 (8.8–29.1)$^{+++}$
HOMA-IR	1.7 (1.0–2.7)	3.5 (1.8–6.9)$^{+++}$
Resistin (ng/ml)	3.7 ± 1.5	4.3 ± 1.6**
SBP (mmHg)	115 ± 4.9	125 ± 15.5***
DBP (mmHg)	63.1 ± 9	68 ± 8.8***

Results are expressed as mean ± S.D. analysed by Student's t-test (*$P < .05$, **$P < .01$, ***$P < .001$) or as median (25th–75th percentile) and Mann Whitney U test ($^{+}P < .05$, $^{++}P < .01$, $^{+++}P < .001$) depending on the distribution of data.
BMI, body mass index; BMI-SDS, BMI-standard deviation score; HOMA-IR, homeostatic model assessment-insulin resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; carotid IMT, carotid intima-media thickness.

Table 2: Neopterin, body fat, HDL cholesterol, resistin, oxLDL, and C-reactive protein concentrations (mean ± S.D.) before and after 12 months of intervention therapy.

	Start of intervention	End of intervention (12 months)
n	31 (male 13, female 18)	31
Neopterin (nmol/L)	4.4 ± 0.38	5.0 ± 1.7*
Body fat (%)	36.8 ± 7.0	29.5 ± 5.1***
HDL cholesterol (mg/dl)	42.4 ± 7.6	47.2 ± 9.3*
Resistin (ng/ml)	4.7 ± 1.6	3.9 ± 0.9*
Oxidized LDL (mg/dl)	59.7 ± 10.8	53.1 ± 16.3*
C-reactive protein (mg/dl)	2.8 ± 2.6	3.2 ± 3.2

*$P < .05$, **$P < .01$, ***$P < .001$ (paired t-test).

to act as a proinflammatory biomarker in atherogenesis [36]. Neopterin appears to play an important role in the pathogenesis of CVD. However, to our surprise this seems to be true only in adults but not in juvenile obesity. There is already information available from adults that CAD activity more than the size of lesions is important for the elevation of neopterin, but in contrast, despite detectable CAD activity in juvenile obesity, neopterin concentrations tended to even lower levels than in nonobese controls of similar age.

Increased neopterin concentrations are characteristic for an activated Th1-type immune response [22]. Accordingly, the decreased or low-neopterin concentrations found in our study cohort of obese adolescents even if rather small in absolute terms might indicate a suppressed Th1-type immunity and thus lowered IFN-γ production [37]. In fact, only the neopterin concentrations found in the obese groups were lower than normal, the mean value of neopterin concentrations measured in the nonobese control population (5.3 ± 1.9 nmol/L; Table 1) was almost identical with the one found in larger populations of adult blood donors and healthy controls as 5.3 ± 2.7 nmol/L [21, 22, 38]. At present, increased neopterin concentrations are well established in patients with several diseases which are known to be associated with immune activation such as virus infections, autoimmune syndromes, and various types of cancer [21, 22]. However, subnormal or suppressed neopterin release in clinical disease is rather rare. Only from *in vitro* studies, it is known that several antioxidant compounds are able to slowdown neopterin production in peripheral blood mononuclear cells. Among them are antioxidant food supplements like preservatives and colorants, but also high dose of antioxidant vitamins C and E suppresses Th1-type cytokines and inducees a Th2-type immunity pattern *in vitro* [39].

Whereas antioxidant compounds were observed to suppress Th1-type immunity and neopterin production *in vitro*, physical exercise and sports like long-distance running and hiking were found to induce a transient increase of neopterin concentrations [40, 41]. An increase of neopterin concentrations was also observed in a rather small subgroup

of our patients who attended an intervention therapy during one year that comprised improved dietary habits and the increase of exercise. This increase of neopterin indicates a proinflammatory response which is induced by exercise and corresponds nicely to the above-mentioned earlier findings in healthy individuals. Thus, the most direct and consistent interpretation would be that physical exercise was responsible for the increase of neopterin concentrations.

One may conclude that low or even subnormal neopterin concentrations may relate to a low incidence of physical activities in obese juveniles. Moreover, in diabetes patients, antioxidants have been recently found to prevent the health-promoting effects of physical exercise [42]. In conclusion, low frequency of physical exercise and sports as well as antioxidant food supplements could contribute to the low neopterin levels in obese juveniles, and it may contribute to weight gain. However, this is merely speculation and still the increase of body weight and the decrease of neopterin might well develop in parallel and are not necessarily linked directly.

Unfortunately, the findings of the intervention program appear somewhat hampered by the fact that those individuals who were willing to enter this program presented with baseline levels somewhat different from the whole study group, for example, neopterin concentrations were slightly lower and resistin and CRP levels were slightly but not significantly higher. The intervention program increased neopterin concentrations, but the average neopterin level reached was only slightly higher as compared to the whole group without any special sports activities. Still the difference of neopterin concentrations before and after intervention was significant when paired samples of the subgroup were compared. Certainly a larger study will be necessary to adequately address this issue, but it is not easy to get.

According to the findings on neopterin concentrations, obesity in adolescents might be classified as a condition linked with Th2-type immunity. It will be interesting to investigate in future whether the balance between Th1- and Th2-type immunity is essentially involved in the observed neopterin kinetics and in obesity development. It can be speculated that the early low-grade inflammation, as found in our obese juveniles in context with an already increased IMT, may be a more Th2-driven process, whereas the advanced atherosclerosis of adulthood is associated with a stronger Th1-type immune activation. There might be a role of mast cells which are known to be strongly engaged in the allergic response but also have the potential to promote atherosclerosis by releasing proinflammatory cytokines which could become relevant with older age [43].

In conclusion, the finding of low-serum neopterin concentrations in obese juveniles forces one to consider that the pathogenesis of cardiovascular disease development in children and juveniles may differ from adults. Further studies should help to better define age-dependent differences and to define optimized preventive strategies, not only the increase of physical exercise and sports but also avoidance of antioxidant food supplements such as preservatives or extra vitamins might be of benefit.

Abbreviations

BMI:	Body mass index
CVD:	Cardiovascular disease
CRP:	C-reactive protein
ELISA:	Enzyme-linked immunosorbant assay
HDL:	High-density lipoprotein
HOMA-IR:	Homeostatic model assessment-insulin resistance
IFN-γ:	Interferon-γ
IMT:	Carotid intima-media thickness
LDL:	Low-density lipoprotein
MI:	Myocardial infarction
oxHDL:	Oxidized high-density lipoprotein
oxLDL:	Oxidized low-density lipoprotein
STYJOBS:	STYrian Juvenile Obesity Study.

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Clinical Study

Perspectives of CB1 Antagonist in Treatment of Obesity: Experience of RIO-Asia

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Rimonabant, a selective cannabinoid-1 (CB1) receptor antagonist, has been shown to reduce weight and enhance improvements in cardiometabolic risk parameters in Western populations. This study assessed these effects of rimonabant in Asian population. A total of 643 patients (BMI 25 kg/m^2 or greater without diabetes) from China, Republic of Korea, and Taiwan were prescribed a hypocaloric diet (600 kcal/day deficit) and randomized to rimonabant 20 mg ($n = 318$) or placebo ($n = 325$) for 9months. The primary efficacy variable was weight change from baseline after 9 months of treatment. Results showed that rimonabant group lost more weight than placebo, (LSM ± SEM of −4.7 ± 0.3 kg vs. −1.7 ± 0.3 kg, $P < .0001$). The 5% and 10% responders were 2 or 3 folds more in the rimonabant group (53.0% vs. 20.0% and 21.5% vs. 5.7%, resp.) ($P < .0001$). Rimonabant also significantly increased HDL-cholesterol, decreased triglycerides and waist circumference,by 7.1%, 10.6%, and 2.8 cm, respectively ($P < .0001$). This study confirmed the comparable efficacy and safety profile of rimonabant in Asian population to Caucasians. Owing to the recent suspension of all the CB1 antagonists off the pharmaceutical market for weight reduction in Europe and USA, a perspective in drug discovery for intervening peripheral CB1 receptor in the management of obesity is discussed.

1. Introduction

Overweight and obesity, a global phenomenon, affects more than 1 billion adults, with 300 million being clinically obese [1]. With changing life styles and dietary patterns, obesity is rapidly increasing in epidemic proportions globally. In the national nutritional surveys of the 3 countries that participated in the RIO-Asia study, the obesity prevalence rates were at 14.7% in China [2], 26.6% in Taiwan [3], and 30.6% in Korea [4] in the year 2001-2002.

Obesity is a complex metabolic disorder characterized by an imbalance in energy homeostasis [5], abnormal development of adipose tissue, and deregulation of hormones and cytokines including adipocytokines [6]. This chronic metabolic imbalance is associated with comorbidities such as cardiovascular disorders [7], hypertension [8], sleep apnea [9], diabetes mellitus [1], and certain types of cancer [10] and related morbidity/mortality. Besides weight loss and favorable cardiometabolic profile [11], pharmacological interventions for obesity should address reduction in abdominal obesity and lower the risk of developing diabetes mellitus.

G protein-coupled cannabinoid receptor, CB-1 of the endocannabinoid system (ECS), plays a crucial role in regulating feeding pattern, lipid metabolism, and energy homeostasis. CB-1 receptors are located in the central nervous system and peripheral tissues including adipocytes, pancreas, gut, liver, and muscle [12]. Rimonabant is a selective CB-1 antagonist drug to be developed for weight loss. Results of in vitro experiments have suggested the involvement of fourth and fifth transmembrane domains of the CB-1 receptor for high-affinity binding of rimonabant [13, 14]. Rimonabant

has been shown to exert a peripheral effect on food intake by regulating Acrp30 (i.e., adiponectin) and insulin, hormones involved in lipid and glucose metabolism, respectively [5]. The mechanism of the long-lasting weight loss through rimonabant may be associated with an increase in energy expenditure and/or metabolic activities [5, 15].

Animal studies [16, 17] and human trials have demonstrated the efficacy of rimonabant in inducing weight loss and improving dyslipidemia and insulin sensitivity. The RIO-Europe [18] and RIO-North America [19] clinical trials studied the weight loss in obese or overweight Caucasians treated with 20 mg rimonabant. At the end of a 1-year period, the weight loss was significantly greater in patients treated with rimonabant (−6.3 to −6.6 kg; $P < .001$ versus placebo, −1.6 to −1.8 kg). The proportion of patients who achieved 5% or greater decrease in body weight was 48.6–50.9% while a 10% or greater decrease was noted in 25.2–27.4% patients. A reduction in waist circumference (3.6 cm) and triglycerides (13.2%), as well as an increase in HDL-cholesterol (7.2%), was also observed in the rimonabant group. After 2 years of treatment with 20 mg rimonabant, the weight loss was maintained at 7.4 kg. Similar results were observed in the RIO-Lipid study [20] which analyzed the efficacy of rimonabant 20 mg in overweight or obese Caucasians with dyslipidemia. A significant suggestion of the study was that in addition to weight reduction, rimonabant also mobilized abdominal fat and improved cardiovascular risk profile. Furthermore, although the adiponectin-elevating effect was indirect, it was independent of weight loss.

The efficacy of rimonabant in Asian ethnicity has not yet been evaluated. Evidence shows that the incidence of obesity is increasing rapidly in Asian countries [1, 21, 22]. As stated above, Asian populations have a greater body fat content at a lower body mass index (BMI) compared with Caucasians [23, 24]. Consequently, obesity-related metabolic disorders like dyslipidemia, hypertension, and type 2 diabetes are more frequently observed at lower BMI in Asians than in Caucasians [22]. Hence, the WHO has redefined the classification of obesity based on BMI in Asians. The BMI cutoff was lowered in Asians to ≥23.0 kg/m^2 for overweight and ≥25.0 kg/m^2 for obesity compared to 25–30 kg/m^2 for overweight and ≥30 kg/m^2 for obesity in Caucasians [1, 22, 25].

The RIO-ASIA study evaluated the efficacy and safety of rimonabant 20 mg, along with a hypocaloric diet, in reducing weight and improving cardiometabolic risk factors in obese Asian population.

2. Methods and Procedures

2.1. Study Design. This phase 3, randomized, double-blind, placebo-controlled, parallel-group, multinational study was carried out in 32 centers in China ($n = 240$), Republic of Korea ($n = 200$), and Taiwan ($n = 203$) between April 2006 and April 2007.

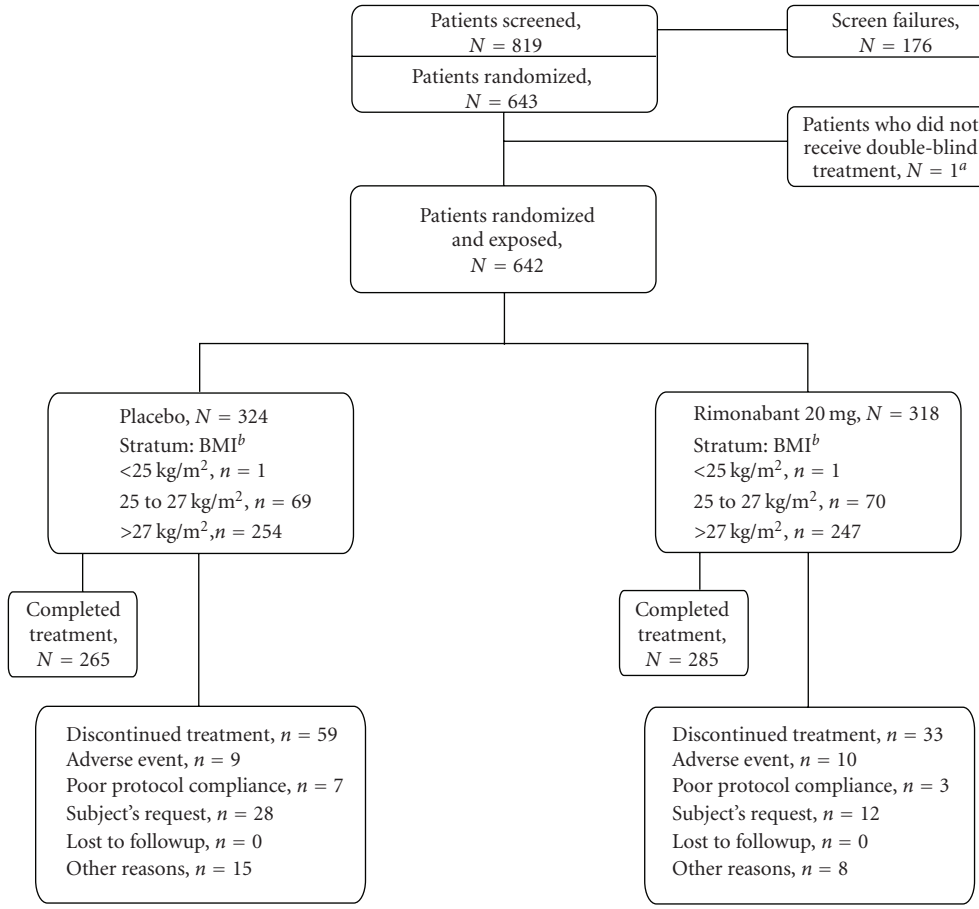
After a screening period of 7 to 14 days, obese patients were randomized in a 1 : 1 ratio to treatment with rimonabant 20 mg ($n = 318$) once daily or placebo ($n = 325$) for a 9-month period. Randomization was stratified based on BMI status at baseline as 25 to 27 kg/m^2 or >27 kg/m^2. Follow-up visits were scheduled every 28 days throughout the study duration. Study participants were prescribed at baseline a hypocaloric diet for the study duration. The calorie requirement was calculated based on basal metabolism rate and physical activity, from which 600 kcal/day was subtracted to calculate the recommended diet. A food intake assessment was carried out on day 84, day 168, and day 252 from diet diary entries. The protocol, approved by the Institutional Review Boards/Ethics Committees for each centre, was conducted in compliance with the Helsinki Declaration and with an independent, unblinded data monitoring committee. All patients provided written informed consent.

2.2. Patients. Eligible patients were aged ≥18 years with a BMI ≥25 kg/m^2. Exclusion criteria were a body weight fluctuation of at least 5 kg in the previous 3 months, a systolic blood pressure >165 mm Hg and/or diastolic blood pressure >105 mm Hg at screening and baseline, presence of type 1 or type 2 diabetes, presence of any clinically significant disorders (endocrine/metabolic/neurological/psychological disorders; presence/history of cancer).

2.3. Measures and Assessments. The primary efficacy endpoint was the absolute weight change from baseline (randomization) to 9 months in the intent-to-treat (ITT) population. Secondary efficacy endpoints were waist circumference (as a marker of change in abdominal obesity), high-density lipoprotein (HDL)-cholesterol, triglycerides, fasting insulin, and glucose.

Measurement of body weight was carried out at each visit using a calibrated scale. Based on established protocol [26], 3 waist measurements were taken in centimeters at the midpoint between the lower rib margin and the iliac crest. The variations in these measurements were expected to be ≤1.0 cm. Failure to fit within these criteria would lead to a fourth measurement. Metabolic parameters, including glucose, insulin, glycosylated hemoglobin (HbA1c), total cholesterol, low-density lipoprotein (LDL)-cholesterol, HDL-cholesterol, and triglycerides, were measured under fasting conditions on day 84, day 168, and day 252 in a central laboratory. Safety assessments consisted of spontaneously reported adverse events, clinical examinations, laboratory tests, vital signs, and electrocardiograms.

2.4. Statistical Analysis. The sample size calculations for the study were derived from the RIO-Europe study and were based on a test to compare the mean change in weight (primary variable) and HDL-cholesterol (secondary confirmatory variable) from baseline between the 2 groups. The calculations of sample sizes were done using the sample size software nQuery Advisor 4.0. Thus with a standard deviation (SD) of 6.5 kg, 200 randomized patients (100 patients/group) were required to detect a mean treatment difference of 3 kg in weight. For HDL-cholesterol change, with a common SD of 25%, 414 randomized patients (207 patients/group) were required to detect a mean treatment difference of 8% in HDL-cholesterol. Finally, to fulfill the country/region requirements for the number of exposed



[a]One patient who was randomized to the placebo group was not exposed to the investigational product owing to a diagnosis of pregnancy at the time of randomization.

[b]Baseline BMI was used.

Figure 1: Flow of patients in RIO Asia study.

patients, a total of 640 patients had to be randomized (320 patients/group).

Statistical analysis was performed using SAS version 8.2 or higher. All statistical tests were 2-sided at the 5% significance level. Efficacy analyses were performed in ITT population, which consisted of all randomized patients and was the primary population for efficacy analyses.

The absolute change in body weight from baseline to 9 months was analyzed using a 3-way analysis of covariance (ANCOVA) model, which included 3 fixed effects—treatment, country/region, and randomization stratum—and 1 covariate, baseline weight. Rimonabant 20 mg was compared with placebo using a Student's t-test within the framework of the ANCOVA model. In addition, a 95% confidence interval (CI) was constructed for the difference in mean absolute weight changes between the groups.

Patients who achieved a 5% or 10% reduction in body weight from baseline at 9 months were classified as 5% or 10% responders. The proportion of 5% or 10% responders in the rimonabant 20-mg group was compared with placebo using a logistic regression model with 3 fixed effects—treatment, country/region, and randomization stratum—and 1 covariate, baseline body weight.

Secondary efficacy endpoints were analyzed at 9 months using the ITT population. With the exception of the lipid parameters, continuous secondary efficacy endpoints were assessed as the absolute change from baseline at 9 months. Lipid parameters, except total cholesterol/HDL-cholesterol ratio, were assessed as relative change from baseline and analyzed as described for the other secondary end points.

In the efficacy analyses of endpoints for the ITT population, the last observation carried forward method (LOCF) was used to account for missing assessments or patients who prematurely discontinued. These endpoints were replaced by the last available postbaseline on-treatment observation.

3. Results

3.1. Patients. A total of 643 patients were randomized (318 in the rimonabant 20 mg group and 325 in the placebo group) from 32 centers in China ($n = 240$), Republic

Table 1: Demographic characteristics and comorbidities.

	Placebo ($N = 324$)	Rimonabant 20 mg ($N = 318$)	Overall ($N = 642$)
Age (years)			
Overall, mean (SD)	35.3 (10.5)	36.7 (10.8)	36.0 (10.7)
Age group, *n* (%)			
<18	0	1 (0.3%)	1 (0.2%)
[18–49]	294 (90.7%)	273 (85.8%)	567 (88.3%)
[50–64]	27 (8.3%)	41 (12.9%)	68 (10.6%)
≥65	3 (0.9%)	3 (0.9%)	6 (0.9%)
Gender, *n* (%)			
Male	103 (31.8%)	99 (31.1%)	202 (31.5%)
Female	221 (68.2%)	219 (68.9%)	440 (68.5%)
Country, *n* (%)			
China	121 (37.3%)	119 (37.4%)	240 (37.4%)
Republic of Korea	101 (31.2%)	99 (31.1%)	200 (31.2%)
Taiwan	102 (31.5%)	100 (31.4%)	202 (31.5%)
Comorbidities, *n* (%)			
Hypertension	42 (13.0%)	51 (16.0%)	93 (14.5%)
Dyslipidemia	73 (22.5%)	73 (23.0%)	146 (22.7%)

of Korea ($n = 200$), and Taiwan ($n = 203$)—1 patient randomized to the placebo group did not receive the study treatment. A higher percentage of patients in the rimonabant 20 mg group ($n = 285$, 89.6%) completed the study treatment period compared with the placebo group ($n = 265$, 81.5%) (Figure 1). 11 patients were excluded from the ITT population (1 for nonexposure and 10 for missing postbaseline weight assessment); thus, the ITT population consisted of 632 patients (317 and 315 patients on placebo and rimonabant 20 mg groups, resp.). Treatment compliance was similar in both treatment groups: 98.7% and 96.9%; patients in the rimonabant and placebo groups, respectively, achieved 80% treatment compliance target (data on file).

Demographic and baseline characteristics were similar between the 2 treatment groups, with equal distribution of patients across treatment in the 3 countries (Tables 1 and 2). 14.5% patients had hypertension, and 22.7% were dyslipidemic. Mean age was 36 ± 10.7 years with more than two thirds of patients being women (68.5%). Mean BMI was 30.26 ± 4.23 kg/m^2 with a baseline weight ranged from 54.3 to 161.5 kg and a mean weight of 81.48 ± 15.67 kg. Majority of patients (78.0%) had BMI > 27 kg/m^2. More than one third of patients had low HDL-cholesterol (34.1%), high triglycerides (31.3%), and high LDL-cholesterol (32.7%), and 17.4% had impaired fasting glucose.

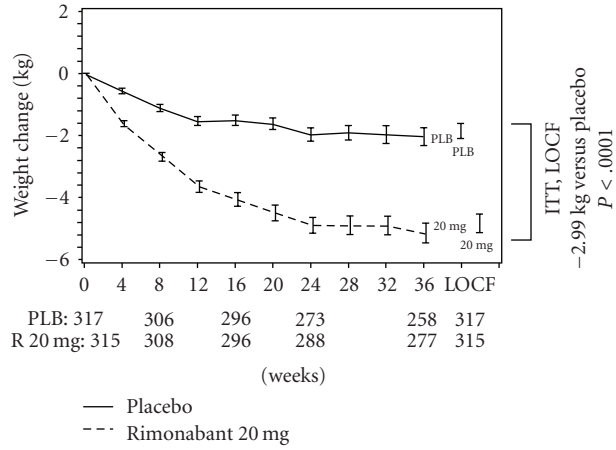
3.2. Weight. In the ITT population, weight loss was significantly greater in the rimonabant group (least square (LS) mean ± SEM: −4.7 ± 0.3) than in the placebo group (LS mean ± SEM: −1.7 ± 0.3) at 9 months, leading to a placebo-subtracted weight loss of approximately 3.0 kg ($P < .0001$) (Figure 2(a)). When expressedas a percentage, rimonabant-treated patients lost 6.0% of their baseline body weight versus 2.3% in the placebo group.

The percentage of patients who achieved at least a 5% reduction and 10% reduction from their baseline body weight at 9 months in the rimonabant 20-mg group (53.0% and 20.0%, resp.) was more than 2-fold and 3-fold higher compared with the placebo group (21.5% and 5.7%, resp.) ($P < .0001$) in the ITT population (Figure 2(b)).

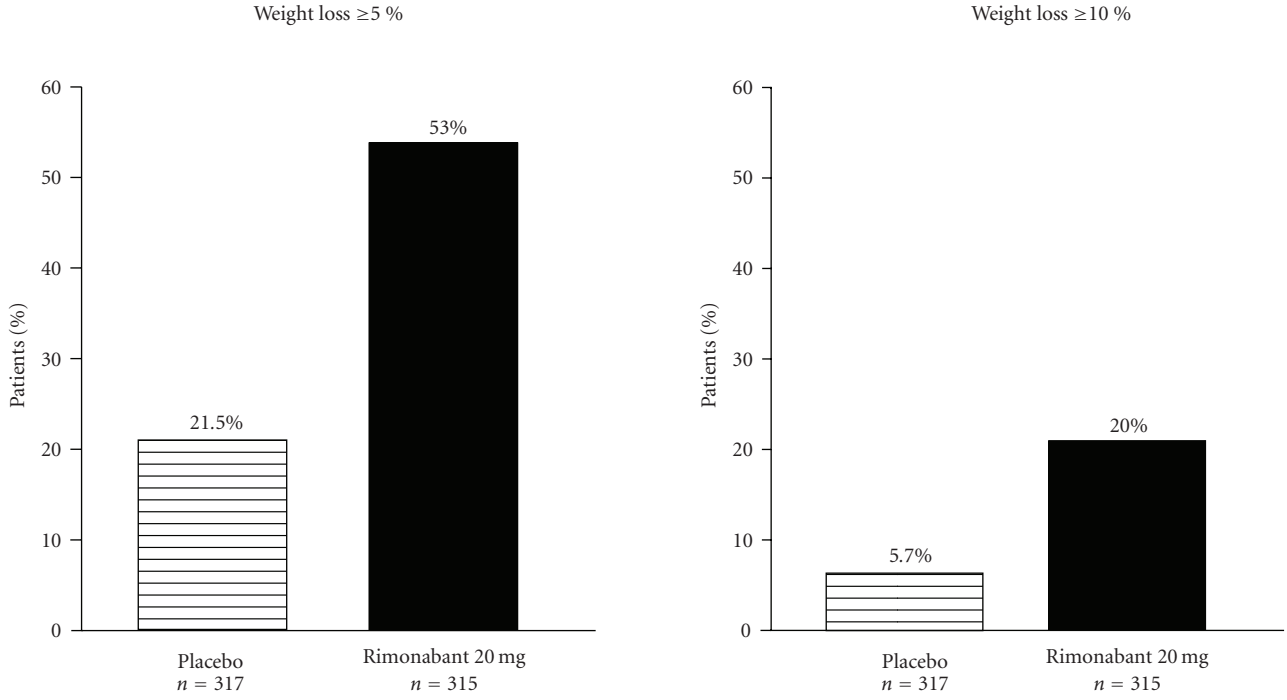
Treatment with rimonabant resulted in a significant ($P < .001$) decrease in weight in both BMI strata. In patients with BMI of 25 to 27 kg/m^2 and >27 kg/m^2, the percentage of weight decrease was 5.61% and 6.11%, respectively, with rimonabant 20 mg while the corresponding changes in the placebo group were 2.05% and 2.31% (Table 3 and Figure 3).

3.3. Changes in Secondary Efficacy Variables. At 9 months, the percentage change in HDL-cholesterol revealed a steady increase in both treatment groups (Figure 4(a)). Rimonabant increased HDL-cholesterol by 7.1% compared with placebo ($P < .0001$). Rimonabant reduced triglyceride levels by 10.6% compared with placebo ($P = .0047$). The mean waist circumference decreased from baseline by 5.93 ± 0.33 cm in the rimonabant 20-mg group compared with 3.2 ± 0.33 cm in the placebo group (−2.8 cm versus placebo; $P < .0001$) (Figure 4(c)). In this nondiabetic population, minimal, nonsignificant changes were observed in fasting glucose and fasting insulin in both groups at 9 months, with, however, a favorable trend for rimonabant 20 mg, especially in fasting insulin.

3.4. Safety Events. The incidence of treatment-emergent adverse events (TEAEs) was higher in the rimonabant group



(a)



[a] $P < .0001$ versus placebo.
ITT, intent to treat; LOCF, last observation carried forward;
PLB, placebe; R, Rimonabant.

(b)

Figure 2: Effect of rimonabant on (a) weight at each visit and at 9 months (b) 5% and 10% weight responders.

(66.0%, $n = 210$) compared with the placebo group (56.8%, $n = 184$) (Table 4). The percentage of patients who permanently discontinued due to TEAEs was low and comparable between the placebo (2.8%, $n = 9$) and rimonabant (3.1%, $n = 10$) groups. Serious TEAEs were more frequently reported in the placebo group compared with the rimonabant 20 mg group (4.6% versus 3.1%) with no deaths reported during the study period. The most frequent adverse events (≥0.5%) leading to treatment discontinuation were dizziness in the rimonabant 20 mg group (0.9% versus 0.6% in placebo) and headache (0.6% versus 0.0% in rimonabant 20 mg) in the placebo group (data on file).

In the rimonabant group (Table 4), the common adverse events reported with a ≥2% incidence (≥1% over placebo patients) were upper respiratory tract infections (10.7% versus 9.0%), nausea (11.6% versus 4.0%), diarrhea (7.5%

Table 2: Baseline characteristics: randomized and expose patients.

	Placebo ($N = 324$)	Rimonabant 20 mg ($N = 318$)	Overall ($N = 642$)
Weight (kg)			
Mean (SD)	82.00 (16.40)	80.95 (14.90)	81.48 (15.67)
Waist (cm)			
Male, *n* (%)			
>90 cm	97 (94.2%)	92 (92.9%)	189 (93.6%)
Female, *n* (%)			
>80 cm	209 (94.6%)	211 (96.3%)	420 (95.5%)
BMI (kg/m²)			
Mean (SD)	30.45 (4.41)	30.07 (4.04)	30.26 (4.23)
BMI group, *n* (%)			
<25	1 (0.3%)	1 (0.3%)	2 (0.3%)
[25–27]	69 (21.3%)	70 (22.0%)	139 (21.7%)
>27	254 (78.4%)	247 (77.7%)	501 (78.0%)
HDL-cholesterol (mmol/L)			
Mean (SD)	1.35 (0.29)	1.35 (0.29)	1.35 (0.29)
HDL-cholesterol group, *n* (%)			
M: <1.036, F: <1.295	100 (30.9%)	119 (37.4%)	219 (34.1%)
Triglycerides (mmol/L)			
Mean (SD)	1.57 (1.01)	1.59 (0.98)	1.58 (0.99)
Triglycerides group, *n* (%)			
≥1.69	99 (30.7%)	101 (31.9%)	200 (31.3%)
LDL-cholesterol (mmol/L)			
Mean (SD)	3.08 (0.72)	3.01 (0.72)	3.04 (0.72)
LDL-cholesterol group, *n* (%)			
≥3.36	113 (34.9%)	97 (30.5%)	210 (32.7%)
Total/HDL-cholesterol			
Mean (SD)	3.70 (0.96)	3.65 (0.97)	3.67 (0.97)
Fasting glucose (mmol/L)			
Mean (SD)	5.07 (0.48)	5.17 (0.56)	5.12 (0.52)
Fasting glucose group, *n* (%)			
IFG: ≥5.55 and <6.99	43 (13.4%)	68 (21.5%)	111 (17.4%)
Fasting insulin (pmol/L)	92.04 (57.41)	98.30 (158.08)	95.17 (118.8)
Mean (SD)			
HbA1c (%)			
Mean (SD)	5.56 (0.39)	5.61 (0.41)	5.59 (0.40)

BMI: body mass index. HDL: high-density lipoprotein. IFG: impaired fasting glucose. LDL: low-density lipoprotein. HbA1c: glycosylated hemoglobin.

versus 4.3%), dyspepsia (3.1% versus 0.6%), vomiting (2.8% versus 0.9%), dizziness (10.7% versus 9.6%), and depression (4.1% versus 0.9%).

4. Discussion

This 9-month RIO-Asia study showed that rimonabant 20 mg is effective in reducing body weight in obese Asians. The 9-month treatment period with rimonabant was considered to be sufficient to compare the efficacy and safety of rimonabant with placebo, based on the results from previous RIO studies. A significantly greater reduction in weight was observed in the rimonabant 20-mg group compared with the placebo group (−2.99 kg, $P < .0001$).

The percentage of patients who achieved at least 5% (53.0%) and 10% (20%) reduction of their baseline body weight with rimonabant treatment at 9 months was more than twice (21.5%) and four times (5.7%) compared with placebo. In obesity, a 5% decrease in body weight is associated with an improved cardiometabolic profile [11].

The reduction in waist circumference followed the same pattern as body weight reduction. Obesity guidelines for Asia-Pacific have recommended a waist circumference of ≥90 cm in men and ≥80 cm in women [25]. Since rimonabant decreased the mean waist circumference by 2.8 cm, reduction in abdominal obesity would also lower the risk of developing related metabolic disorders. International Day for the Evaluation of Obesity (IDEA) study established a graded

Table 3: Change in weight (last observation carried forward) by BMI stratum in intent-to-treat population.

	BMI: 25 to 27 kg/m^2		BMI: >27 kg/m^2	
Weight (kg)	Placebo ($N = 74$)	Rimonabant 20 mg ($N = 71$)	Placebo ($N = 251$)	Rimonabant 20 mg ($N = 247$)
Baseline				
Mean (SD)	67.42 (6.21)	69.28 (7.45)	86.19 (16.22)	84.23 (14.81)
Month 9 (LOCF)				
Mean (SD)	66.06 (6.93)	65.47 (8.51)	84.20 (16.50)	79.14 (15.33)
Change from baseline				
Mean (SD)	−1.37 (2.95)	−3.81 (3.59)	−1.99 (4.55)	−5.09 (5.37)
LS Mean (SEM)	−1.35 (0.384)	−3.84 (0.387)	−2.02 (0.315)	−5.18 (0.316)
LS Mean Difference (SEM)	—	−2.49 (0.548)	—	−3.16 (0.446)
95%CI	—	(−3.570 to −1.403)	—	(−4.034 to −2.283)
P value	—	<.0001	—	<.0001
Percent change from baseline				
Mean (SD)	−2.05 (4.58)	−5.61 (5.33)	−2.31 (4.94)	−6.11 (6.22)

BMI: body mass index. CI: confidence interval. LS: least square. LOCF: last observation carried forward.

Table 4: Most common adverse events (≥2% in rimonabant-treated patients and ≥1% in placebo).

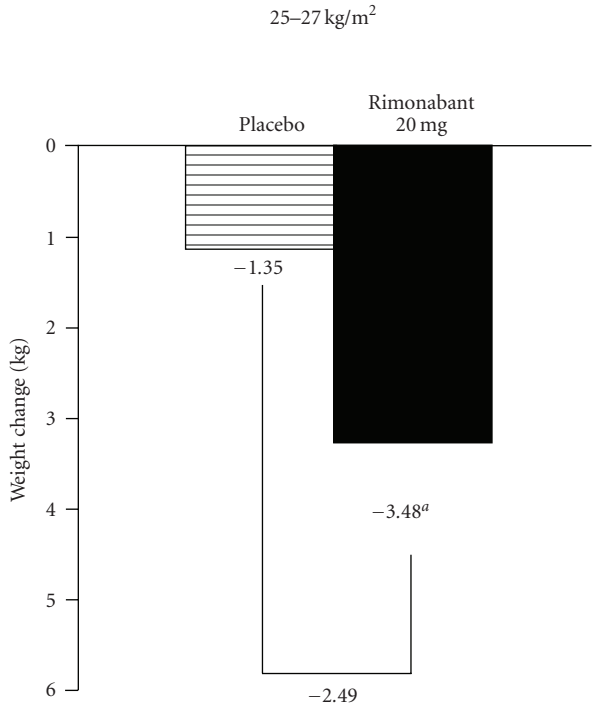
	Placebo ($N = 324$)	Rimonabant (20 mg) ($N = 318$)
Patients with any TEAE	184 (56.8%)	210 (66.0%)
Patients with any serious TEAE	15 (4.6%)	10 (3.1%)
Patients with any TEAE leading to death	0	0
Patients permanently discontinued due to TEAE	9 (2.8%)	10 (3.1%)
Most common adverse events		
Gastrointestinal disorders		
Nausea	13 (4.0%)	37 (11.6%)
Diarrhea	14 (4.3%)	24 (7.5%)
Dyspepsia	2 (0.6%)	10 (3.1%)
Vomiting	3 (0.9%)	9 (2.8%)
Nervous system disorders		
Dizziness	31 (9.6%)	34 (10.7%)
Psychiatric disorders		
Insomnia	11 (3.4%)	14 (4.4%)
Depression	3 (0.9%)	13 (4.1%)
Anxiety	5 (1.5%)	12 (3.8%)
Skin and subcutaneous tissue disorders		
Hyperhidrosis	3 (0.9%)	7 (2.2%)
Cardiac disorders		
Palpitations	3 (0.9%)	15 (4.7%)
General disorders and administration site conditions		
Fatigue	3 (0.9%)	8 (2.5%)
Infections and infestations		
URTI	29 (9.0%)	34 (10.7%)

TEAE: treatment-emergent adverse event; URTI: upper respiratory tract infection.
Note: TEAEs included all adverse events with an onset date during treatment exposure and up to 75 days following the last study drug intake.

relationship between waist circumference, diabetes mellitus, and cardiovascular disease [27].

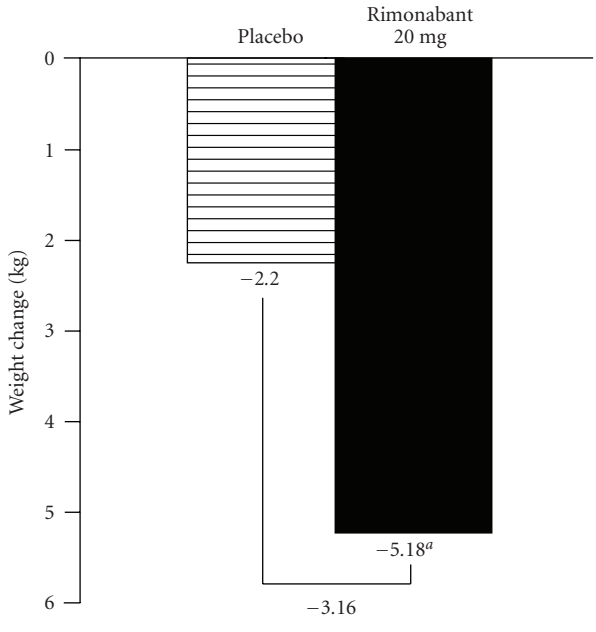
Significant improvements in HDL-cholesterol and triglycerides, associated with the reduction in weight and waist circumference, were seen in the rimonabant 20 mg group compared with the placebo group ($P < .001$, $P = .0047$, resp.). Hence, the results of this study indicate that treatment with rimonabant would have favourable effects on body weight and cardiometabolic risk profile in obese Asians who are known to have a greater incidence of obesity-related metabolic disorders at a lower BMI as compared with Caucasians [22].

The results in this RIO-Asia study are consistent with those in the 4 previous double-blind, placebo-controlled RIO studies (RIO-Lipids, RIO-Europe, RIO-Diabetes, and RIO-North America) conducted in more than 6000 patients, where rimonabant 20 mg had shown a significant reduction in body weight at 1 year compared with placebo ($P < .001$



[a]$P < .0001$ versus placepo

(a)



[a]$P< .0001$ versus placepo

(b)

Figure 3: Changes in weight in intent to treat population stratified by BMI (last observation carried forward).

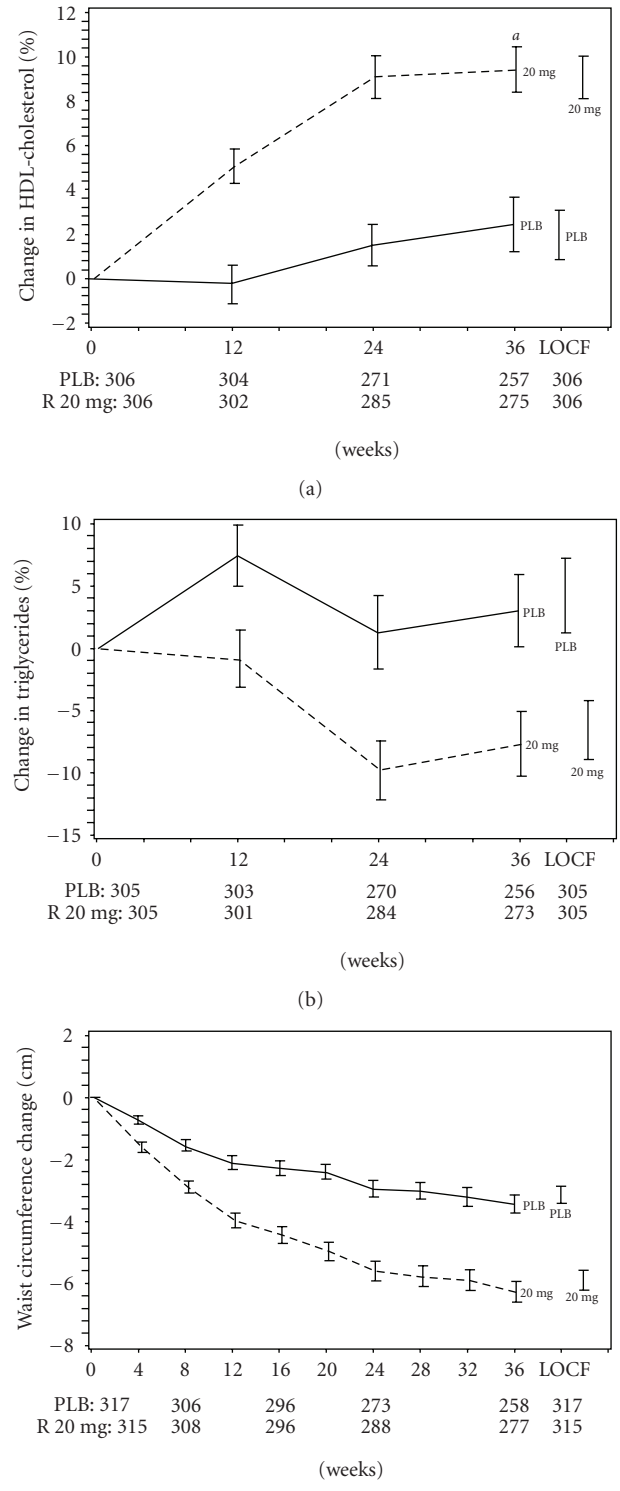


Figure 4: Effect of rimonabant on HDL-cholesterol, triglycerides, and waist circumference at each visit and at 9 months.

versus placebo). In Rio-Asia, the mean decrease in weight induced by rimonabant, compared with placebo, in the ITT population was 2.99 kg which was lower than RIO-North America (5.2 kg), RIO-Europe (5.3 kg), and RIO-Lipids (6.0 kg) on day 252 (data on file). The lower mean loss in body weight in the RIO-Asia study could be attributed to the lower baseline weights in Asian study population (81.48 kg) as compared with the Caucasian populations in the other studies (93.2–102.7 kg), but the percentage change in the weight loss was comparable among the different studies.

In this study, similar results for weight loss was observed across both BMI strata (25–27 kg/m^2 and >27 kg/m^2). Rimonabant decreased weight by 5.61% in the group with a BMI 25 to 27 kg/m^2 and by 6.11% in the group with a BMI > 27 kg/m^2, which can lower the risk of developing related metabolic disorders. Metabolic disorders are initiated at a lower BMI in Asians compared with Caucasians [22]. The BMI cut-off points for overweight and obesity are different in the Asian population as compared with the Caucasian population [1, 22, 25]. In the IDEA study, which involved 177, 345 patients in 63 countries, one half to two thirds of the study population were overweight or obese (BMI ≥ 25 kg/m^2). The frequency of diabetes mellitus and coronary vascular disease increased with waist circumference in all categories of BMI (<25 kg/m^2, 25–30 kg/m^2, and >30 kg/m^2) [27]. In adult Korean population, the incidence of diabetes, hypertension, and dyslipidemia was 2 times more at a BMI of 23.0 to 24.0 kg/m^2 and 3 times more at a BMI of 26.0 kg/m^2 [28]. In Chinese population, the inclination to accumulate truncal body fat is associated with metabolic complications, which occur at a lower BMI than in European/North American populations [21, 29].

Safety evaluation in this study found that rimonabant 20 mg was generally well tolerated in the Asian study population. The most common adverse events reported with an incidence ≥2% in the rimonabant group were upper respiratory tract infection, nausea, diarrhea, dyspepsia, vomiting, dizziness, depression, anxiety, insomnia, hyperhidrosis, palpitations, and fatigue. In addition, a lower number of patients discontinued treatment due to adverse events and this number was comparable between the 2 treatment groups (3.1% versus 2.8%, in rimonabant and placebo group, resp.). Among neuropsychiatric events, depression (4.1%, $n = 13$) and anxiety (3.8%, $n = 12$) were higher in the rimonabant 20 mg compared to placebo (0.9%, $n = 3$ and 1.5%, $n = 5$). A meta-analysis of randomized trials involving rimonabant 20 mg/day has proposed that rimonabant increases the risk of psychiatric adverse events including depression and anxiety. Patients were 2.5 and 3.0 times more likely to discontinue rimonabant due to depression and anxiety [30]. An overview of the RIO programme with rimonabant was published recently, which reviewed and summarized the overall efficacy and side effects of RIO trials including RIO-Europe, RIO-North America, RIO-Lipids, and RIO-Diabetes [31]. The vast majority of the test populations are western ethnics. The overall TEAE-induced drug discontinuation rate was higher in the rimonabant than the placebo groups either without (13.6% versus 7.7%, resp.) or with diabetes (15.0% versus 5.5%, resp.). The reported AE of depression was increased in the rimonabant group, either in all obese cohort (3.9% versus 1.7%, resp.) or in diabetes programme (2.5% versus 1.4%, resp.). The TEAE-induced drug discontinuation rate in RIO-Asia seems to be much lower than that of other RIO trials. Although the RIO-Asia has relatively a smaller sample size and shorter duration than other RIO trials, the reported AE of depression rate is still significantly increased in the rimonabant group in Asian population. Hence, monitoring of rimonabant-induced psychiatric adverse events is essential.

The endocannabinoid system (ECS), encompassing endocannabinoids including anandamide and 2-arachidonoylglycerol (2-AG), two cannabinoid receptors, type 1 (CB1) and type 2 (CB2), and enzymes responsible for endogenous ligand synthesis (phospholipase D) and degradation (fatty acid amide hydrolase (FAAH)), is a complex physiological system involved in metabolic homeostasis such as modulating energy fluxes and nutrients regulation [32]. Recently, substantial clinical evidence revealed that CB1 antagonists might result in risks of severe psychiatric problems, including depression, anxiety, and stress disorders, especially in those with underlying or susceptible psychological problems. These findings have made rimonabant (SR14176A), the first CB1 inverse agonist approved and launched in Europe in 2006, withdrawn from the market in 2008, and thereof, several CB1 target-related candidates including taranabant (MK-0364) and otenabant (CP-945598) were suspended at the late clinical development stage (phase III). In the meanwhile, increased evidence indicates that CB1 receptors present in the peripheral tissue, including fat and liver, might regulate food intake and energy balance as effectively as those present in the central nervous system (CNS) [33]. This provides a rationale for development of peripherally preferred or restricted CB1 antagonists which possess minimized CNS adverse reactions and preserve antiobesity effects. Recently, this strategy of developing CB1 antagonists without penetrating blood-brain barrier (BBB) was found to meet with a certain degree of success. Sufficient weight-reduction efficacy and less CNS toxic profile have been observed with romonabant-mimicking analogues acting exclusively on peripheral CB1 receptors [34]. However, these compounds have been tested only in animals.

5. Conclusion

The RIO-ASIA study of 9-month duration confirmed the efficacy of rimonabant 20 mg on weight reduction in the Asian population. Besides the favourable effect on weight, significant improvements were also observed in waist circumference, HDL-cholesterol, and triglycerides. The drug was associated with a good safety profile in the Asian study population, which was consistent with the pattern observed in the Caucasian population. However, in patients with a history of psychiatric disorder, especially those with a past history of depression, the usage of rimonabant is not recommended owing to mild yet considerable increase of

psychiatric untoward side effects observed in this trial as well as other such similar trials. In addition, the psychiatric status of patients taking rimonabant has to be monitored regularly, preferably using a practical risk evaluation form such as the Columbia classification algorithm of suicide assessment (C-CASA) [35]. Perceptively, to avoid untoward CNS side effects in drug discovery for the intervention of the endocannabinoids system, an appealing strategy of development of peripherally restricted and highly CB1/CB2 selective antagonists may be desired. Some compounds of this kind have been shown to have promising results in lowering propensity to pass the blood-brain barrier with preserved weight-reducing effects in DIO mice [36, 37]. Another peripheral-acting CB1 neutral antagonist has been shown to have weight-independent effects on improving cardiometabolic risks and fatty liver in mouse models of obesity [38]. However, the clinical efficacy and safety of these new antiobesity compounds are yet to be seen.

Conflict of Interests

The authors declared no conflict of interests.

Acknowledgments

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Review Article

Treatment of Obesity-Related Complications with Novel Classes of Naturally Occurring PPAR Agonists

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The prevalence of obesity and its associated comorbidities has grown to epidemic proportions in the US and worldwide. Thus, developing safe and effective therapeutic approaches against these widespread and debilitating diseases is important and timely. Activation of peroxisome proliferator-activated receptors (PPARs) α, γ, and δ through several classes of pharmaceuticals can prevent or treat a variety of metabolic and inflammatory diseases, including type II diabetes (T2D). Thus, PPARs represent important molecular targets for developing novel and better treatments for a wide range of debilitating and widespread obesity-related diseases and disorders. However, available PPAR γ agonistic drugs such as Avandia have significant adverse side effects, including weight gain, fluid retention, hepatotoxicity, and congestive heart failure. An alternative to synthetic agonists of PPAR γ is the discovery and development of naturally occurring and safer nutraceuticals that may be dual or pan PPAR agonists. The purpose of this paper is to summarize the health effects of three plant-derived PPAR agonists: abscisic acid (ABA), punicic acid (PUA), and catalpic acid (CAA) in the prevention and treatment of chronic inflammatory and metabolic diseases and disorders.

1. Introduction

During the past two decades, the prevalence of obesity has risen to epidemic proportions in the United States, and current estimates indicate that approximately 67% of the population is overweight and one-third is obese. This burgeoning problem has brought with it numerous health and fiscal concerns. Obesity is associated with a number of chronic diseases such as type II diabetes (T2D), cardiovascular disease (CVD), chronic kidney disease (CKD), nonalcoholic fatty liver disease (NAFLD), obstructive sleep apnea (OSA), gallbladder diseases, and various types of cancers [1], and obesity-related spending is currently estimated at $147 billion per year [2]. This number has been anticipated to rise to $344 billion, or 21% of all health care spending, in 2018 if rates continue on their current trajectory [3].

PPARs are the receptors for endogenous lipid molecules (i.e., prostaglandins or hydroxy-containing PUFA such as 12/15-hydroxyeicosatetraenoic (HETE), 13-hydroxyoctadecadienoic (HODE)) molecular targets for drugs against type 2 diabetes [4–6] and represent promising new targets for the treatment and prevention of inflammatory disorders such as IBD [7, 8]. PPARs belong to the superfamily of nuclear hormone receptors with 48 members identified in the human genome. There are three known PPAR isoforms; α, β or δ, and γ, which differ in their tissue distribution and functional activity [9]. Functionally, PPARs regulate inflammation, immunity, and metabolism [10]. PPAR activation and expression is controlled by a diverse set of natural and synthetic molecules, including nutrients, nonnutrient endogenous ligands, and drugs (i.e., thiazolidinediones (TZDs) and fibrates).

Activation of the nuclear receptor peroxisome proliferator-activated receptor γ (PPAR γ) has shown efficacy in the treatment of type II diabetes [11], inflammatory bowel disease [12, 13], colorectal cancer [14, 15], and influenza virus-induced pulmonary inflammation [16]. Thiazolidinediones (TZDs) are highly efficacious in activating PPAR γ, but the key representative of this class of drugs, rosiglitazone (Avandia), is associated with significant adverse side effects,

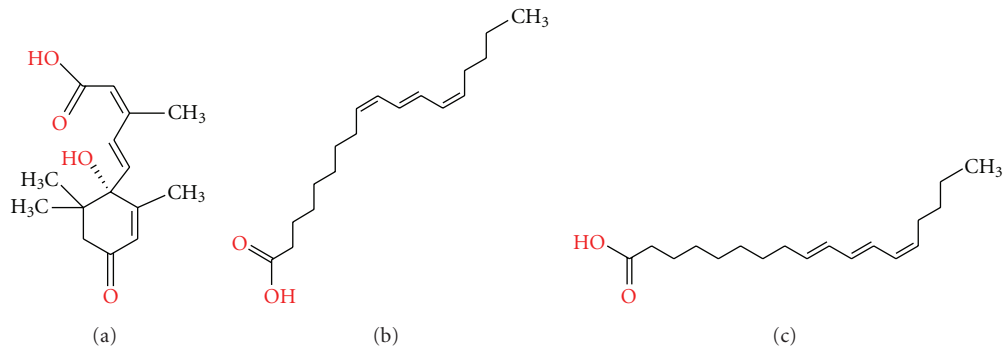

Figure 1: Chemical structures of the isoprenoid abscisic acid (a) and the conjugated linolenic acid stereo- or regioisomers punicic acid- (9*Z*, 11*E*, 13*E*-) octadeca-9, 11, 13-trienoic acid (b) and catalpic acid- (9*E*, 11*E*, 13*Z*-) octadeca-9, 11, 13-trienoic acid (c).

including weight gain, congestive heart failure, and fluid retention [6]. Although the results of the recent action to control cardiovascular disease in diabetes (ACCORD) trial did not report side effects associated with the use of the fibrate class of PPAR α agonists [17], other studies have reported that they can increase the risk for myopathy, cholelithiasis, and venous thrombosis [18]. Therefore, based on the concerns regarding cardiovascular safety of rosiglitazone, in 2010, the European Medicines Agency took rosiglitazone off the market in Europe. Moreover, the Food and Drug Administration restricted the use of rosiglitazone in the United States to situations in which other medications are not effective, thereby minimizing its use. Thus, there is an urgent need to develop novel and safer agonists of PPAR α and γ as therapeutics for obesity, diabetes, inflammation, and cancer. Our group has already developed a pipeline of naturally occurring compounds that activate PPARs [12, 19–26] and are both efficacious and safe, thereby confirming that in spite of the side effects of some synthetic agonists, PPARs remain valid drug/nutraceutical discovery targets. We have also set up the systems for virtually screening large compound libraries [27]. This paper describes the mechanisms of action of abscisic acid (ABA), punicic acid (PUA), and catalpic acid (CAA) (Figure 1) and their effects in mouse models of obesity, diabetes, and atherosclerosis.

2. Abscisic Acid

In 2009, the story of the isoprenoid phytohormone abscisic acid (ABA) and its elusive receptors were selected as runner up for "breakthrough of the year" by Science magazine [28]. This distinction awarded to ABA-research is attributable in part to the importance that ABA holds as a signaling compound. In plants, ABA is involved in numerous developmental and adaptive stress responses, including those related to stomatal opening and closing, pathogen defense, embryo and seed development, germination, promotion of seed desiccation tolerance and dormancy, and general growth and reproduction [29]. Researchers over the past 30 years have identified over 100 loci and numerous second messengers involved in ABA-induced responses, messengers such as calcium, reactive oxygen species (ROS), cyclic nucleotides, and phospholipids [29].

During the past few years, our laboratory and others have been investigating the potential of ABA as a bioactive compound for the prevention and treatment of diseases. In 2007, Bruzzone et al. found that ABA can be produced by human granulocytes and proposed that it may function as an endogenous signaling compound in mammals [30]. The signaling pathway identified by Bruzzone et al. is remarkably similar to that described in plants. The components of the pathway include a pertussis-toxin-sensitive G-protein-coupled receptor on the plasma membrane, cAMP overproduction, protein kinase A- (PKA-) induced activation of ADP-ribosyl cyclase CD38, cADP-ribose generation, and an increase in intracellular calcium [30]. The lanthionine synthetase C-like protein 2 (LANCL2), a protein which shares high-sequence homology to the ABA-binding protein GCR2, was recently recognized as being critical for ABA-induced responses in the mammalian cells [31].

Research in our laboratory has focused on *in vivo* effects of ABA administration. We initially determined that ABA increases the activity of the nuclear receptor peroxisome proliferator-activated receptor γ (PPAR γ) in 3T3-L1 preadipocytes [22]. Because it has been shown to be an influential protein in a number of cells and tissues integral to glucose homeostasis, uncovering how PPAR γ activation improves the insulin response has been a complex task. In white adipose tissue (WAT), a region which expresses high levels of PPAR γ, TZDs increase the differentiation of new adipocytes, thereby preventing adipocyte hypertrophy and lipid efflux into peripheral tissues [32]. PPAR γ activation also inhibits macrophage and lymphocyte-induced inflammation [33], a hallmark of obesity and its comorbidities, and its presence has been shown to be important in the proper function of key metabolic regions such as skeletal muscle [34], the liver [35], and pancreas [36].

Despite the benefits associated with PPAR γ activation, TZDs are associated with unwanted side effects, such as weight gain, fluid retention, and congestive heart failure, which diminish their appeal as therapeutic agents [6]. Recently, the TZD rosiglitazone was associated with increased risk of myocardial infarction and cardiovascular mortality although another TZD, pioglitazone (Actos), appears to be safer [37]. An objective in our research with ABA was to determine whether it could replicate some of the

beneficial effects associated with PPAR γ activation without causing adverse side effects.

3. ABA in Type II Diabetes

Our laboratory's first study with ABA was designed to assess its efficacy in a mouse model of obesity and diabetes [22]. Db/db mice were fed high-fat diets containing a racemic ABA mixture at 0, 100, 200, 400, and 800 mg/kg diet. After a 5-week ABA supplementation, we observed significant improvements in fasting blood glucose at all doses [22]. There were no side effects noted with any of the ABA concentrations. Further testing with ABA continued with the lowest-effective dose measured (100 mg/kg). At this concentration, ABA supplementation significantly improved glucose tolerance, inhibited WAT macrophage infiltration, and reduced tumor necrosis factor α (TNF-α) mRNA. Expressions of PPAR γ and PPAR γ-responsive genes CD36 and aP2 were also significantly elevated in WAT of ABA-fed mice in comparison to control-fed mice, indicating that ABA mimicked TZDs in their capacity to activate PPAR γ gene regulatory mechanisms in the WAT. Also in line with increased PPAR γ activity, the number of small adipocytes in WAT was increased, and interscapular brown adipose tissue fat pads were greater in ABA-fed mice. ABA-fed mice also had significantly small liver weights [22], an effect that may be due to increased expression of hepatic adiponectin receptors.

We next sought to further dissect the cells and tissues affected by ABA by assessing its effect on the two main fractions of WAT, adipocytes and stromal vascular cells (SVCs). The SVC fraction of the WAT, composed of immune cells, preadipocytes, and endothelial cells and fibroblasts, is a mainly non-energy storing portion of WAT that plays an important role in the tissue's homeostasis. Gene expression analyses revealed that oral ABA supplementation exerted a significantly more substantial affect on the SVCs. Levels of PPAR γ and its responsive gene CD36 were more greatly enhanced in the SVCs than in adipocytes, and SVCs from ABA-fed mice showed a marked reduction (~15-fold) in monocyte chemoattractant protein 1 (MCP-1) mRNA and protein levels [21]. In line with decreased MCP-1, the infiltration of $CCR2^+CD11b^+F4/80^{hi}$ macrophages into WAT was significantly decreased by ABA [21]. Chemokine receptor 2 (CCR2) is the receptor for MCP-1, and both proteins have been associated with obesity-related inflammation and insulin resistance [38, 39].

To determine whether ABA's affects were dependent on PPAR γ, control and ABA-supplemented diets were fed to tissue-specific PPAR γ knockout mice. The transgenic mice used in this study, PPAR γ flfl; MMTV Cre^+ mice, lacked PPAR γ in all hematopoietic and epithelial cells [12]. After 28 weeks of high-fat feeding, we found that ABA's ability to normalize glucose tolerance and reduce WAT inflammation was significantly impaired in the MMTV Cre^+ mice [21]. There were also no adverse side effects observed with this long-term feeding trial.

Our results show that ABA's antidiabetic effect is mediated through a PPAR γ-dependent mechanism though *in vitro* studies in human and rat pancreatic islet cells demonstrate that ABA-induced increases in insulin secretion are mediated through cAMP/PKA [40]. Interestingly, recent findings in our lab link PPAR γ activation with the cAMP/PKA signaling pathway. More specifically, by using obese db/db mice, we showed that ABA and the TZD rosiglitazone acted synergistically, rather than competitively, to reduce WAT macrophage infiltration [20]. Further, ABA's *in vitro* activation of PPAR γ in 3T3-L1 preadipocytes was inhibited by the cAMP-inhibitor 2′5′ dideoxyadenosine or the PKA inhibitor 14–22 myristolated PKA-inhibitor fragment [20]. There have been previous reports indicating that PKA activation increases PPAR γ activity [41], and together these findings indicate that both cAMP and PPAR γ may be linked to ABA's antidiabetic effects.

4. ABA and Cardiovascular Disease

In addition to T2D and obesity-related inflammation, our laboratory has also investigated whether dietary ABA may reduce cardiovascular disease (CVD) risk. When fed to ApoE-deficient mice as part of a high-fat diet for 12 weeks, ABA treatment significantly improved atherosclerosis lesions and atherosclerosis-related hypertension, reduced $F4/80^+CD11b^+$ macrophage infiltration and $CD4^+$ lymphocyte infiltration into the aortic root, decreased aortic root inflammatory lesions, and decreased aortic expression of VCAM-1 and MCP-1 [23]. ABA also increased aortic eNOS expression, both *in vivo* and *in vitro* in human aortic endothelial cells (HAECs) [23]. Short-term (10 minute) stimulation with ABA in HAECs also increased intracellular cAMP and NO release [23], an effect that is likely independent of binding to and activation of PPAR γ.

Despite our consistent anti-inflammatory *in vivo* findings, the effect of ABA on inflammation is somewhat ambiguous. *In vitro* studies from Bruzzone et al. [30], Magnone et al. [44], and Bordrato et al. [45] suggest that ABA has proinflammatory effects as well. In their work which principally dealt with ABA's potential effect on atherogenesis, Magnone et al. show that treatment of monocytes with ABA activates NF-κB and increases MCP-1 and MMP-9 release [44]. ABA also increased aortic smooth muscle cell proliferation and migration and was found at 10-fold higher concentrations in human arterial plaques compared to normal arterial tissue [44]. An alternative explanation is that ABA levels are greater in atherosclerotic plaques as a part of an endogenous regulatory mechanism designed to minimize inflammatory lesion development during atherosclerosis. This alternative explanation would be consistent with our *in vivo* findings in ApoE mice [23], a well established model of atherosclerosis. Ultimately, the discrepancy between both reports as to whether ABA treatment is anti- or proinflammatory highlights an incomplete understanding of the mechanisms of immune modulation by ABA and suggests possible opposing effects depending on cellular and environmental conditions.

Our most recent studies with ABA in a model of colitis suggest that its anti-inflammatory effects may depend on

Table 1: Summary of activities of novel classes of peroxisome proliferator-activated receptor (PPAR) agonists.

	Punicic acid	Catalpic acid	Abscisic acid
PPAR α reporter activity[1]	Yes	Yes	No
PPAR γ reporter activity[1]	Yes	No	Yes
PPAR δ reporter activity[1]	No	Unknown	No
PPAR γ ligand-binding activity[2]	Yes	Yes	No
PPAR γ *in silico* Docking[3]	Yes	Yes	No
Changes in PPAR-responsive genes *in vivo*[4]	PPAR α in adipose tissue PPAR γ in skeletal muscle	PPAR α in adipose tissue	PPAR γ in adipose tissue
Efficacy in tissue-specific PPAR γ null mice	Impaired	Unknown	Impaired
PPAR-independent Mechanisms	Modulation of eicosanoid synthesis	Decreases cyclooxygenase-2 expression	Lantionine synthetase component C-like 2, cAMP, and protein kinase A
Proposed utilities	Gut Anti-inflammatory Blood sugar control Immune modulator	Antiobesity Lipid-lowering Anticancer	Systemic anti-inflammatory Blood sugar control Antiatherosclerotic Immune modulator

[1] PPAR α, γ, and δ reporter activity assays were conducted as previously described [22].
[2] PPAR γ ligand-binding assay was performed using a commercially available competitive tracer displacement kit as previously described [42].
[3] Molecular modeling and docking studies were performed as previously described [27, 43].
[4] PPAR-responsive gene expression was measured *in vivo* as previously described [12].

T cells, and such findings may also hold relevance in its attenuation of CVD risk in ApoE-deficient mice. In the DSS model of inflammatory bowel disease (IBD), we have found that 5 weeks of ABA supplementation significantly improves colitis severity and decreases cellular adhesion molecule and proinflammatory cytokine expression in the colon [46]. The values we observed in this model were much more substantial than those found in the aortas of ApoE-deficient mice. Moreover, these beneficial effects of ABA in this model were impaired in T-cell-specific PPAR γ knockout mice. This outcome occurred despite fully functional PPAR γ in macrophages and epithelial cells; the main cell types involved in the immunopathogenesis of IBD and in the DSS model of colitis. $CD4^+CD25^+FoxP3^+$ regulatory T cells appeared to be important in ABA's anti-inflammatory mechanism in these IBD studies, as well as the protein CTLA-4, which was enhanced on $CD4^+$ T cells by ABA *in vitro* through a PPAR γ-dependent mechanism [46]. These recent findings in our colitis model corroborate earlier results indicating the importance of immune cells in ABA's anti-inflammatory mechanism and link T cell PPAR γ with ABA's systemic anti-inflammatory effects.

5. Conjugated Linolenic Acid Isomers in Obesity-Related Complications

Conjugated linolenic acids (CLnAs) or conjugated triene fatty acids are found as triglycerides in the seed oils of some plants belonging to the Punicaceae, Bignoniaceae, Rosaceae, Curcubitaceae, and Euphorbiaceae families [47]. Glycerides from these plant sources provide an easily accessible source of these unusual types of fatty acids, including but not limited to punicic (PUA), jacaric acid (JAA) catalpic (CAA), and eleostearic acids (ESA); all of which have demonstrated some promising health effects by acting as dual or pan-agonists of PPARs [12, 25, 26]. Of note, PUA is a conjugated octadecatrienoic acid containing c9, t11, and c13 double bonds that resembles the cis-9, trans-11 conjugated linoleic acid (CLA) isomer, a predominant CLA isomer in nature, ranging from 0.34 to 1.07% of the total fat in dairy products [48, 49]. Interestingly, CLA has shown efficacy both as an immune modulatory nutraceutical [49] as well as a possible antiobesity therapeutic [50]. Although CLA is beyond the scope of the present paper (see [50] for a thorough review), some of the health effects of CLnAs resemble those of CLA.

6. PUA in Type II Diabetes and Obesity-Related Inflammation

PUA also known as trichosanic acid is a conjugated triene fatty acid naturally found at high concentrations in the seed of *Punica granatum* (Punicaceae, Pomegranate) [51] and *Trichosanthes kirilowii* [47]. PUA constitutes 64–83 percent of the pomegranate seed oil (PSO) [52, 53], but it also contains minor amounts of ESA, CAA, and JAA [51]. Phytosterols (i.e., beta-sitosterol, campesterol, and stigmasterol) were also found in quite high concentrations in the PSO (4,089–6,205 mg/kg), about 3-4-fold higher than in soybean oil [53]. We recently demonstrated a dose-dependent increase in the ability of PUA to activate PPAR α and γ reporter activity in 3T3-L1 cells and to bind to PPAR γ and δ ligand-binding domain [25]. In line with these *in vitro* findings, we also demonstrated that oral PUA administration ameliorated fasting plasma glucose concentrations and as well as the

glucose normalizing ability during a glucose tolerance test in db/db mice, suggesting that PUA prevents or ameliorates type 2 diabetes. These improvements in disease markers were accompanied with upregulation of PPAR α-responsive genes in skeletal muscle and both PPAR α- and γ-responsive genes in intra-abdominal white adipose tissue [25]. Like CLA, PUA also suppressed the expression of inflammatory cytokines such as TNF-α and NF-κB activation. Furthermore, the deletion of the PPAR γ gene from immune cells abrogated the beneficial effect of PUA on glucose normalization and impaired PUA's anti-inflammatory effect, thereby suggesting that PUA ameliorates metabolic and inflammatory changes associated with obesity, in part, through a PPAR γ-dependent mechanism. We have also demonstrated that PUA is safe in acute toxicity studies in rats [54]. We also demonstrated that PUA treatment increased the expression of PPAR δ in pancreatic tissue (unpublished observation from our group), suggesting that this isoform may be a potentially interesting mediator of some of the insulin-sensitizing effects of PUA. Based on the phenotype observed during PUA treatment, the response of skeletal muscle could also be PPAR δ mediated, although no evidence of such effect is available at the moment.

7. CAA in Obesity and Type II Diabetes

Catalpic acid (CAA) is a conjugated triene fatty acid (trans-9, trans-11, cis-13 CLnA) naturally found at high concentrations in the seed of plants in the *Catalpa* Scop. Genus, belonging to the Bignoniaceae family. CAA-producing plants comprise 11 species of trees and shrubs native to East Africa and America, including *Catalpa ovata, Catalpa speciosa, Catalpa bungei, and Catalpa bignonioides* (southern catalpa) of the family Bignoniaceae. CAA constitutes approximately 60% of the oil of the catalpa seed oil. Even though the putative medicinal properties of catalpa trees have been alleged for centuries as a part of popular folklore, little is known about the effects of CAA on chronic conditions afflicting today's developed societies such as obesity and type 2 diabetes. For instance, the biological activities of extracts from *Catalpa bignonioides* have been investigated in recent years [55].

We found that oral CAA administration decreased abdominal fat accumulation, improved glucose homeostasis, increased plasma HDL cholesterol concentrations, increased plasma HDL cholesterol concentrations, and decreases plasma TG levels in mice fed high-saturated fat diets [26]. The lipid-lowering actions of CAA are consistent with the PPAR α agonistic actions of fibrates [56]. Of note, fibrates increase HDL cholesterol through a PPAR α-induced transcriptional activation of liver X receptor, a nuclear receptor that increases reverse cholesterol transport through promotion of the ATP-binding cassette transporters ABCA-1 and ABCG-1 [57–59]. Additionally, real time RT-PCR results demonstrated that PPAR α and PPAR α-responsive genes are upregulated in WAT of mice administered CAA, whereas PPAR γ and δ expression were not significantly affected by CAA in WAT. These data suggest that the metabolic effects of CAA on glucose and lipid metabolism may be mediated through a PPAR α-dependent mechanism. In contrast to ABA and PUA that modulate obesity comorbidities (i.e., insulin resistance, diabetes, or systemic inflammation), CAA has a direct effect in reducing adipose tissue accumulation.

8. Concluding Remarks and Future Directions

While diet and exercise remain the recommended approaches to prevent and treat obesity and its comorbidities, nutraceuticals and drugs may play a role as adjunct therapeutic interventions for controlling certain aspects of the disease etiopathogenesis. Mounting evidence demonstrates that naturally occurring compounds such as ABA, PUA, and CAA can act on well-established pharmacologic targets such as PPARs and exert their antidiabetic, anti-inflammatory, or antiobesity properties in animal models. In contrast to synthetic agonists of PPARs, these plant-derived compounds are safer and may act through multiple molecular targets. Intervention studies in humans are necessary to determine whether the beneficial effects reported in mice translate into clinical improvements in obese, diabetic, or insulin-resistant individuals.

With regard to the further developments of these compounds, based on the results reported here, CAA will be further developed for antiobesity and lipid-lowering applications, PUA will be developed for regulating blood sugar levels as well as controlling intestinal inflammation, and ABA will be developed for improving insulin sensitivity as a potential safer alternative to TZD and a systemic anti-inflammatory compound. Additionally, we will investigate the possibility of synergistic interactions between these compounds and drugs or other natural products to maximize their efficacy.

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Clinical Study

Impact of Orlistat-Induced Weight Loss on Diastolic Function and Heart Rate Variability in Severely Obese Subjects with Diabetes

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Objective. Determine the impact of Orlistat-induced weight loss on metabolic profile and cardiovascular function in severely obese patients with type 2 diabetes. *Methods*. Twenty-nine patients were randomized either to a nonplacebo control group or to a treatment group with Orlistat thrice a day. Metabolic profile, anthropometric parameters, heart rate variability indices, and echocardiographic variables were measured before and after a 12-week treatment period. *Results*. Treatment with Orlistat induced a modest but significant weight loss compared to controls (3.7 ± 3.0 versus 0.5 ± 2.2 kg, resp.; $P = .003$). There was significant decrease in fasting glycemia (7.9 ± 3.0 versus 6.7 ± 2.2 mmol/L; $P = .03$) and significant improvements in left ventricular diastolic function ($P = .03$) and in the sympathovagal balance (LF/HF ratio) ($P = .04$) in the Orlistat group. *Conclusion*. These results suggest that a modest weight loss improves fasting glycemia, left ventricular diastolic function, and sympathovagal balance in severely obese patients with type 2 diabetes.

1. Introduction

Severe obesity is defined as a body mass index (BMI) >40 kg/m^2 which is associated with comorbidities such as insulin resistance, diabetes mellitus, systemic hypertension, dyslipidemia, and cancer. Obesity is an independent risk factor for increased cardiovascular morbidity and mortality [1]. Furthermore, severely obese subjects have an increased total mortality with a concomitant increased risk of sudden death, which may be caused by fatal arrhythmias [1].

Heart rate variability (HRV) which is the fluctuation of heart rate around mean heart rate that may be assessed with a 24-hour cardiac Holter monitoring provides valuable information on the activity of the cardiac autonomic nervous system (ANS). The ANS is an important contributor to the regulation of both the cardiovascular system and energy expenditure and it is assumed to play a role in the pathophysiology of obesity and related complications [1, 2]. In obese subjects, many studies have observed abnormalities in the sympathetic and the parasympathetic ANS activity, which could partly explain the relation between obesity, comorbidities, sudden death, and arrhythmias [1, 3]. Available data regarding the metabolic and ANS impacts of weight loss in severely obese subjects by other methods than gastric bypass [4, 5] and hypocaloric diet [3, 6–9] are sparse.

Excess body fat also directly influences heart function [1]. Obesity is associated with decreased left ventricular (LV) systolic function and impaired LV diastolic function [1, 10]. Whereas some studies using surgical procedures have reported that substantial weight loss induces significant improvements in LV diastolic function [10–14], the effect of modest weight loss on LV diastolic dysfunction in obese subjects has been less extensively investigated but has never been reported in severe obesity. Diet and exercise programs are associated with disappointing long-term results on weight loss in severely obese subjects [15]. Weight loss medication is recommended for subjects with a BMI >30 kg/m^2 or with a BMI >27 kg/m^2 associated with ≥1 risk factor of cardiovascular disease [16]. Orlistat is a gastrointestinal lipase inhibitor, reducing fat absorption, which may result in weight loss of approximately 5%–10% of the initial weight after one year [17]. It may be relevant to investigate the

impact of modest weight loss on LV diastolic function and on HRV since these parameters may be associated with increased cardiovascular risk [18, 19]. This pilot study aimed to determine the impact of weight loss induced by Orlistat on HRV and on LV diastolic function in severely obese patients with type 2 diabetes.

2. Methods

2.1. Subjects. A total of 38 severely obese patients with type 2 diabetes were recruited from the waiting list of bariatric surgery in our Institution. The experimental protocol was approved by the ethics committee of IUCPQ and we certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. All the subjects gave their written informed consent. Subjects 18 years or older and treated with antidiabetic medication and/or insulin were included. Subjects were excluded if they used medication known to influence HRV or if they had documented chronic heart failure and/or kidney failure. Subjects were randomized to a control group or to a treatment group, which consisted of 120 mg of Orlistat (Xenical Roche, Nutley, NJ, USA) thrice a day, 30 minutes before meal, for 12 weeks. The medication was provided at no cost. For both treated and control groups, no reinforced specific nutritional intervention was recommended during this study other than the usual nutritional advice regarding the medication, reflecting a real clinical context. Follow-up was systematically conducted by phone for all subjects at 4 and 8 weeks into the study, in order to ensure subject's compliance with the medication and to note any significant changes in lifestyle. Nine subjects did not complete the study. In the control group, (1) one underwent bariatric surgery, (2) one had medication change within follow-up period, and (3) five did not attend their follow-up visit. In the treated group, (1) one had clinically significant side effects on the medication, and (2) one did not attend the follow-up visit.

2.2. Anthropometric Measurements. Total body mass, lean and fat mass, height, and BMI have been determined with an electrical bioimpedance balance (Tanita TBF-350, Tokyo, Japan). Waist circumference was measured using standardized method with an inelastic tape. Blood pressure and resting heart rate were measured following a 30-minute resting period while subjects were lying on their side during the echocardiogram study. Blood pressure was measured using an adapted size blood pressure cuff and an electronic sphygmomanometer (Welch-Allyn, 5200 series, Arden, NC, USA).

2.3. Biochemistry. Blood sample was taken after a 12-hour fast. Glycemia was determined using an enzymatic method (Hitachi, 717 Auto analyzer; Roche, Laval, Canada) and glycated hemoglobin (HbA_{1c}) was measured by binding affinity (Abbot IMX, Mississauga, Canada). Plasma cholesterol and triglycerides concentrations were measured using previously described methods (Hitachi, 717 Auto analyzer; Roche, Laval, Canada) [20, 21]. Serum HDL-cholesterol was analysed with an enzymatic precipitation of LDL-cholesterol and VLDL-cholesterol, using phosphotungstate and $MgCl_2$. LDL-cholesterol concentration was calculated with Friedewald's formula [22].

2.4. Echocardiography. Echocardiographic measures were performed with a commercial ultrasound system (Sonos 5500; Hewlet Packard, Andover, Massachusetts). Standard parasternal, short-axis, and apical views were performed in accordance with the recommendations of the American Society of Echocardiography and the same observer, who was blinded to randomisation, obtained all recordings and measurements. Left ventricular diastolic dysfunction (LVDD), using transmitral and pulmonary veins recordings, was evaluated using well-standardized criteria as previously reported [20, 21]. Tissue Doppler was not performed.

Left ventricular mass (LVM) was calculated according to the following formula [23]: $\text{LVM (g)} = 0.8 \times 1.04[(\text{LVEDD} + \text{IVST} + \text{PWT})^3 - (\text{LVEDD})^3] + 0.6$, where LVEDD represents the left ventricle end diastolic dimension, IVST the interventricular septal thickness, and PWT the posterior wall thickness.

2.5. Heart Rate Variability. Heart rate variability (HRV) was derived from a 24-hour Holter monitoring system (Marquette Electronics Inc., Milwaukee, WI) in all subjects during normal daily life activity [21]. Numerous indices in the frequency and the time domains were determined [7, 21]: mean value of all RR intervals (mean NN), standard deviation of the RR intervals (SDNN), standard deviation of the mean NN intervals for each 5 minutes period (SDANN), square root of the mean squared difference of successive RR intervals (rMSSD), percentage of differences between adjacent normal RR intervals exceeding 50 milliseconds (pNN50), high frequency (HF), and low frequency (LF). Within the 24-hour evaluation, three periods were assessed: (1) 24 hours, (2) daytime period defined as 8:00 AM to 8:00 PM, and (3) night time period defined as 12:00 AM to 6:00 AM. The separation into daytime and night time was arbitrary [7].

2.6. Statistical Analysis. Data are presented as mean ± standard deviation unless otherwise specified. Comparisons between normal, spontaneous, and pseudonormal pattern of LV filling pre and posttreatment were conducted with a mixed ANOVA design. This analysis allowed us to first compare groups (treatment versus control) in regards to baseline data and to determine the treatment effect as well as interactions between groups and treatment. The factor with random effect was linked to subjects. The dependent variable associated to LV diastolic function was analysed using a cumulative multinomial distribution with an independent covariance structure. Thereafter, if significant differences occurred, *a posteriori* Tukey's comparison technique was performed to determine differences. Relationships among variables were measured using Pearson's correlation coefficients. In order to test the normality distribution of the data,

Table 1: Baseline characteristics of type 2 diabetic severely obese subjects before and after treatment.

	Control group		Treatment group	
	Pre	Post	Pre	Post
N	13	13	16	16
Age (years)	48 ± 11	—	47 ± 9	—
Duration of diabetes (years)[1,3]	4.8 ± 4.6	—	7.2 ± 6.1	—
Men/Women (number)	7/6	7/6	7/9	7/9
Weight (kg)	142.6 ± 34.6	142.1 ± 35.0	129.6 ± 17.4	125.9 ± 17.5*‡
BMI (kg/m^2)	51.2 ± 9.2	51.2 ± 9.0	45.9 ± 7.3	44.8 ± 7.5*‡
Waist circumference (cm)	145.8 ± 20.8	144.3 ± 20.6	138.4 ± 14.7	133.9 ± 17.0*
Fat percentage (%)[2]	48.5 ± 5.4	48.8 ± 4.1	45.7 ± 8.3	45.6 ± 8.7
Fat mass (kg)[2]	67.3 ± 13.6	66.2 ± 13.8	59.1 ± 13.1	57.4 ± 13.5
Fat free mass (kg)[2]	72.3 ± 18.9	70.0 ± 17.8	70.4 ± 15.4	68.5 ± 15.2*
Resting HR (beat/min)	83 ± 7	84 ± 12	80 ± 12	78 ± 11
SBP (mmHg)	127 ± 20	126 ± 15	137 ± 18	127 ± 13
DBP (mmHg)	71 ± 10	70 ± 9	74 ± 12	70 ± 11
Fasting glycemia (mmol/L)[1,3]	8.0 ± 2.5	8.1 ± 2.5	7.9 ± 3.0	6.7 ± 2.2*
HbA_{1c} (%)[1]	7.3 ± 1.1	6.7 ± 0.9*	7.3 ± 2.1	6.6 ± 1.3*
Total cholesterol[4]	4.8 ± 0.8	4.7 ± 0.7	4.5 ± 0.7	4.3 ± 0.8
HDL-cholesterol (mmol/L)[4]	1.3 ± 0.2	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.3
LDL-cholesterol (mmol/L)[4]	2.7 ± 0.8	2.5 ± 0.7*	2.5 ± 0.6	2.2 ± 0.8*
Triglyceride (mmol/L)[4]	1.8 ± 0.5	2.0 ± 0.7	1.7 ± 0.6	1.9 ± 0.8

Mean ± standard deviation; *$P < .05$: pre versus post; ‡$P < .05$: control versus treatment; BMI: Body mass index; DBP: Diastolic blood pressure; HR: Heart rate; MBP: Mean blood pressure; SBP: Systolic blood pressure.
[1]One subject missing in the control group.
[2]Two subjects missing in the control group.
[3]One subject missing in the treatment group.
[4]Two subjects missing in the treatment group.

the Shapiro-Wilk test was performed. Brown and Forsythe's variation of Levene's statistics test was used to verify the homogeneity of variances. A P value inferior to .05 was considered statistically significant. Data were analysed using the statistical packages Sigma Stat (Chicago, IL, USA) and SAS (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Clinical Parameters. Table 1 presents clinical characteristics of patients in both groups before and after treatment. There were no significant statistical differences between the two groups for all parameters at the beginning of the study (Table 1). After 3 months of treatment with Orlistat, there was a significant reduction in body weight ($P < .001$), BMI ($P = .003$), and waist circumference ($P = .005$). When compared to the control group, mean weight loss (3.7 ± 3.0 versus 0.5 ± 2.2 kg; $P = .003$), corresponding to a percentage of weight loss of 2.9 ± 2.5 versus 0.3 ± 1.5% ($P = .003$), was statistically different. Weight loss in the treatment group was associated with improvements in fasting glycemia, which decreased only in the group treated with Orlistat ($P = .03$). However, there was a significant decrease in HbA_{1c} (controls: $P < .001$ and treated: $P = .02$) as well as in the LDL-cholesterol levels in both groups (controls: $P = .05$ and treated: $P = .03$). In the treatment group, fat-free mass decreased significantly ($P = .03$). No significant difference was observed between the two groups after treatment for the other anthropometric and metabolic parameters (Table 1).

3.2. Heart Rate Variability

3.2.1. Time Domain. In the 24-hour HRV indices, there was a significant increase in rMSSD ($P = .02$) as well as in pNN50 ($P = .03$) in the treated group following weight loss (Table 2). While there was no significant change in the HRV daytime indices, night time measures showed a significant improvement in rMSSD in the treated group only ($P = .023$).

3.2.2. Spectral domain. Following weight loss, high-frequency (HF) power measured during the 24-hour period increased significantly ($P = .05$), but during daytime and night time, increments did not reach statistical significance (daytime: $P = .056$ and night time: $P = .1$) (Table 2). The LF/HF ratio decreased significantly in the treated group in the 24-hour assessment ($P = .038$) as well as during daytime ($P = .006$).

3.3. Echocardiography. Echocardiographic parameters measured in both groups as well as normal values are shown in Table 3. There were no statistically significant differences in heart dimensions or parameters of LV diastolic function between the control group and the Orlistat-treated group at

Table 2: Heart rate variability indices in severely obese subjects with type 2 diabetes before and after treatment.

(a) 24 hour

	Control group		Treatment group	
	Pre	Post	Pre	Post
N	13	12	16	16
Mean NN (ms)	768 ± 86	786 ± 111	775 ± 102	796 ± 101
SDNN (ms)	106 ± 34	106 ± 30	102 ± 25	112 ± 23
SDANN (ms)	93 ± 38	91 ± 35	88 ± 22	97 ± 22
rMSSD (ms)	26 ± 15	28 ± 16	24 ± 10	30 ± 10*
pNN50 (%)	7 ± 10	8 ± 10	6 ± 7	9 ± 8*
HF (ln)	4.4 ± 1.0	4.6 ± 0.9	4.5 ± 0.9	4.9 ± 0.7*
LF (ln)	5.6 ± 0.9	5.7 ± 0.7	5.7 ± 1.0	5.9 ± 0.7
LF/HF ratio	3.7 ± 1.9	3.4 ± 1.7	3.3 ± 1.1	2.8 ± 1.3*

(b) Daytime

	Control group		Treatment group	
	Pre	Post	Pre	Post
N	13	12	16	16
Mean NN (ms)	733 ± 100	762 ± 133	734 ± 104	742 ± 105
SDNN (ms)	80 ± 29	85 ± 30	80 ± 22	84 ± 22
SDANN (ms)	68 ± 23	69 ± 26	63 ± 18	66 ± 21
rMSSD (ms)	21 ± 18	26 ± 20	21 ± 8	25 ± 9
pNN50 (%)	5 ± 13	7 ± 13	4 ± 5	6 ± 7
HF (ln)	3.8 ± 1.2	4.3 ± 1.2	4.1 ± 1.0	4.6 ± 0.8
LF (ln)	4.9 ± 0.9	5.4 ± 0.8	5.5 ± 1.0	5.7 ± 0.7
LF/HF ratio	3.8 ± 2.4	3.7 ± 2.5	4.0 ± 1.8	3.2 ± 1.2*

(c) Night time

	Control group		Treatment group	
	Pre	Post	Pre	Post
N	13	12	16	16
Mean NN (ms)	840 ± 86	827 ± 96	851 ± 112	890 ± 105
SDNN (ms)	88 ± 45	86 ± 30	83 ± 25	85 ± 23
SDANN (ms)	57 ± 39	60 ± 31	57 ± 18	53 ± 16
rMSSD (ms)	32 ± 17	30 ± 11	31 ± 15	36 ± 16*
pNN50 (%)	10 ± 12	10 ± 10	11 ± 12	15 ± 14
HF (ln)	5.1 ± 1.1	4.9 ± 0.9	5.0 ± 1.0	5.4 ± 1.0
LF (ln)	6.2 ± 1.1	5.9 ± 0.9	6.0 ± 1.0	6.2 ± 0.9‡
LF/HF ratio	3.7 ± 2.6	3.2 ± 2.0	2.9 ± 1.4	2.6 ± 1.7

Mean ± standard deviation; *$P < .05$: pre versus post; ‡$P < .05$: control versus treatment; ln: logarithmic transformation; HF: High frequency; LF: Low frequency; Mean NN: Mean value of all RR intervals; pNN50: Percentage of intervals differing of ≥50 ms than the preceding interval; rMSSD: square root of the mean squared difference of successive RR intervals; SDANN: Standard deviation of the mean NN intervals for each 5 minutes period; SDNN: Standard deviation of the RR intervals.

the beginning of the study. Despite normal baseline values, we observed a nonstatistical increment in ejection fraction after treatment with Orlistat ($P = .054$), the values after treatment being also significantly greater than the ejection fraction values of the control group ($P = .04$). There were 11 out of 16 subjects with impaired LV diastolic function (7 spontaneous and 4 pseudonormal LVDD) in the treatment group while there were 7 out of 13 subjects (5 spontaneous and 2 pseudonormal LVDD) in the control group (Figure 1). Isovolumetric relaxation time, A wave velocity, E/A ratio, deceleration time, as well as the A wave duration were not statistically different following treatment. However, E wave velocity significantly increased in the treatment group ($P = .046$). During the Valsalva manoeuvre, we observed a significant decrease of the A wave velocity in the treatment group ($P = .007$), concomitant to a significant increase in the E/A ratio ($P = .024$). This increment was statistically different to the effect observed in the control group ($P = .027$). Accordingly, for the patients with a pseudonormal diastolic function, using the E/A value assessed during the

Table 3: Echocardiographic parameters in severely obese subjects with type 2 diabetes before and after treatment.

	Normal values	Control group		Treatment group	
		Pre	Post	Pre	Post
N		13	13	16	16
Aortic root (mm)	20–37	33 ± 4	35 ± 4*	33 ± 4	33 ± 4‡
IVS (mm)	6–11	11 ± 1[1]	11 ± 1[1]	11 ± 2[1]	11 ± 2
PW (mm)	6–11	10 ± 2[1]	10 ± 1[1]	11 ± 1[1]	10 ± 1*
LA (mm)	19–40	42 ± 4	41 ± 5	42 ± 4	41 ± 3
RV diastolic (mm)	7–23	26 ± 4	26 ± 4	26 ± 3	26 ± 3
LV diastolic (mm)	35–57	53 ± 8	54 ± 9	50 ± 4	51 ± 3
LV systolic (mm)	20–39	34 ± 9[2]	35 ± 10[1]	29 ± 4[1]	30 ± 4
LV mass (g/m)[4]	M < 163 W < 121	226 ± 74[2]	233 ± 61[2]	209 ± 53[3]	198 ± 46[2]*
Ejection fraction (%)	>50	60 ± 11	60 ± 13	64 ± 5	67 ± 4

Mean ± standard deviation; *$P < .05$: pre versus post; ‡$P < .05$: control versus treatment; IVS: Interventricular septum; LA: Left atrium; LV: Left ventricle; PW: Posterior wall; RV: Right ventricle.
[1]One missing subject.
[2]Two missing subjects.
[3]Three missing subject.
[4]Normal values: <163 g/m for men and <121 g/m for women [24].

Valsalva manoeuvre in order to account for the increased pressure of LV filling, there was a significantly enhanced LV diastolic function in the treatment group ($P = .014$). There was no difference in the other indices of LV diastolic function.

Figure 1 shows the influence of weight lost on LV diastolic function. Thirty-height percent of the subjects (6/16) in the treatment group significantly improved their LV diastolic function ($P = .03$). In contrast, in the control group, there was no improvement ($P = .35$) except for one subject while 3 subjects worsen their LV diastolic function. The amelioration of LV diastolic function in the treated group was significantly different to the control group ($P = .026$).

3.3.1. Correlations. As expected, a positive correlation was found between body weight and BMI and LV mass ($r = 0.68$ and $r = 0.44$, resp.; both $P < .001$). An inverse correlation was also observed between body weight, BMI, and LV ejection fraction ($r = -0.48$ and $r = -0.52$, resp.; both $P < .001$). There was positive correlation between *E/A* ratio and 24-hour measurement of HF ($r = 0.361$; $P < .01$), rMSSD ($r = 0.456$; $P < .001$), and pNN50 ($r = 0.458$; $P < .001$). Changes in *E/A* ratio and delta LF/HF ratio during 24-hour and daytime also showed correlations ($r = 0.377$; $P = .048$ and $r = 0.458$; $P = .014$, resp.).

4. Discussion

We observed that modest Orlistat-induced weight loss enhances sympathovagal balance and cardiac function in severely obese patients with type 2 diabetes. After a 3-month treatment period with Orlistat, subjects lost significantly more weight than subjects in the control group.

Akehi et al. reported that very-low calorie diet-induced weight loss in moderately obese subjects improved NN, SDNN, SDANN, rMSSD, HF, and LF/HF ratio values [6]. This was interpreted as an improvement in the sympathovagal balance. Similarly, Poirier et al. [7] reported that a 10% diet-induced weight loss in 8 severely obese subjects was associated with significant improvement in autonomic cardiac modulation through enhancement of parasympathetic modulation which translates clinically into decreased heart rate and increased HRV. In our study, rMSSD, pNN50, and HF values of the 24-hour HRV were significantly improved in the treated group. All these indices are considered to reflect the parasympathetic system and being protective from a cardiovascular disease viewpoint [25]. Furthermore, another significant improvement was the decrease in the LF/HF ratio assessed during 24 hours and during daytime in the treatment group. Accordingly, Poirier et al. observed that the improvement in HRV after weight loss occurred mainly during the daytime period [7]. Our results show similar improvement in the parasympathetic modulation and in the sympathovagal balance, particularly during daytime. Studies [10, 14, 26] reported that, following bariatric surgery which induced important weight loss in severely obese subjects, LV mass was significantly reduced. In our study, weight loss in the treatment group was accompanied with a significant decrease in the posterior wall thickness and in LV mass as also reported by others [27–29].

To our knowledge, there are no data available in severely obese subjects investigating the impact of weight loss on the pseudonormal pattern of LV filling using the Valsalva manoeuvre. We observed an improvement of LV diastolic function in over half of the treated subjects compared to only one patient who had an improved LV diastolic function in the control group. Of note, the relations between HRV and echocardiographic parameters revealed that rMSSD and HF values correlate with *E/A* ratio. Interestingly, we also found a significant positive correlation between changes in *E/A* ratio and changes in LF/HF ratio during 24-hour and

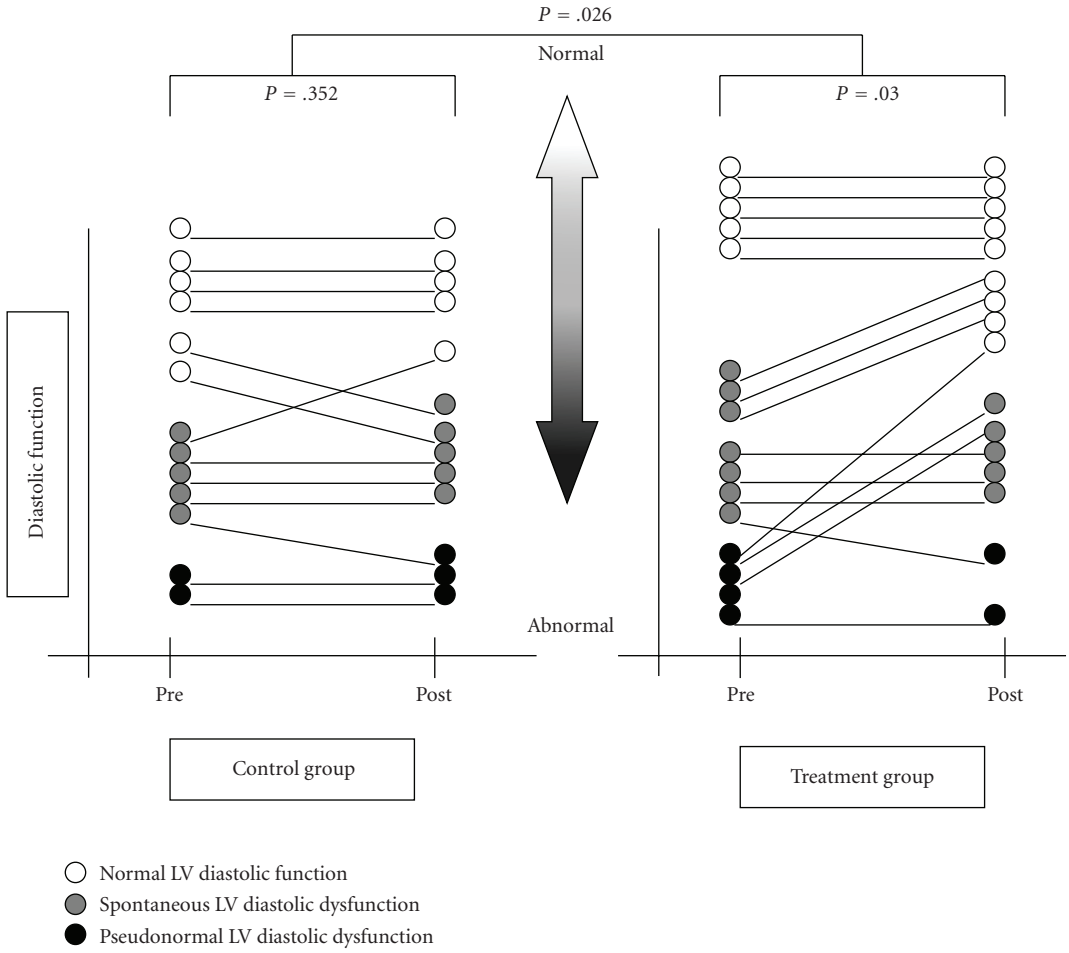

Figure 1: Changes in left ventricular (LV) diastolic function in both groups pre and posttreatment.

daytime. These associations suggest that improvement in the parasympathetic function is related to improvement in the cardiac LV diastolic function parameters, as we previously reported in overweight subjects with diabetes [21]. Of note, there are only few reported studies using pharmacological approach in severely obese patients [30–32] and HRV and diastolic function has not been evaluated within the same study in this population.

The magnitude of weight loss induced by the medication is smaller (2.9 ± 2.5%) but may be comparable to findings previously reported, accounting for the time-treatment period. Sjöström et al. have reported a 10% weight lost (10 kg) after a year of treatment with Orlistat in a cohort of overweight to severely obese subjects [33]. Other studies using Orlistat for a longer period (12 to 24 months) have also reported similar results [34–39]. Taking into account that our study only lasted three months, we may speculate that subjects of our study could have possibly lost comparable amount of weight if had our study lasted 12 months. Indubitably, the definitive treatment in this obesity category is bariatric surgery, but clinicians aim at a 5%–10% weight loss in order to achieve better metabolic profile in obese subjects [1, 15]. Fasting glycemia significantly decreased in the treated group in contrast to the control group. Accordingly, Fujioka et al. [40] reported that every 4.5 kg reduction in weight should result in a 1.1 mmol/L decrement in fasting glycemia and to a 0.5% reduction in HbA_{1c}. Accordingly, HbA_{1c} values have significantly decreased in the treated group. However, we also observed a significant decrease in HbA_{1c} in the control group, which was not different to the decrease observed in the treated group. We also observed a significant decrease in LDL cholesterol in the treated group as well as in the control group. This is not unusual and it is often encountered in placebo groups of weight loss studies. Indeed, similar results have been reported in obese subjects after a 4-week run-in period before treatment with Orlistat [34, 35]. Studies [38, 39] in overweight to obese type 2 diabetic patients with longer treatment duration (6 to 12 months) resulting to greater weight loss report greater changes in metabolic profiles, leading after 12 months to significant differences between Orlistat-treated group and control group.

We acknowledge that this study included a small number of subjects. Therefore, it should be considered as a pilot study. However, weight loss was significant in the treated group whereas it was not in the control group. Also, a

few echocardiographic measurements, mainly those regarding the pulmonary veins assessment, were not adequately recorded because of technical reasons due to the corpulence of the subjects. Mitral inflow velocities are known to be load-dependent. Other indices of LV diastolic function, such as tissue Doppler-derived indices, may be less load-dependent. However, Dumesnil et al. [41] reported that tissue Doppler-derived indices are not totally preload independent and should be interpreted in light of the other Doppler parameters and the use of Valsalva's manoeuvre.

5. Summary

The aim of this study was to determine the impact of Orlistat-induced weight loss on the metabolic profile and cardiovascular function in severely obese type 2 diabetes patients. Twenty-nine severely obese patients with type 2 diabetes were randomized either to a nonplacebo control group or to a treatment group with Orlistat thrice a day. Metabolic profile, anthropometric parameters, heart rate variability indices, and echocardiographic variables were measured before and after a 12-week treatment period. Our results suggest that a modest weight loss improves fasting glycemia, diastolic function, and sympathovagal balance in severely obese patients with type 2 diabetes.

6. Conclusions

Despite modest but significant weight loss, we observed that subjects treated with Orlistat showed improvements in fasting glycemia. We also observed improvements in HRV as well as in the LV diastolic function. The improvement in the cardiac parasympathetic ANS modulation was associated with enhancement in LV diastolic function. Our results emphasize the benefit of even modest weight loss in severely obese patients with diabetes.

Disclosures

Roche Company had no role in the design, collection, analysis, or interpretation of the data nor in the decision to submit the study for publication.

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Review Article

AMPK as Target for Intervention in Childhood and Adolescent Obesity

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Childhood obesity is a major worldwide health problem. Intervention programs to ameliorate the rate of obesity have been designed and implemented; yet the epidemic has no end near in sight. AMP-activated protein kinase (AMPK) has become one of the most important key elements in energy control, appetite regulation, myogenesis, adipocyte differentiation, and cellular stress management. Obesity is a multifactorial disease, which has a very strong genetic component, especially epigenetic factors. The intrauterine milieu has a determinant impact on adult life, since the measures taken for survival are kept throughout life thanks to epigenetic modification. Nutrigenomics studies the influence of certain food molecules on the metabolome profile, raising the question of an individualized obesity therapy according to metabolic (and probably) genetic features. Metformin, an insulin sensitizing agent, its known to lower insulin resistance and enhance metabolic profile, with an additional weight reduction capacity, via activation of AMPK. Exercise is coadjutant for lifestyle modifications, which also activates AMPK in several ways contributing to glucose and fat oxidation. The following review examines AMPK's role in obesity, applying its use as a tool for childhood and adolescent obesity.

1. Introduction

Obesity is considered a new pathology in the history of Humankind, being the new food security tendency the one to blame for such a rising wave [1]. In the last century, technological advances and cutting edge science have modified human lifestyle, changing diet regimes and physical activity and therefor ecreating an imbalance between caloric ingestion and an energy expenditure that is not able to compensate the caloric excess ingested. This spill-over energy is accumulated in the adipose tissue manifesting itself as obesity which is considered a step closer to the new evolved man: *Homo obesus* [1, 2].

The World Health Organization (WHO) has labeled obesity as the *new epidemic* of the 21st century. According to WHO projections for 2005, around 1,600 billion adults worldwide were overweight and at least 400 million were obese; by 2015, more than 2,3 billion adults will be overweight and 700 million will be obese [3]. The values in the pediatrics population are even less encouraging, with at least 20 million overweight children of less than 5 years of age [3]. In the United States, the prevalence has risen in the last 30 years, with a 3,8-fold for the 6–11 years. old group (from 4% to 15,3%), and 2,6-fold for the adolescent group (from 6% to 15,5%) [4]. Sekhobo et al. [5] published their results based on an analysis in overweight/obesity tendency in the low-income prescholars who were part of the New York State Special Supplemental Nutrition Program for Women, Infants, and Children, 2002–2007. The prevalence of obesity raised in 2003 [3, 6], later declining from 2003 to 2005, finally stabilizing itself at 14,7% by 2007. Nevertheless, there was an increasing prevalence of overweightness during the whole study. There is no doubt that overweight and obesity have become a major health problem [5].

Defining obesity in the pediatric group is a real challenge due to growth (weight and height) variations in childhood and adolescence. The International Obesity Task Force

(IOTF) has established that obesity and overweight will be defined by corresponding percentiles of Body Mass Index (BMI) 25–30 at 18 years of age, and that the percentiles will be conserved throughout the age groups. Some authors use only BMI values to differentiate between overweight and obesity in adolescents; nevertheless Cole et al. [7] published the percentile tables according to age and sex. With these tools, the Center for Disease Control (CDC) has defined overweight inetary group 2–18 years of age as BMI equal or above 95th percentile according to age and sex. The term *risk of overweight* is applied to circumscribe children with BMI between 85th and 95th according to age and sex, since CDC prefers not to use the term obesity for psychological/social reasons [8, 9].

The Metabolic Syndrome has evolved from its first definition back in 1998 by the WHO [10]. The first published consensus was meant to detect those high-risk patients, diabetic or not, with any degree of glucose intolerance, hypertension, dyslipidemia, and/or microalbuminuria. In 1999 the European Group for the Study of Insulin Resistance (EGIR) issued another set of variables for the diagnosisexcluding microalbuminuria [11]. By 2001, the Adult Treatment Panel III (ATPIII) announced their criteria suggesting that insulin resistance was not necessary for the diagnosis [12]. The International Diabetes Federation (IDF) participated in this worldwide debate when in 2005 they published their own definition, giving particular interest to the influence of ethnicity in the proper diagnosis of the disease and the cut-offs being set for the patients, particularly since abdominal obesity was now proposed as a fundamental element for its identification [13]. In 2005, the American Heart Association together with the National Institutes of Health, Heart, Lung, Blood Institute (AHA/NIHHLBI) revised the ATPIII definition, modifying normal fasting glucose levels lowered to 100 mg/dl, in accordance with the American Diabetes Association's new cut-off [14]. Finally, the IDF with AHA/NIHHLBI issued the newest MS definition in 2009 [15], emphasizing need for proper individualization of anthropometric reference values in every ethnic population. See Table 1.

Table 1: Metabolic Syndrome criteria according to International Diabetes Federation/American Heart Association/National Institutes of Health, Heart, Lung, Blood Institute, 2009.

IDF/AHA/NHLBI 2009[14]
Three of the following:
(i) *Abdominal Obesity*:
Elevated waist circumference according to a population and country-specific definition.
(ii) *Lipid Profile*:
Triglycerides ≥150 mg/dL or treatment for elevated triglycerides.
(iii) *Lipid Profile*:
HDL-c <40 mg/dL in men or <50 mg/dL in women.
Or treatment for reduced HDL-c.
(iv) *Blood Pressure*:
≥130 mmHg on Systolic Pressure.
Or ≥85 mmHg on Diastolic Pressure.
Or on anti-hypertensive drug treatment in a patient with a history of HTA.
(v) *Glycemia*:
Fasting glucose ≥100 mg/dL.
Or on drug treatment for elevated glucose.

Overweight/Obesity is the most studied variable of MS in children, and there is no official statement on the cut-off points for its definition in this population; in fact there is no consensus on the existence of MS in childhood due to its limited capacity for predicting outcomes like Type 2 Diabetes Mellitus (T2DM) and Cardiovascular disease (CVD). Goodman et al. [16] demonstrated in a 3-year follow-up study that 61,1% of the children with MS lost at least one of the variables during the trial, while 25,5% of the children without MS acquired at least one risk factor, defined by the authors as "*the instability in the diagnosis of metabolic syndrome.*" Nonetheless, one of the outcomes that have been explored is the risk of adult obesity in the already obese child. Around 30% of adulthood obesity starts in infancy, with even worst consequences than compared to obese adults who were lean. Overweight adolescents have 50%–70% chance to become overweight/obese adults, while only 30% of the lean children will become obese in adulthood [6, 17]. This only ensures the importance of a proper and early intervention to minimize or completely prevent future metabolic complications.

In 1973, two research groups described at the same time the qualities of a protein kinase that was involved in lipid metabolism, which was able to inhibit Acetil~CoACarboxilase (ACC) and 3-Hydroxi-3-MethylGlutaryl~CoA (HMG-CoA) Reductase [18, 19]. Later on, this kinase was labeled AMPK (*AMP activated protein kinase*) since it has been activated by increased AMP concentrations. The enzyme has been the topic of several studies, which have revealed its effect on energy balance, mitochondrial biogenesis, regulation of lipid/carbohydrate metabolism, and modulation of genetic expression [20, 21].

The role of AMPK as an energy thermostat puts it at crossroads for energy homeostasis, making it fundamental to analyze the signaling pathways involved in energy metabolism, not only for academic purposes but also for therapeutical goals in childhood obesity. The purpose of this review is to analyze the role of AMPK as aninterventiontargetin childhood obesity.

2. Nutrition of the Fetus: Under versus Over

2.1. Food Intake Control. The regulation of food intake is a very complicated network involving peripheral signals and central processing, which renders behavioral patterns that lead to weight gain or weight loss [22]. The processing of all input signals is done between the "satiety centre" in the ventro-medial nuclei and the "hunger centre" in the lateral hypothalamic area, arcuate, and paraventricular nuclei. The adiposity signals come from the pancreas and the adipose tissue, represented by insulin and leptin; both of them are destined to stop eating behaviour and food intake once

energy stores have been filled. These signals activate the Pro-Opio-Melanocortin/Cocaine and Amphetamine-Regulated Transcript neurons (POMC/CART) inducing satiety (see Figure 1).

On the other hand, gut signals usually modulate hunger and feeding behavioral patterns [23].Glucagon-like peptide 1 (GLP-1) is an incretin which is released while eating, inducing insulin release from the β-cellsand haltering food consumption. Peptide YY—a member of the NPY peptide family—is a 34-aminoacid peptide secreted by the intestine which hinders food intake and modifies gut motility (ileal brake), and its release depends on the amount of carbohydrate or lipids in the ingested meal. This molecule inhibits NPY neurons and activates POMC population, lowering ~ 30% of food intake using the Y2Receptor. PYY is negatively correlated with the degree of adiposity with reduced values when compared with normal weight subjects [24]. Ghrelin is a peptide synthetized in the stomach, and it is associated with hunger. It exerts its effects through the Growth hormone secretagogue receptor in the hypothalamus and brain stem, with the activation of Neuropeptide Y/Agouti related Peptide (NPY/AgRP) neurons, which induces ppetite while inhibiting POMC/CART secreting cells. Obestatin is a 23-aminoacid byproduct of the ghrelin gene breakdown, also synthetized in the A-cells in the stomach [25] whose physiological role is still under consideration; yet certain studies have linked this molecule with antiapoptotic properties in β-cell, and with inhibition of food consumption via upregulation of GLP1 mRNA [26]. Other factors include pancreatic peptide (PP) and oxyntomodulin (Oxm)both of which are food intake inhibitors.

Ghrelin has been one of the most researched gut-derived molecules in childhood obesity. Disturbances of its pathway have been proposed as basic pathophysiology for several diseases like: growth hormone deficiencies, anorexia nervosa, cachexia, chronic heart failure, gastrointestinal motility disorders, osteoporosis, obesity, and Prader-Willi syndrome [27]. Ghrelin enhances AMPK activity in the hypothalamus, lowering malonyl~CoA levels, inducing carnitine palmitoyl transferase-1, elevating long chain fatty acids, releasing NPY, and activating hunger [28]. As basic rule of thumb, ghrelin levels are inversely correlated to BMI, and there is a negative association between insulin and this hormone. James et al. [29] reported that ghrelin levels are associated with slow weight gain from birth to 3 months of age, which relates it to postnatal catch up growth. In a study using 208 preterm children, Darendeliler et al. [30] reported that at prepubertal ages those preterm newborns—either small (SGA) or adequate for gestational age (AGA)—had higher ghrelin levels compared to those at-term newborn children. This sustained elevation of ghrelin might be needed for the compensatory growth which they are subjected, but it does not correlate to the degree of catch-up growth achieved nor with the levels of insulin found. Similar results were published the following year by Darendeliler et al. [31] analyzing prepubertal children who were born large for gestational age, reporting that nonobese prepubertal youngsters had lower ghrelin levels compared to AGA born counterparts, proposing that birth weight is in fact a fundamental determinant in ghrelin levels during childhood.

Maffeis et al. [32] proposed that meal-induction of insulin secretion promotes a fall in ghrelin levels, and that in insulin resistance states this blunting is reduced. Now, even though this is true, this inhibiting effect of feeding is lost in childhood suggesting that ghrelin acts as an anabolic hormone meant to provide the necessary substrates for growth [33]. This feeding reducing effect on ghrelin depends on insulin availability, and its deficiency can explain the observed hyperphagia in Type 1 Diabetes Mellitus [34]. Bacha and Arslanian [35] conducted a trial using overweight children as subjects to evaluate insulin's power of inhibition, reporting that fasting ghrelin is determined by insulin sensitivity regardless of adiposity.

Obesity is a multifactorial disease which has no clear genetic cause. It *is* known that obesity is a heritable syndrome, with a heritability of 0.7 to 0.8 [36]; yet the genes at play are still pending confirmation. Lessons have been taken from monogenic syndromes like the Congenital Leptin Deficiency [37–39] which is characterized by hyperphagia and early-onset obesity. The mutations causing this disease render a functional protein that cannot mediate appetite control, and the patients develop hyperphagia very early on. Subjects with this mutation are candidates for leptin replacement since it is the defective molecule. Gibson et al. [40] reported the 4-year treatment of a subject (with the Δ133G mutation) with subcutaneous recombinant leptin, providing beneficial and long lasting controlling effects on hyperinsulinemia, hyperlipidemia, fat mass distribution, and TSH levels. Not all mutations are located on one gene nor do they have such a profound effect like the former Mendelian syndrome; most of the genes associated with obesity [41] interact with each other to express a phenotype that will end with abdominal obesity. Genomewide scans have revealed several gene candidates [41], including adiponectin (3q27) [42, 43], adrenergic receptor α-2A (10q24-q26) [44, 45], leptin (7q31.3) [46, 47], glucocorticoid receptor (5q31) [48], PPARγ (3p25) [49], serotonin receptor (Xq24) [50], and melanocortin 4 receptor (18q21) [51]. The interaction of these genes with environmental factors affect any check point in the appetite network, either in the behavioral aspects or in the metabolic adaptations, as can be seen in Figure 2.

The *Thrifty Phenotype* theory proposed by Barker [52] tried to explain the relationship between intrauterine growth retardation and premature death due to cardiovascular disease or T2DM complications (see Figure 3). He postulated that according to the insult enforced on the fetus, the reprogramming of several axes would determine the fetus' survival during the pregnancy phase; yet the necessary measures imprinted for salvationare deleterious during adult life. Fetal malnutrition can be achieved by several ways, but the overall outcome is always the same: hypoglycemia and hypoxia. Small placentas, which have not acquired enough spiral arteries remodeling, have trouble oxygenating and nurturing the *conceptus*, developing a hypoxic placental milieu [53]. It has been proposed that modifications in the adipoinsular axis of the fetus promote hyperinsulinism and hyperleptinemia and confer the necessary epigenetic

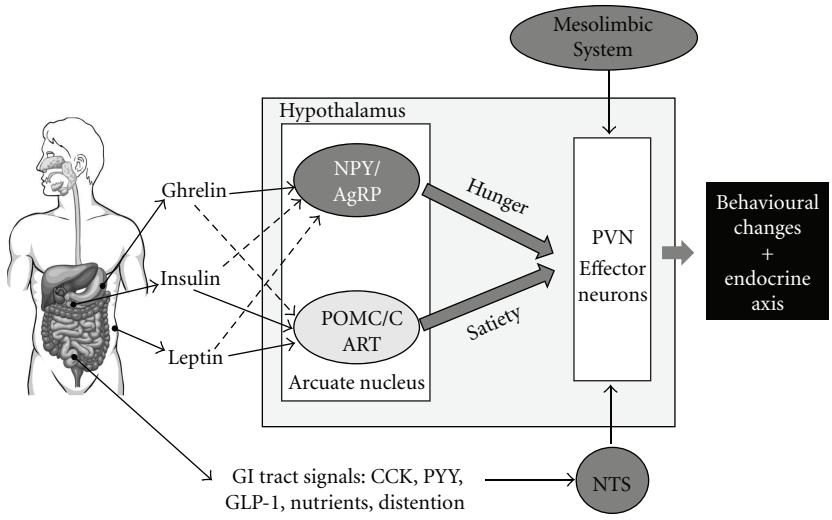

Figure 1: The Food Intake network is a complex system compromising several levels. See text for further information. CCK: cholecystokinine; GLP-1, Glucagon-like peptide 1, NTS, nucleus of the solitary tract, PVN, paraventricular nucleus; PYY, Peptide YY.

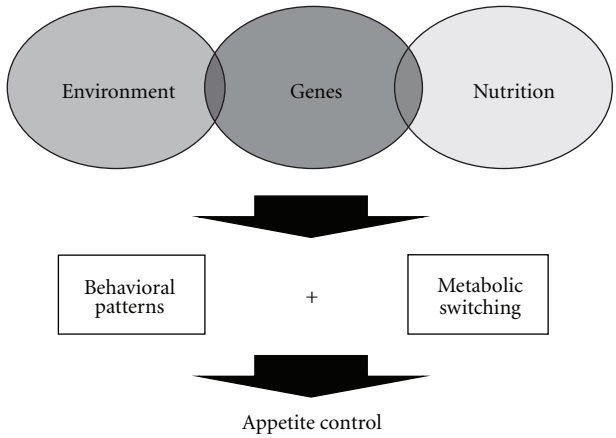

Figure 2: Appetite control is a very complex network, which has to intertwine genetic factors, nutritional signals, and environmental determinants. The making of metabolic switches and acquiring food-seeking related behavioral patterns is the final combination to manage food intake.

work-up to maintain this during postnatal life [54]. Fetuses small for their age are usually dysmorphic, with small bodies compared to the proportion of their head (*brain sparing*) [55], low fat mass concentrations, and blunted response to hyperglycemia apparently from small β-cell ontogeny and mass [56].

Leptin can be detected as early as 17-week gestation [57], modulating adipose tissue development. Leptin acts as a *lipostat* in fetal life, giving information about fat maturity and proportions. In animal models—as in humans—fetal fat has brown and white adipose tissue characteristics, and it goes along with the very fundamental fact of being born with a matured hypothalamic-pituitary axis [58]. It is noteworthy to remark that adipose tissue grows in locules (uni and multiple), and there is always a dominant unilocular tissue which correlates with leptin concentrations. These unilocular spots have peculiar (transitional) adipocytes with abundant mitochondria, uncoupling protein 1 (UCP1), and Prolactin receptor-long type that are brown tissue characteristics; yet they are capable of secreting leptin (white adipose tissue trait). Lipid synthesis is a very expensive process, using 39 MJ/kg (compared with carbohydrates which consumes 15–25 MJ/kg), and in the fetus this pathway depends on oxygen and metabolic substrates supply [58]. In light of this, if the mother modifies the amount of food and the quality of it, the proper maturation of fetal adipose tissue can be modulated. Indeed, several animal models have shown that maternal chronic hypoxia, hypoglycemia, and hypoinsulinemia are related to low levels of mRNA for leptin [59, 60].

Vickers et al. [61] published their findings on the fetal origins of hyperphagia and obesity using Virgin Wistar rats that were mated randomly and subsequently were divided into 2 groups: those with food restriction and those with food *ad libitum*. The pups born from the malnourished group were smaller at birth and had higher food intake in the immediate postnatal period, and this behaviour was enhanced with a hypercaloric diet. They concluded that hyperinsulinism and hyperleptinemia are responsible for fetal programming and adult hyperphagia, obesity, and high blood pressure. During that same year, Ekert et al. [62] reported that maternal nutrition during pregnancy in fact reprograms leptin secretion and this pattern is maintained even in adulthood. The research group used the pig model, whose mothers were fed a restricted diet during the whole pregnancy (whole pregnancy undernourishment), and half of these were then fed 35% more food during the second quarter of the pregnancy (a migestation malnutrition and late pregnancy recuperation). Leptin's expression was measured by determining mRNA of the protein in the subcutaneous adipose tissue of the mothers, reporting a negative correlation between birth weight and leptin levels

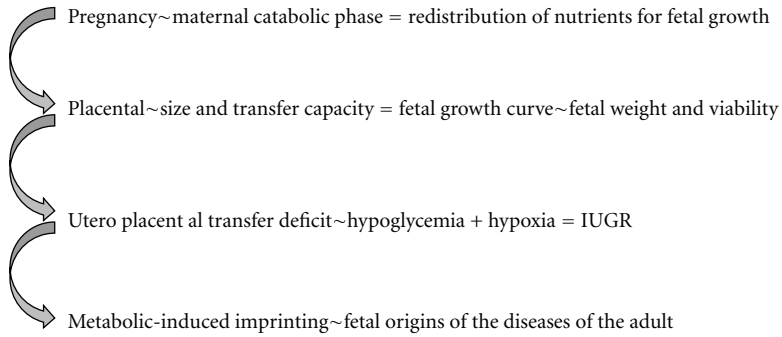

Figure 3: The intrauterine life is very delicate balance between fetal growth and placental transport capacity which depends on the mother's vascular capacity. If the placenta is not fully developed (anomalous insertion, second wave invasion incomplete, or other maternal morbidity like long-term diabetes, hypertension or other related disease with vasculopathy), decreased blood flow through the umbilical cord will generate several survival responses in the fetus, all with the aim of reaching a viable weight and enough lung maturation to survive outside the womb. The mechanisms are energy-savers and try to protect the brain from damage. This metabolically induced imprinting lasts very well into adulthood, being the key feature in the development of chronic diseases like Obesity, Type 2 diabetes, Stroke, Cardiovascular disease, among others.

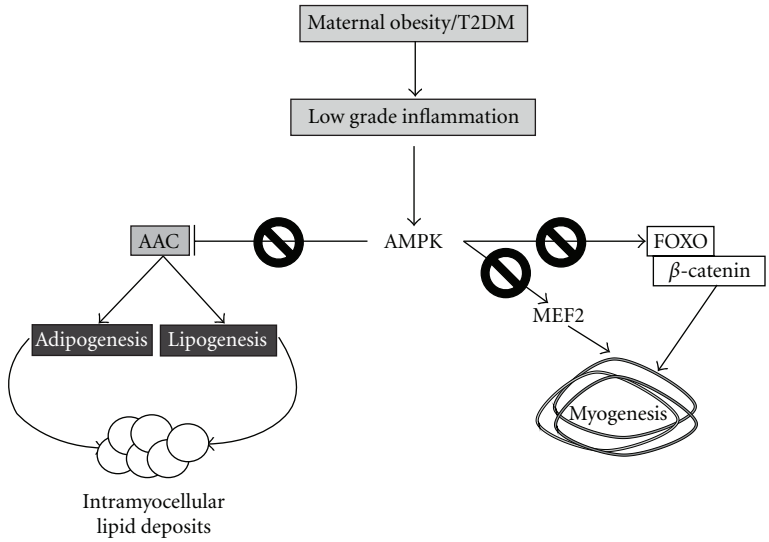

Figure 4: If the baby develops in a proinflammatory milieu, it is another study. The Barker hypothesis comes with an inflammatory component but it is basically aimed at the mother due to placental hypoxia (it is basically responsible for the hypertension and prothrombotic features of Preeclampsia). In the Macrosomic fetus low-grade inflammation takes it is toll on AMPK activation, which due to it is inhibition 2 basic pathways are modified. First, Acetyl~CoA loss of inhibition, which lets loose adipogenesis and lipogenesis with increased intramyocellular lipid deposits. Second, while preadipocyte is differentiated to mature adipocytes, myogenesis is being stalled due to loss of activation of expression of genes that regulate myocyte development. *FOXO*: nuclear transcription factor; *MEF2*: myocyte enhancer factor-2.

in subcutaneous fat; yet they were higher in the pigs born from mothers with late pregnancy recuperation suggesting that leptin is programmed in utero.

By 2001, Thomas et al. [63] used the ewe adolescent model to analyze the patter of secretion of leptin during pregnancy. The ewes were subjected to a dietary regime from moderate to high, or high to moderate food intake. Leptin expression and protein were higher in those which were in the higher overfed group, suggesting that the produced leptin was a reflection of fat deposition in the mother due to overfeeding. Moreover, there was a negative correlation between maternal leptin levels and fetal and placental birth weight. In the fetal sheep, leptin has been known to modulate energy-expensive processes like angiogenesis and hematopoiesis. It is expressed mostly in the brain and liver, but it can also be found in fetal skeletal muscle, kidney, and perirenal adipose depot. Mothers might have a negative correlation with fetal weight; yet neonatal leptin levels do have a positive correlation between birth weights in humans, and while maternal nutrition modification reduces serum leptin, it does not affect fetal concentrations [64]. These findings were confirmed by Mühlhäusler et al. [65], who analyzed the effect of nutrient intake on leptin levels in pregnant ewes during the second half of pregnancy. They reported that increasing

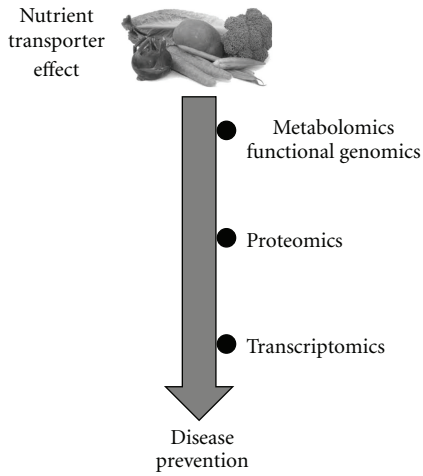

Figure 5: Nutrigenomics is a science that studies how food components exert their influence on metabolism pathways and manipulate genetic transcription, which can be used for disease prevention.

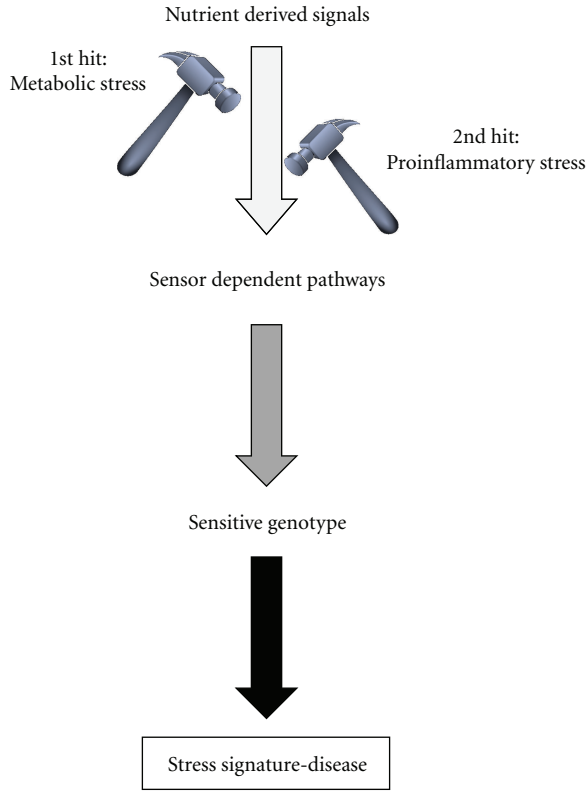

Figure 6: The basic idea with nutrigenomics is to determine a patient's polymorphisms and formulate a diet that ameliorates these genetic factors to minimum, regulating and maintaining weight loss.

maternal food intake elevated glucose but not leptin levels; yet leptin was a good surrogate for fetal adiposity and poses as a moderator of endogenous energy expenditure in the fetus. Once more, in 2003 the same research team published [66] that a moderate increase in maternal and fetal supply modulates leptin and UCP1 expression, explained by the progressive capacity of the unilocular adipose mass to synthetize the proteins; these results propose that lipid storage capacity is established during the prenatal period of life.

Sheep models have been used to also evaluate the in utero programming of glucose metabolism. Gardner et al. [67] studied the effects of early and late malnutrition in sheep and evaluated its effects on glucose metabolism at 1 year of age in the offsprings of such animal subjects. The study concluded that late gestation undernutrition (50% less of requirements) influences glucose homeostasis, especially when the maximal fetal growth is being achieved. Perhaps one of the most interesting findings is that if the fetus was under malnourishment since the beginning, there was no significant alteration in insulin sensitivity or in glucose tolerance. Yet, those who starved during the second half of the pregnancy had reduced GLUT4 expression, suggesting that metabolic modifications are tissue-specific. The previous trial was conducted during late gestation showing nonsubstantial findings on early pregnancy interventions, but Ford et al. [68] published otherwise. This group reported that malnourishment from early to midgestation in sheep is related to increased body weight, fat deposition, and glucose dysregulation compared to its counterparts in adolescence. Another conclusion was the biphasic effect of undernutrition which associates with late gestation fetal and postnatal catch-up growth, all consistent with the thrifty phenotype and the accumulating data on fetal low-weightness in relation to the onset of Diabetes Mellitus, associated with a rise in the adipogenesis signaling cascade previous to the onset of obesity [68].

The epigenetics involved in the "Small Baby" model include methylation changes in fundamental genes which control β-cell ontogeny and functional differentiation [39–41, 69–72]. The Pancreatic and Duodenal Homeobox 1 (*Pdx1*) is a transcription factor which regulates the developing growth of the bud that will become the pancreas. This factor shows progressive declining of transcription in intrauterine growth retardation (IUGR), and it is associated with epigenetic regulation via histone methylation. The GTP cyclohydrolase 1 (*Gch1*) is part of the folate and biopterin biosynthesis pathways and has been positively related to endothelial dysfunction observed in diabetes. PDX1 is in charge of modulating the expression of fibroblast growth factor receptor 1 (*Fgfr1*) which is involved in glucose homeostasis. In IUGR this gene is upregulated and it is related to vessel malfunction and fibrosis of the pancreas. Finally, IUGR is associated with lengthening of β-cell cycle, decreasing the number of mitosis, contributing to insufficient insulin production in the postnatal life. Survival of the fittest fetus requires downregulation and shut-down of several stress and energy sensors in liver and skeletal muscle, which is advantageous during pregnancy, but has deleterious effects after birth, because nutrient sensing and insulin sensitivity are compromised [73].

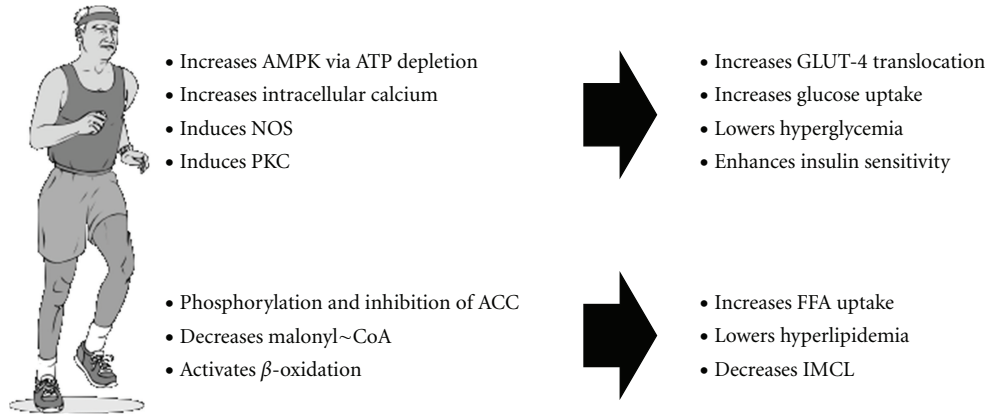

Figure 7: A few of the Metabolic benefits obtained through exercise.

On a final note, undernutrition in the fetus can alter the food network "wiring" providing the tools for metabolic disorders in the postnatal life. IUGR induces low levels of leptin, which is involved in the nerve fiber projecting towards the paraventricular nucleus from the POMC neuron population, which are the anorectic ones [74, 75]. On the contrary, maternal hypoxia and glucocorticoids exposure of the fetus enhances Neuropeptide Y (NPY) expression and secretion, having a role in fetal stress management and setting up the hypothalamus network to orexigenic signals [76, 77], especially since increased expression and release on NPY is maintained postnatally [78]. In simpler words, nutritional and hormonal factors may disturb the proper development of the hypothalamus and its subsequent function manifesting itself in eating habits-related disorders [79, 80].

There is another theory which was developed to explain the teratogenic ability of glucose—"*The Fuel-induced teratogenesis*". Freinkel et al. [81] proposed that fuel in the form of hyperglycemia, is the very cause of diabetic embryopathy and fetopathy associated with fuel excess. Various clinical findings have proven this theory, especially in certain aboriginal population like the Pima Indians [82–85] and the Pacific Islanders [86–88] who have the highest prevalence of Gestational Diabetes and macrosomia. Overnutrition is known to enhance physiological and epigenetic effects of glucose, resulting in chronic hyperglycemia, hyperinsulinemia and hyperleptinemia [89]. Fuel-induced cases are associated with maternal obesity and/or diabetes rendering a specific metabolic profile: maternal hyperglycemia, hyperinsulinemia, and low-grade inflammation.

Since insulin cannot cross the placental barrier, glucose is the main secretagogue in the fetal pancreas circa the 27th weeks of gestation. Skeletal muscle development is crucial to adult life since it is responsible for the majority of glucose and fatty oxidation rates. Macrosomic newborns have visceromegaly and high amounts of adipose tissue, but scarce development of the skeletal muscle, especially type II fibers which are responsible for the energy production/aerobic capacities of the muscle. In a fuel-inducing environment there is a mismatch between myogenesis and adipogenesis, with chronic inflammation as the culprit for this switch in differentiation [90–92] via inhibition of AMPK, downregulation of WNT pathways, and epigenetic modifications (see Figure 4).

2.2. Reversal of Fortune. Leptin has been considered the pivotal molecule according to animal and human analysis; yet its place was secured once it was proven that treatment with it reversed the developmental programming that occurs after maternal undernutrition during pregnancy [93]. Vickers et al. used 2.5 μg/g/day of leptin on rat pups born from malnourished mothers, from day 3 to 13 of life, resulting in normalization of caloric intake, voluntary motor activity, body weight, glucose, insulin, and leptin levels, reversing the prenatal programming [93], which seems to be gender-specific and dependent of pre- and postnatal nutritional status [94]. Alimentary intervention trials have also proven to be effective in reducing and/or preventing the adverse outcomes of reprogramming, as it was shown by Wyrwoll et al. [95] by using ω-3 fatty acids, and Zambrano et al. [96] by applying a dietary modification strategy prior to pregnancy.

In summary, the intrauterine environment is the key phase for acquiring the metabolic tools for surviving *inside the maternal womb*; the dilemma is what happens when the baby is born? Interestingly, these survival methods are not without risk for the unborn fetus, since each of the situations—IUGR and Macrosomia—carries a high risk for stillbirth and immediate neonatal death [97–99]; every change in the epigenetic make-up has a cost, and in the pediatrics world, it is a high one. Growth restricted babies are small for their gestational age, with small β-cell masses, hypoleptinemia, and showing signs of "*chronic hunger*", while the Overfed fetuses are chubby, above the 90th weight for their age, with elevated degree of fatness and decreased muscle development. Both situations revolve around leptin levels and AMPK intracellular signaling networks.

3. First Arrow: Nutrigenomics

> "*Nutrigenomics focuses on the effect of nutrients on the genome, proteome, and* ***metabolome***" [100].

Diet and exercise are fundamental pillars in the treatment of overweight and obesity and related conditions such as insulin resistance, cardiovascular disease, cancer, among others. Several types of diets have been developed focusing on determining a favorable macronutrient composition in order to reach certain metabolic states that induce body weight loss; see Figure 5 [101]. Initially, diet recommendations implied low-fat, moderate-protein, and relatively high carbohydrate content diets, based on the fact that high-fat diets induce less satiety [102] and that a diminishment in diet fat consumption reduced significantly the risk of cardiovascular disease by lowering circulating lipids [103]. However, a paradoxical phenomenon was observed; subjects on this diet began to gain weight, instead of losing it, especially in Western countries.

In response to the lack of effectiveness of these nutritional recommendations, new dietary alternative schemes are been developed. Dr. Atkins is one of the pioneers of the widely popularized low-carbohydrate diets, with a low glycemic index and high-fiber content [104]. These diets induce rapid body weight loss, increased satiety [105], and an associated reduction of cardiovascular disease and diabetes risk [106]. However, there are only a few long-term and adequately randomized studies to recommend this type of diet [107, 108]. In the past decade, several studies have been carried out to analyze the effect of a high-protein content diet in weight loss, demonstrating body weight reduction along with a higher maintenance of this weight loss [109, 110]. Interest in the development of these types of diets largely derives from the theoretical effect that diet composition can have on energy consumption as well as on food consumption [111]. Studies on the physiological effects of dietary composition in human population turned out to be complicated because of lack of compliance of the diet as well as accurate reporting, which is why several animal models have been developed in order to determine the effects of diet on metabolism and are the main source of information on this topic.

Recognizing the central role of AMPK in controlling energy balance, as a sensor of cellular energy quantum [112] the following question is formulated: *What are the effects of dietary components on the activity of AMPK?*

3.1. Lipids and AMPK. Although the exact mechanism linked to this phenomenon remains unknown, there is substantial evidence that high-fat diet is a risk factor that promotes obesity development, glucose homeostasis alterations, and cardiovascular system disorders [113]. The main metabolic manifestations of this diet are elevated free fatty acids, decreased intracellular fatty acid oxidation, and lipid accumulation on insulin-targeted organs [114]. A high-fat diet is correlated with a decreased expression of mRNA for the AMPK-α2 isoform as well as AMPK phosphorylation with consequent decreased activity of this enzyme in skeletal muscle, leading to decreased glucose uptake, meanwhile in adipose tissue it promotes preadipocyte differentiation, lipolysis and the secretion of adipokines (TNFα),perpetuating the process [115, 116].

AMPK has a crucial role in the hypothalamus' food intake control tower, constituting the signaling pathway of several hormones—including leptin—to regulate satiety. A high-fat diet induces hyperleptinemia which is associated with both peripheral and central leptin-resistance [117]. Reduced hypothalamic levels of leptin activity may be due, at least in part, to the constitutive alteration in the signaling pathway of AMPK. In the paraventricular nucleus of mice with diet-induced obesity, AMPK activity is constitutively diminished, and in the arcuate and medial nucleus of the hypothalamus leptin fails to suppress the activity of AMPK [118]. It has also been shown that long-chain fatty acid esters are able to inhibit AMPK kinase (AMPKK) and thus downregulate the signaling cascade of this pathway [119].

Eicosapentanoic Acid (C20:5 ω3, EPA) and Docosahexanoic acid (C22;6 ω3, DHA) exert prophylactic effects in cardiovascular disease, protect against insulin resistance and obesity in mice with high-fat diets, and improved insulin response in humans. The administration of polyunsaturated fatty acids has been shown to reduce the insulin resistance caused by high levels of saturated fats [120, 121]. Ingestion of diets rich in polyunsaturated fatty acids (PUFAs) has shown to suppress hepatic lipogenesis, lower TAG-rich lipoproteins synthesis in the liver, and increase fatty acid oxidation and induction of genes that regulate fatty acid oxidation (i.e., CPT1). A similar situation occurs in skeletal muscle where PUFAs increase thermogenesis, fatty acid oxidation, and glucose uptake. All these events are modulated by the action of AMPK [122, 123]. The mechanism by which PUFA may activate AMPK remains to be elucidated, but several hypotheses have been proposed such as an increase in the AMP/ATP ratio, decreased dephosphorylation of AMPK by control over the activity of protein phosphatase 2A (PP2A), or enhanced AMPK activity secondary to elevated plasma levels of adiponectin, IL-6, leptin and others [124, 125].

Conjugated linoleic acid (CLA), a group of linoleic acid isomer, has several physiological functions including anticancer properties, decreased atherosclerotic process, and modulation of the immune system [126, 127]. The CLA decreases the expression of AMPK-α2 and satiety and consequently decreases body weight [128].

Recent evidence suggests that short-chain fatty acids produced by fermentation of carbohydrates in the intestinal lumen could be absorbed and affect hepatic glucose metabolism [129]. The regulation of hepatic AMPK activity could play a critical role in this process; yet there is little data available on the effect of short-chain fatty acids on the activity of AMPK. In hepatocytes culture, acetate activates AMPK activity probably by increasing the rate AMP/ATP [130, 131]. Butyrate supplementation can prevent the development of insulin resistance in mice by promoting energy expenditure through the induction of mitochondrial function [132].

3.2. Carbohydrates and AMPK. The deleterious effect of a high-carbohydrate diet on health is well known. Using the current knowledge previously discussed here and the lack of effectiveness of low-fat diets, low-carbohydrate diets with

or without caloric restriction have been developed [133]. There is currently much interest in the potential role of foods glycemic index in the management of obesity and other metabolic disorders. It has been shown that foods with low glycemic index may be beneficial in regulating body weight in two ways: first by promoting satiety and secondly by increasing fatty acid oxidation, both explained by the action of AMPK [134]. However, a number of mechanisms that can be triggered by the glycemic index of foods consumed in the diet may explain the variability in the results of studies of weight loss using the glycemic index [135]. Glucose restriction provokes AMPK activation, resulting in the inhibition of anabolic processes which consume ATP such synthetic pathways for fatty acids, proteins, and cholesterol, and activation of catabolic processes which generate ATP to maintain cellular energy deposits like fatty acid and glucose oxidation [135].

AMPK regulates myogenesis and differentiation of skeletal muscle and preadipocyte differentiation into mature adipocytes, through SIRT1 (NAD-dependent deacetylase sirtuin-1 [*silent mating type information regulation 2 homolog 1*]) [136]. The loss of differentiation due to cellular starvation can be explained as a simple adaptation of a cell that is incapable to sustain the energy demanding activities that come with differentiation. Nevertheless, recent evidence suggests that there is a pathway activated in low-calorie cellular microambients, and it involves AMPK/Visfatina/SIRT1. Visfatin is a new adipokine which exerts insulin-like actions [137]. The pathway is characterized by AMPK-dependent—SIRT-mediated induction of Visfatin, resulting in elevation of the NAD+/NADH ratio, which induces genetic transcription shutdown [138, 139]. The beneficial effects of a low-carbohydrate diet have been observed in short-term trials, which warrant the need for long-term studies to fully evaluate and fully recommend this type of nutritional recommendation [140].

3.3. Proteins and AMPK. In the last 10 years, low-carbohydrate diets with high protein intake have become really popular. The evidence suggests that the main mechanism for its success is that high protein ingestion promotes weight loss by inducing thermogenesis and satiety [141]. Of course, it is not only the percentage of ingested protein but also the quality and aminoacid proportion which determines the loss-weight property [142, 143]. Proteins exert their effect in different manners, from the intestinal lumen with activation of chemoreceptors which respond to aminoacid/peptide presence releasing cholecystokinine (CCK), Glucagon-like peptide-1 (GLP-1), or peptide YY to a higher central level, modulating neurotransmitter release in middle cellular levelsregulating AMPK activity [143, 144].

A high-protein diet is capable of controlling food intake due to enhanced POMC expression and repression of NPY in the hypothalamus, via activation of mTOR and low phosphorylation rates of AMPK [145, 146]. Leucine affects AMPK pathway by inhibiting it, and in doing so, it activates the mTOR signaling pathway. Intraventricular injection of leucine in rats reduces food intake in a dose-dependent manner, and this effect is not observed with other aminoacids. Although this is true, weight reduction and food intake magnitude observe with the leucine treatment was similar to that achieved by a high-protein diet, which can explain why leucine is the most abundant aminoacid in most of the protein-rich formulated diets [146]. The exact mechanism for leucine AMPK-inhibiting activity is unknown; yet it probably relates to allosteric activation of the Glutamate Dehydrogenase (GDH) resulting in elevated substrate flux towards Krebs cycle (via glutamate conversion to α-ketoglutarate), lowering AMP/ATP ratio, and reducing AMPK phosphorylation [147].

Alternative medicine has been a huge source of natural products now used in obesity and insulin resistance treatment, but the vast majority of the cases lack scientific evidence which can vouch for its efficiency and the mechanism of action is usually unknown. The bitter melon, *Momordicacharantia* (Cucurbitaceae), is an Asian cultivated plant that is used as a herbal medicine and has gained fame for its hypoglycemic effects in animal models and humans [148]. Triterpenoids are the main constituent of the fruit but the active principle has not been found yet. In a recent study by Tan et al. [149], they reported that curbitanetriterpenoids (*Mormodicoside S*, and *karavilosede XI*) are capable of stimulating AMPK activity, favoring GLUT4 translocation, weight loss, and metabolic control. The Phytoalexin resveratrol (*trans*-revestrol) is naturally produced by bacteria or some fungi species. This polyphenol is capable of enhancing AMPK, SIRT1, and Peroxisome proliferator activated-receptor gamma coactivator 1-α (PGC1-α), reducing Insulin-like growth factor 1 levels enhancing insulin sensitivity [150, 151]. Berberine is an alkaloid found in a plant cultivated in Asia, used mainly in Korea and China for several diseases including diabetes treatment in humans [152]. Berberine acutely stimulates AMPK in myocytes and adipocytes, inducing GLUT4 translocation and lowering fat storage in adipose tissue. This substance also decreases the differentiation rate of preadipocytesby of PPARγ by $p38^{MAPK}$ phosphorylation, whose activity seems to be enhanced by berverine [153, 154].

There is no doubt that nutrition is fundamental to obesity development and other major chronic diseases like CVD and T2DM, and this is especially true in the pediatric population where scientists have seen a rise in caloric ingestion based on saturated fat and cholesterol with associated low energy expenditure. Several diet regimes have been devised; yet there are not enough trials to validate them [155, 156]. It is extremely necessary to design and undertake randomized trials to evaluate the nutrigenomic properties of food and apply them to individualized diets [157]; see Figure 6.

4. Second Arrow: Metformin

Even though there is growing evidence published every day concerning childhood obesity, the use of pharmacological treatment in them is still in controversy. The complexity

of this disease has delayed data gathering, most of which is extrapolated—not without difficulty—from large general population trials. It is noteworthy to add that there is no magical drug for weight loss, and most of the guidelines recommend pharmacological treatment after lifestyle modification and diet regime have failed to meet the primary goals, and that implementation of drug therapy has to be accompanied by diet and exercise [158, 159].

There are only 2 drugs FDA (Food and Drugs Administration) approved for obesity treatment in the pediatric population: Orlistat and Sibutramine [160]. Orlistat is a gastric and pancreatic lipase inhibitor, which lowers fat absorption favoring weight loss. Small pilot studies were undertaken in children and adolescents, measuring it is safety and tolerability. This drug was approved by the FDA in 2003 as treatment for obesity in children beyond 12 years of ages [161, 162]. On the other hand, Sibutramine is a serotonin-norepinephrine-dopamine reuptake inhibitor, which induces weight loss via appetite suppression, which is FDA approved for adolescents above 16 years old [163, 164]. Metformin is a molecule widely used in the treatment of T2DM in children above 10 years old. The drug's mechanism of action is still partially understood; yet in 2001 there was a major breakthrough when Zhou et al. [165] observed that AMPK plays a fundamental role in this puzzle. Since neither Orlistat nor Sibutramine modulates AMPK, we will analyze the weight reduction effect of metformin applied to obese children and adolescents.

As was mentioned previously, metformin is used in T2DM due to its insulin sensitizing effects, which are efficient in the treatment of Polycystic Ovary Syndrome, T2DM, and Hepatic Steatosis. These applications have revealed that patients obtain and maintain weight reduction, making the science community take a closer look at metformin and its influence in appetite control [166, 167]. Freemark and Bursey [168] was one of the first researchers to acknowledge metformin's efficiency on weight loss in a double-blind case-controlled trial with 29 adolescents who had fasting hyperinsulinemia and T2DM family history, using 500 mgs twice a day versus placebo tablets for a period of 6 months. The study reported a 0,12 decrease SD in BMI, while there was an increase Standard Deviation (SD) of 0,23 in the placebo group. Similar findings were published by Srinivasan et al. [169] who conducted a randomized controlled trial comparing metformin (1 gram twice a day) versus placebo in 28 obese patients between 9–18 years old. The metformin group had the highest reduction in BMI ($-1.26\,\text{Kg/m}^2$), in weight ($-4.35\,\text{kg}$), waist circumference ($-2.8\,\text{cms}$), and in fasting insulin levels ($-2.2\,\text{mU/L}$). In 2008, Burgert et al. [170] published their results on the cardiometabolic benefits of metformin in 28 morbidly obese adolescents patients, who were randomized and divided into 2 groups, one receiving metformin 1,500 mgs daily ($n = 15$), and the placebo group for 4 months. The placebo group had elevation of BMI ($1.1\,\text{kg/m}^2$); meanwhile, the treatment group had reduction in this criteria ($-0.9\,\text{kg/m}^2$). Compared with placebo, the metformin group had enhanced insulin sensitivity, subcutaneous (but not visceral) fat reduction—data which dissents from the one reported by Srinivasan—and obtained cardiovascular function improvement.

Love-Osborne et al. [171] investigated the effects of metformin in a lifestyle modification program in insulin resistant adolescents. This was a randomized, controlled double-blind trial with 85 obese adolescents with insulin resistance who were divided into 2 groups, placebo and treatment group who received 850 mgs twice a day. The patients who finished the study and who maintained the treatment had the highest BMI reduction, with an estimated BMI reduction of 5% or more. Casteels et al. [172] studied 42 obese teenagers with motor deficit who had low physical fitness and elevated body fat. This study was conducted to analyze metformin's weight control properties compared to placebo. Six months of therapy offered weight and BMI reduction, due to lower visceral fat. Finally, earlier this year The Glaser Pediatric Research Network Obesity Study Group [173] did a 48-week Metformin Extended Release (1 gram twice a day) trial in 77 obese adolescents(13–18 years old), concluding that the drug caused significant BMI reduction, and this effect lasted after 12–24 weeks of treatment cessation.

Even though the studies are promising, they have the disadvantage of being conducted with small patient samples for short periods of time. In this matter, the REACH-Activity and Metformin Intervention in Obese Adolescents study is underway [174], applying a 2-year protocol with metformin (1,500 grams daily) and with physical activity. The recruitment is focused on obese patients between 10–16 years old alongside their parents, which will be randomly controlled and assigned drug or placebo. The preliminary results are expected in the end of 2010 with the complete results of this intervention to be published in 2012.

5. Third Arrow: Physical Activity/Exercise

Perhaps the grayest area in childhood obesity is how much is enough physical activity in the child and adolescent because several factors are at play here: age, gender, geography and seasoning, race, psychological factors, ecological factors, sociocultural determinants, and school activities, among others [175]. Physical activity in the pediatrics population is not only beneficial to cardiometabolic health, but it also serves as a psychological tool to improve behavioral well-being and lower the incidence of drugs, smoking, early sexual activity, and other problematic habits, shaping the lifestyle pattern the child will have as a grown adult. Overweight children and adolescents are prone to acquire risk factors associated with CVD, are likely to become obese adults, predisposed to inappropriate dieting practices like anorexia or bulimia, physical inactivity, alcohol, and tobacco use, have obesity-related health issues like asthma, sleep apnea, T2DM and nonalcoholic hepatic steatosis, and finally, are at high risk for long-term chronic disease, like stroke, cancer, and gall bladder disease [176].

Physical activity can be defined as any movement produced by the skeletal muscles which ends in energy consumption and can be measured in kilocalories [177].

Another definition is any daily activity of at least 30 minutes (occupational, leisure, home-related), which can be vigorous or moderate, planned or unplanned, and is inserted in the daily lifestyle of the subject [178]. Exercise is immerged in the concept of physical activity, and the difference between them relies in the very fact that exercise has a physical fitness purpose [177, 178]. How much or what type of exercise can be recommended for the patients is still in controversy, since it depends on comorbidities, age, psychological status, and aerobic capacity [179].

5.1. Growing Up. One of the main issues about pediatric obesity is the age of appearance and growth sprouts throughout adolescence until adulthood. We have already discussed the intrauterine milieus in previous sections, and 3 outcomes can be concluded: the growth restricted, a normal weight, and the macrosomic newborns. The small baby and the big baby are at high risk for early infant growth which is associated with subsequent obesity in adulthood [180]. In babies fed with milk-formulas, rapid weight gain during the first weeks of life is determinant for obesity several decades later [181]; meanwhile breast-fed babies are inversely correlated with adult obesity regardless of the mother's weight or glucose tolerance, highlighting the protective effect of breast milk [182]. BMI increases steadily and rapidly as the baby grows into a toddler and towards infancy reaching a minimum plateau at around 5–6 years of age, which is denominated *BMI rebound* (or adiposity rebound (AR)). Several studies have linked early rebound to increase risk of obesity in later life [183–186], especially in children who were tall at 3 years of age [187]. The third critical period phase is adolescence, which is elementary to determine the probability of adult obesity. During this phase, gender takes its toll in risk assessment, and girls are in a bit more danger than the boys to become obese, since sexual maturation comes with fat increase. Around 80% overweight adolescents will become obese adults, and in light of this data, intervention measures must be installed.

Adiposity rebound has gathered a fair amount of attention since the mid 80s when the first associations were made with obesity [188]. The phenotype of early rebounders is characterized by different velocities for height and weight gaining, varying in each child, with basic features as advanced bone age [189], early menarche [190], and later obesity [191]. Whitaker et al. [192] conducted a retrospective cohort study with 390 patients and their parents, reporting that the mean age for AR was 5,5 years, and by adulthood, 15% of the subjects were obese—with higher rates in those who were earlier and heavier rebounders and those who had heavier parents. The following year, Eriksson et al. [193] published that the highest death rates occurred in boys who were SGA at birth but had an early catch-up phase with normal or above average rates when compared to normal counterparts. Later, Velasquez-Mieyer et al. [194] undertook a cohort study analyzing 25 risk factors for AR, determining that 8 were the most prevalent among 909 subjects from Great Britain: parental obesity, very early AR (by 43 months), more than 8 hours spent watching television per week at the age of 3, presence of catch-up growth, weight gain during the first year, birth weight, short sleep duration at age 3, and standard deviation score at age 8 and 18 months. These features are in agreement with factors used to identify high-risk youngsters, as published by Velasquez-Mieyer et al. [194]: placental insufficiency, gestational diabetes, maternal overweight/obesity and weight gain during pregnancy, SGA, LGA, infant overnutrition, bottle feeding, drug induced weight gain, early AR, and overweightness during adolescence.

Yet, the question rises, during which phase must intervention be applied? Several studies have proven that BMI rebound is in fact the most critical and determinant phase of the 3, and measures should be taken to improve adiposity in this children [187, 189, 192, 195–197], since early intervention is the best option to void adolescence obesity, and later adult obesity. According to the Physical Activity and Health CDC report [198] all people above 2 years of age should do at least 3 minutes of endurance-type of physical activity, at least of moderate intensity, preferably every day of the week. The problem in this statement is that children below 6 years old might not comprehend the object of physical activity; so planned sequential exercise should be substituted with programmed school activities which guarantee moderate activity for over 30 minutes. Young children are not physically adept to be subjected to adolescent or adult physical activity regimes; therefore the guidelines must be developed in accordance with this fact. The Framingham Children' Study [199–201] revealed several aspects that need to be addressed during this period: parental involvement in the physical activities and nutritional intervention, elevated TV viewing time, and nutrient tracking.

5.2. The Benefits of AMPK. Exercise has several advantageous properties which makes it appropriate to include it in the day-to-day life of a high-risk child/adolescent. The primary effects of exercise include enhanced GLUT4 translocation via insulin-independent pathways which include Nitric Oxide synthesis and AMPK activation (see Figure 7). AMPK is activated via phosphorylation of Thr^{172} by AMPK kinase or Calmodulin dependent kinase (CaMKK). Once this enzyme is activated, it phosphorylates atypical Protein Kinase C (PKC) which stimulates phosphatase that will act upon IRS1, leaving the insulin pathway unopposed to induce Akt and glucose transporter translocation. Moreover, AMPK induces the expression of GLUT4 gene through MEFA2A and MEFA2D (factor 2a myocyte enhancer). During AMPK activation, it will inhibit AcetilCo~ACarboxilase 2 (ACC2), lowering Malonyl~CoA concentrations, spiking β-oxidation of fatty acids [202].

It has also been suggested that exercise exerts anti-inflammatory effects which are effective in aiding the low-grade inflammation that characterizes adult obesity [203], and it is not different in childhood/adolescent obesity [204, 205]. Weiss et al. [206] published that adiponectin inversely correlates with C-Reactive Protein (CRP) levels, while the latter and IL-6 were in direct relation to BMI. In children

between 8–16 year olds, being overweight is associated with elevated white blood count and CRP suggesting low-grade inflammation in these children [164]. Hypoadiponectinemia has been independently associated with metabolic syndrome in teenagers, which has upgraded adiponectin to a high CVD risk [207, 208], alongside with IL-6, and IL-18 [209–211]. When skeletal muscles exercise, they release a 100-fold of IL-6, with a concomitant release of IL-10 and IL-1 receptor antagonist [212, 213], serving as powerful immune modulators that interfere with the metainflammation that characterizes obesity.

6. Conclusions and Recommendations

Obesity in the pediatrics population has been a major public concern since the late 1970s [214]. Several steps have been taken to undermine the situation, but somehow it has gotten out of hand [215]. The Healthy People Program has a primary goal 5% or less prevalence of obesity in children and adolescents, increases school nutrition education, and increases the number (>75%) of primary care providers who are able to handle overweight reduction services [216]. Behavioral and motivational interventions have to go together with any of the primary tools: diet, exercise, and/or drug. The sticking to the program depends highly on this aspect, since children and adolescents are susceptible of depression, anxiety, impaired family functioning, poor psychological adaptation, and eating disorders behavior [216–218].

Understanding the etiology of a disease is the best way to conquer it: attacking key points in the natural history will help eliminate the progression of the disease. In this light it has been proposed that BMI rebound is the key aspect in high-risk children; nevertheless, the other two stages (prenatal and adolescent) are still important; yet the prenatal state depends on the mother's condition and comorbidities, while the adolescence phase is modulated by sexual hormones which will inevitably take its toll. So, success at halting obesity will be achieved with early interventions during infancy. School-associated programs for physical activity seem to be gaining popularity because they have been shown to reduce weight gain, visceral adipose tissue, and reduces remission of obesity [219–221]. Mallare et al. [222] recommended physical activity of 30–60-minute duration a day and lowering sedentary activities to less than 2 hours a day.

In the era of the "*omics*", each treatment has to be individualized as much as possible, since each patient is a new case. A full history chart and complete blood and anthropometrical work-up will help to assess the main factors involved in the progression of the disease. Nevertheless, AMPK seems to be involved in virtually every aspect of the disease, serving a pivotal role. There is little doubt that common obesity is a multifactorial disease, and targeting the major intracellular components will benefit weight loss management and continuum of the program. More studies are needed to fully approve the use of Metformin as a weight-loss drug, like Orlistat and Sibutramine; yet its pharmacological properties make it the drug of choice in obesity treatment. A nutriogenomically guided diet will aid in the reorganization of the cellular energy status, and exercise will enhance glucose uptake and lipid oxidation, improving the metabolic profile of these youngsters.

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Review Article

Pharmacological Treatment of Obesity in Children and Adolescents : Present and Future

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The prevalence of overweight and obesity is increasing in children and adolescents worldwide raising the question on the approach to this condition because of the potential morbidity, mortality, and economic tolls. Dietetic and behavioral treatments alone have only limited success; consequently, discussion on strategies for treating childhood and adolescent obesity has been promoted. Considering that our knowledge on the physiological systems regulating food intake and body weight is considerably increased, many studies have underlined the scientific and clinical relevance of potential treatments based on management of peripheral or central neuropeptides signals by drugs. In this paper we analyze the data on the currently approved obesity pharmacological treatment suggesting the new potential drugs.

1. Introduction

The report from the International Obesity Taskforce (IOTF) on worldwide prevalence rate shows that the pediatric obesity epidemic has spread globally. The worldwide prevalence of overweight in children and adolescents is approximately 10% with many western countries approaching 30%. In fact, some countries in economic transition have also prevalence rate increase higher than those in the United States (US) [1–3].

Because of the number of the subjects, obesity is now recognized as a healthcare issue on an epidemic scale in both adult and pediatric populations, but it yet remains an unsolved medical problem [4].

The successful management of obesity is theoretically possible through lifestyle changes including diet modifications [5] and increased physical activity. The literature analysis demonstrated, however, that significant results were obtained only in a limited number of subjects and for a relatively short time period: also the management with psychological involvement let the problem substantially unsolved. These considerations recently promoted an interest in pharmacological interventions and bariatric surgery [4].

The renewed interest for a pharmacological approach depends on the knowledge of physiological systems involved in the control of food intake and body weight that has considerably increased over the past decade. A powerful and complex physiological system, based on both afferent and efferent signals, regulating food intake and energy homeostasis, has been elucidated. This system consists of multiple pathways with redundancy signals that are transmitted by both blood and peripheral nerves, which are integrated in brain centres with subsequent regulation of central neuropeptides which in turn modulate feeding and energy expenditure. Appetite includes different aspects of eating patterns, such as frequency and size of eating, choice of high-fat or low-fat foods, energy density of consumed foods, variety of accepted foods, palatability of diet, and variability in day-to-day intake. Feeding behavior is controlled by a series of short-term hormonal, psychological, and neural signals. All signals act at several central nervous system (CNS) sites, but the pathways converge on the hypothalamus, a central region of feeding regulation, containing numerous peptides and neurotransmitters that influence food intake [6]. CNS also regulates energy homeostasis on the basis of peripheral signals from the gastrointestinal tract (GIT) and adipose tissue.

Table 1: Neurotransmitters influencing appetite.

Neurotransmitters that increase food intake	Neurotransmitters that decrease food intake
Agouti-related peptide	Alpha melanocyte-stimulating hormone
Neuropeptide Y	Bombesin-/gastrin-releasing peptide
Melanin-concentrating hormone	Calcitonin gene-related peptide
Orexin	Cholecystokinin
Galanin	Corticotrophin-releasing factor
Ghrelin	Dopamine
Nitric oxide	GABA
Noradrenaline	Glucagon
Opioids (particularly μ and κ agonists)	Glucagon-like peptide 1 (7–36) amide
	Neurotensin
	Serotonin

In this paper, we summarize the currently approved pharmacological treatment for children and adolescents, and we are going to provide an overview of developing drugs.

2. Pharmacological Treatment of Obesity

Current and putative antiobesity drugs share the same fundamental principles as treatment in adults, that is, to decrease caloric intake and increase energy expenditure, miming the effects of some anorectic neuropeptides or contrasting the orectic ones in order to regulate energy balance (Tables 1 and 2).

However, the primary goal of overweight/obesity treatment (i.e., weight reduction or deceleration of weight gain) and the recommended way of intervention are variable and dependent on the child's age and level of overweight, among other considerations. In order to support clinicians in determining the most appropriate form of treatment, pediatric weight management guidelines exist in many countries to promote best practice, but at present many of these recommendations are based on low-grade scientific evidence.

A recent guideline suggests considering pharmacotherapy in

(1) obese children only after failure of a formal program of intensive lifestyle modification;

(2) overweight children only if severe comorbidities persist despite intensive lifestyle modification, particularly in children with a strong family history for type 2 diabetes or premature cardiovascular disease. Pharmacotherapy should be provided only by clinicians who are experienced in the use of antiobesity agents and aware of the potential for adverse reactions [7].

Up to now, only three drugs have been reported to reduce weight and/or body mass index (BMI) in adolescents: (1) *sibutramine*, a neurotransmitter reuptake inhibitor which enhances satiety by inhibiting the reuptake of serotonin, norepinephrine, and dopamine, (2) *orlistat*, a pancreatic lipase inhibitor which reduces fat absorption, and (3) *metformin*, an antihyperglycemic and insulin-sensitizing agent (Table 3).

At present, there are only few drugs approved by the Food and Drug Administration (FDA) for the treatment of adult obesity. The most important ones are sibutramine and orlistat. The FDA in the US approved the latter drug in 2003, and it has recently been approved by the European Union for the treatment of adolescents.

3. Current Available Drugs

3.1. Drugs Affecting Peripheral Mechanisms

3.1.1. Orlistat. Orlistat, approved since 1998, is an inhibitor of pancreatic lipase reducing dietary fat absorption. The compound is a partially hydrated derivative of an endogenous lipstatin produced by *Streptomyces toxytricini* [19].

Orlistat binds irreversibly to the active sites of lipase through covalent binding. Approximately one-third of triglyceride intakes does not undergo digestion and is not absorbed by small intestine, crossing the GI tract and being eliminated. Because of low systemic absorption and first-pass metabolism, the bioavailability of orlistat is <1%; most of the drug being excreted remains unchanged with stools [20].

In adults, orlistat has a good safety profile, is generally well tolerated, has minimal systemic absorption, and determines clinically meaningful and sustained decreases in weight and BMI when combined with a mildly hypocaloric diet and exercise. It is approved for weight management in overweight and obese adults in more than 120 countries, and to date more than 22 million patients have received this drug. Based on clinical and safety characteristics in adult populations, it was considered a logical choice for study in the obese pediatric population because of the nonsystemic mechanism of action.

FDA in December 2003 approved orlistat use in adolescents aged 12 to 18 years old with a BMI (kg/m^2) > 2 units above the reference value at the 95th percentile for age and gender. This conclusion was based on the results of a one-year study evaluating 539 American adolescents submitted to a hypo-caloric diet plus exercise and behavioral therapy and that were randomized to orlistat versus placebo: a significant decrease in BMI was shown in the orlistat group (0.55 versus 0.31 kg/m^2 for placebo; $P < .01$) [8]. Moreover, body composition analysis showed that orlistat did not affect the normal increase in lean body mass physiologically observed in adolescents, and the weight difference between the placebo and orlistat groups was due to a difference in fat mass. The use of orlistat for 1 year in this adolescent population did not raise major safety issues although GIT adverse events, such as fatty or oily stools, oily spotting, increased defecation, cramps, and abdominal pain, were more common in the orlistat group.

Chanoine et al. also measured estradiol levels, cardiovascular effects, gallbladder structure, renal structure, bone

Table 2: Selected GI, pancreatic, and adipose tissue peptides that regulate food intake.

Peptide	Main site of synthesis	Receptors mediating feeding effects	Effect on food intake
CCK	Proximal intestinal I cells	CCK1R	inhibition
GLP-1	Distal intestinal L cells	GLP-1R	inhibition
Oxyntomodulin	Distal intestinal L cells	GLP-1R and others	inhibition
PYY	Distal intestinal L cells	Y2R	inhibition
Enterostatin	Exocrine pancreas	F1-ATPase beta subunit	inhibition
APO AIV	Intestinal epithelial cells	Unknown	inhibition
PP	Pancreatic F cells	Y4R, Y5R	inhibition
Amylin	Pancreatic beta cells	CTRS, RAMPS	inhibition
GRP and NMB	Gastric myenteric neurons	GRPR	inhibition
Gastric leptin	Gastric chief and P cells	Leptin receptor	inhibition
Ghrelin	Gastric x/a-like cells	Ghrelin receptor	stimulation
Insulin	Pancreatic beta cells	Insulin receptor	inhibition
Leptin	Adipocytes	Leptin receptor	inhibition
Adiponectin	Adipocytes	Adiponectin receptor	inhibition

CTRs: calcitonin receptors; RAMPs: receptor activity-modifying proteins; GRP: gastrin-releasing peptide; NMB: neuromedin B; GRPR: GRP receptor.

mineral content/density, and other non-GIT adverse events [8]. Girls in the orlistat group had a statistically significant decrease in estradiol concentrations compared with a slight increase shown in the placebo intervention. Ten participants in the orlistat group and one participant in the placebo group developed abnormalities during the study that were detected on electrocardiograms; none of these was believed to be related to the medication based on review by an independent cardiologist. No other adverse cardiovascular effects were found. At the end of the study, six participants in the orlistat intervention (compared with one participant in the placebo intervention) were found to have asymptomatic gallstones not seen at baseline; five of these patients had lost large amounts of weight (8.2–29.4 kg), and two were siblings. Another patient had multiple gallstones on ultrasound at day 167 after a 15.8 kg weight loss and had a subsequent cholecystectomy. Ultrasound also identified two additional new renal abnormalities in the orlistat intervention group (mild left hydronephrosis and 6 mm echogenic focus without evidence of renal calculus). There were no differences in bone mineral content/density between the two interventions.

Most other non-GIT adverse events were also more prevalent in the orlistat group compared with the placebo group, but the difference between groups was less pronounced than for GIT adverse events; the most common adverse events in this category were headache, upper respiratory tract infection, and nasopharyngitis [8].

As in adults, 120 mg three times daily prescribed with meals are usually necessary to produce a significant effect. Coprescription of a daily multivitamin (A, D, E, and K) is recommended to prevent possible deficiencies of fat-soluble vitamins, due to the interferences with the absorption of them.

Orlistat should be avoided in patients with chronic diarrhea [4, 20, 21]. Moreover, it can reduce the absorption of amiodarone [22], and ciclosporin [23] and can increase warfarin's action [20, 24]. Systemic adverse effects are minimal because of the lack of systemic absorption.

3.1.2. Metformin. Metformin is an effective oral hypoglycemic agent used in the treatment of adults with type 2 diabetes and other conditions with insulin resistance [25–27]. Its hypoglycemic effect is largely caused by inhibition of hepatic gluconeogenesis, increased insulin-mediated glucose disposal, and inhibition of fatty acid oxidation. Reduction of intestinal glucose absorption has been hypothesized as another possible mechanism of action, although data have been inconsistent [28]. The exact mechanism of intracellular action of metformin remains uncertain. Metformin is poorly metabolized, and 90% of the absorbed drug is eliminated as unchanged in urine. Plasma protein binding is insignificant, so the drug is dialyzable.

Metformin therapy for insulin resistance and obesity is safe and well tolerated and has a beneficial effect on weight, BMI, waist circumference, abdominal fat, fasting insulin, and fasting glucose although 6 months of therapy may not be sufficient to have an effect on visceral adipose tissue loss and insulin sensitivity [29]. Metformin is currently being used in more than 90 countries worldwide.

According to recent studies, the major effect of metformin may be a powerful inhibition of appetite under the light of the well-known role of weight excess on hypertension, type 2 diabetes, and dyslipidemia [30]. Because the major component of metabolic syndrome is weight excess, by contrasting it, this drug can also be able to prevent most of its consequences on health.

The potential clinical application of metformin in the pediatric population was first described in the 1970s in a small published study demonstrating the beneficial effect on weight and insulin concentrations in 8–14-year-old obese children [31]. Subsequent pediatric randomized, controlled trial studies have shown improvement in BMI, fasting serum glucose, insulin, and lipid profile in patients on metformin therapy for exogenous obesity associated with insulin resistance [9–11]. Participants received metformin for 6 months at a daily dose of 1 g (0.5 g twice daily) [11] or 2 g (1 g twice daily) [9]. There were no withdrawals due to adverse

Table 3: Summary of studies about orlistat, metformin, and sibutramine in children and adolescents.

Authors and year	Drug	Population treated	Age (years)	Duration	Effects on BMI or other metabolic effects	Adverse events
Chanoine et al. 2005 [8]	Orlistat	357 obese adolescent	12–16	1 year	BMI: −0.55 SD	Mild to moderate gastrointestinal tract adverse events (9%–50%)
Srinivasan et al. 2006 [9]	Metformin	28	9–18	6 months	BMI: −1.26 Kg/m^2 ($P = .002$); waist circumference: −2.8 cm ($P = .003$); fasting insulin: −2.2 mU/liter ($P = .011$)	Nausea
Kay et al. 2001 [10]	Metformin	24	15.6 ± 0.4	8 weeks	Body fat: −6.0 ± 0.62; fat-free mass was similar in metformin group and placebo. *Enhances* insulin sensitivity, significant reduction in plasma leptin, cholesterol, triglycerides, and free fatty acid.	Nausea, dizziness, and stools
Freemark and Bursey 2001 [11]	Metformin	29	12–19	6 months	BMI: decline of 0.12 SD and a 5.5% reduction in serum leptin in girls. Metformin caused a progressive decline in fasting blood glucose and a reduction in fasting insulin levels.	Transient abdominal discomfort or diarrhea
Jones et al. 2002 [12]	Metformin	82	10–16	16 weeks	Improved glycemic control, the adjusted mean change from baseline in fasting plasma glucose was −2.4 mmol/L. Mean HbA_{1c} values was significantly lower.	Gastrointestinal side effects (diarrhea)
Berkowitz et al. 2003 [13]	Sibutramine	82	13–17	6 months	BMI: −8.5%	Elevated blood pressure and/or pulse rate, ventricular premature beats, cholelithiasis, ecchymoses, and rash
Berkowitz et al. 2006 [14]	Sibutramine	498	12–16	12 months	BMI: −2.9 Kg/m^2; body weight: −8.4 Kg ($P < .001$ for both); greater improvements in triglyceride levels, high-density lipoprotein cholesterol levels, insulin levels and sensitivity	Tachycardia
Garcia-Morales et al. 2006 [15]	Sibutramine	46	14–18	6 months	BMI: −9.2%	No significant difference in blood pressure, tachycardia, headache with nausea, or weakness
Godoy-Matos et al. 2005 [16]	Sibutramine	60	14–17	6 months	The mean BMI reduction was greater in the sibutramine group: 3.6 ± 2.5 Kg/m^2	No significant difference in blood pressure or heart rate

Table 3: Continued.

Authors and year	Drug	Population treated	Age (years)	Duration	Effects on BMI or other metabolic effects	Adverse events
Van Mil et al. 2007 [17]	Sibutramine	24	12–17	12 weeks	Effect on BMI-SDS not significant	Abdominal complaints, insomnia, headache, loss of interest, and loss of appetite
Daniels et al. 2007 [18]	Sibutramine	498	12–16	12 months	BMI reduction was > or = 5%	Small mean decreases in blood pressure and pulse rate were seen in both sibutramine and placebo

events in either of the study; however, medication dose was lowered due to nausea in three participants (Freemark (2001) $n = 1$; Srinivasan (2006) $n = 2$). In both studies, there was no adverse effect on serum lactate, measures of liver, or renal function. Freemark et al. reported that there were no episodes of vomiting or lactic acidosis. Srinivasan et al., demonstrated that there was no biochemical evidence of metformin toxicity. In one study, three metformin-treated patients and one placebo patient complained of transient abdominal discomfort or diarrhea that resolved within the first one to two weeks of therapy, and another participant may have had an exacerbation of migraine [11].

Metformin treatment has been approved in children older than 10 years. The beneficial role of this drug in young patients with type 2 diabetes has been demonstrated in a randomized, controlled trial [12].

3.2. Drugs Affecting Central Mechanisms

3.2.1. Serotonin and Noradrenaline Reuptake Inhibitors (SNRIs). Anorectic medications targeting the 5-HT system are typically derived from beta-phenethylamine and mediate their effects by influencing noradrenergic, dopaminergic, and serotonergic neurotransmission [4].

Sibutramine. Sibutramine was originally developed as an antidepressant, and it is a centrally acting monoamine reuptake inhibitor that mainly acts to increase satiety [32]. Its most important mechanism of action is to block norepinephrine and 5-HT re-uptake, but it also stimulates thermogenesis that plays a minor role in weight reduction [33]. Sibutramine undergoes extensive first-pass metabolism, mainly by hepatic cytochrome p450 3A4 enzymes, toactivate primary and secondary amine metabolites that are more potent than the parent compound. The drug and its active metabolites are mainly excreted through the kidney [20].

Sibutramine was approved for adults in the US in 1997 and in the European Union in 1999. Consumer advocacy group Public Citizen campaigned to have sibutramine withdrawn from the market in the US, and two deaths in Italy led to an international investigation in 2002 and temporary removal from the market. In the US, sibutramine may be used in adolescents older than 16 years [34].

The addition of sibutramine to a behavior therapy program resulted in statistically significant improvements in BMI [13–17]. Berkowitz et al. reported that 7–12 months of sibutramine treatment decreased BMI by 2.4%, respect to placebo [13]. In an RCT including 498 adolescents the same authors also showed a reduction of BMI and body weight, and an improvement of metabolic profile in the sibutramine-treated group [14].

Godoy-Matos et al. studied 60 Brazilian adolescents and documented that approximately 25% of adolescents assigned to the sibutramine group reduced their initial weight by at least 15% in comparison to 0% in the placebo group ($P < .001$) [16].

A common landmark is that the efficacy of sibutramine is greatly enhanced when used with intensive lifestyle modification and behavior therapy [13–17].

The most common adverse effects found in adolescents taking sibutramine was tachycardia, even if generally it was not a reason to withdraw from treatment [13–16]. Other common side effects include insomnia, elevation of blood pressure, headache, dizziness, dry mouth, and constipation. Long-term data on the effect of sibutramine on major obesity-related morbidity are lacking. However, the ongoing Sibutramine Cardiovascular Outcomes trial (SCOUT) is assessing the efficacy of sibutramine in reducing myocardial infarction, stroke, and cardiovascular mortality in 9000 obese and overweight patients [35]. It is important to notice that sibutramine effects on heart rate and blood pressure in obese adolescents are generally neutralized by the effective reduction in BMI that seems to be even better than that observed in adults [18].

Phentermine. Phentermines' use is only approved for short-term treatment of obesity in adults of up to 3 months duration due to the lack of long-term studies [36].

It has been demonstrated that, in subjects treated with phentermine, the weight loss was greater (from 2 to 10 kg) than in those receiving placebo. Patients treated with phentermine at the dosage from 15 mg/d to 30 mg/d lost an average of 3.6 kg (95% CI, 0.6 to 6 kg) of additional weight compared with placebo (the duration of treatment with phentermine varied from 2 to 24 weeks). The authors concluded that treatment with phentermine, in addition to lifestyle interventions, resulted in a statistically significant but modest increase in weight loss. In 1961–1963 Lorber carried out a trial including 84 children aged from 3 to 13 years to evaluate the effects of this new drug compared to placebo. Only 68 subjects completed the entire course of 12 weeks, divided into three period, of 4 weeks, during which three

different types of treatment were prescribed (amphetamine resinate, phentermine, and diet with placebo). Authors concluded that phentermine was somewhat more efficient than both diet or the lower dose of amphetamine resinate, with very few side effects; however, the effectiveness tended to wear off after several months of treatment [37].

Side effects, since phentermine is a sympathomimetic amine, can be expected and include insomnia, dry mouth, constipation, restlessness, euphoria, nervousness, increased pulse rate, and blood pressure. The use of noradrenergic agents is thus not indicated in patients with cardiovascular disease, hypertension, and history of drug abuse or in those taking monoamine oxidase inhibitors [21].

3.2.2. Selective Inhibitors of Serotonin Reuptake. Fluoxetine and sertraline are selective inhibitors of 5-HT reuptake despite different chemical structures. Fluoxetine is a phenyl-propanolamin oxy-3-fluorphenyl derivate, and sertraline is a naftilaminic one. They inhibit 5-HT re-uptake at the presynaptic terminal, and their main indication use is in the treatment of depression and bulimia. They are not formally indicated to treat obesity [38], but fluoxetine was used in adult patients to control hyperphagia, and consequently obesity correlated with syndromic condition, such as Prader-Willi syndrome [39].

These agents were found to reduce feeding in experimental animals. In humans, protocol studies, performed to approve these medications as antidepressants, demonstrated weight loss in treated subjects. The key problem in managing these agents is weight regain. Generally, after the first 6 months of treatment, body weight is gradually recovered, although medication is maintained. Fluoxetine therapy for obesity management has also been associated with GIT symptom, sleep disorders, reduced libido, sweating, amnesia, and thirst [40]. Until now no trials exist about their use to treat childhood obesity.

3.2.3. Octreotide. Octreotide is a synthetic, eight-amino-acid analogue of the natural hormone somatostatin. Like somatostatin, octreotide limits β-cell insulin secretion by inhibiting the G0 protein associated with the widening of the voltage-gated calcium channel [41]. It also inhibits secretion of gastric acid, pancreatic enzymes, and bile, prolongs intestinal transit time, and decreases gallbladder contractility. Somatostatin and octreotide inhibit the release of pituitary and gastro-entero-pancreatic hormones such as growth hormone (GH), thyroid stimulating hormone (TSH), glucagon, cholecystokinin (CCK), vasoactive intestinal peptide (VIP), gastrin, and ghrelin [42–44].

Octreotide has been previously shown in both pilot [45] and placebo-controlled trials [46] to be effective in reducing insulin secretion, weight, and BMI in children with hypothalamic obesity. In a pilot study, including obese adults, suppression of insulin secretion was achieved after a 24-week treatment with octreotide long acting (40 mg), and it was associated with both body weight and fat mass loss [47].

The most common adverse events were diarrhea, headache, cholelithiasis, nausea, and abdominal pain [48]. The parenteral administration is one of the major problems in long-term treatment.

3.2.4. Caffeine/Ephedrine. Lipolysis in white and brown adipocytes is activated by sympathetic stimulation of β-adrenoreceptors. In rodents, the β3-adrenoceptor plays the dominant role in these cells. This partly explains why β3-adrenoceptor agonists are highly effective in causing weight loss and improving insulin sensitivity in animal models of obesity and type 2 diabetes. The role of the β3-adrenoceptor in humans is more controversial. The β3-adrenoceptor mRNA is expressed in human adipose tissue, but in lower amounts than in rodents. The receptor is more abundant in multilocular than in unilocular adipocytes. Its abundance is lower in adipocytes of obese subjects than lean ones, but therapy with ephedrine and caffeine increased its expression in obese population.

The ephedrine promotes noradrenaline release as well as causes some direct stimulation of adrenoreceptors, and in association with caffeine it enhances sympathetic activity. Indeed, in humans there is evidence that the thermogenic effect of ephedrine is in part mediated by β3-adrenoceptors [49] and that the β3-adrenoceptor-mediated component of this effect is enhanced by repeated administration of ephedrine or ephedrine plus caffeine [50, 51].

Clinical studies demonstrated that obese subjects' body composition, resting energy expenditure, and lipid metabolism are overall compatible with a more pronounced thermogenic and lipolytic effect by ephedrine plus caffeine plus energy restriction as compared to energy restriction alone [52]. Molnàr et al., in 2000, have published data about a pilot randomized double-blind placebo-controlled trial in 32 obese adolescents for 20 weeks, demonstrating that caffeine/ephedrine can be a safe and effective compound for the treatment of obesity in adolescents, with negligible adverse events [53].

3.3. New and Future Drugs. Recent advances in the understanding of energy balance control have resulted in the exploitation of a large number of new targets, some of which have yielded promising data in clinical trials for weight loss. A second major trend is derived from the hypothesis that improved weight loss efficacy over current therapy is more likely to emerge from treatments targeting multiple mechanisms of energy balance control. Many of these approaches also utilize advances in formulation technology to widen safety margins. Finally, the practicality of peptide therapies for obesity has become better validated in recent studies, and this may allow more rapid exploitation of novel targets, rather than awaiting the development of orally available small molecules [20]. Despite the limitations of current drugs and their declining use in some jurisdiction, anti-obesity drugs still accounted for sales of nearly a half-billion US dollars in the seven largest global markets during 2000 [54, 55]. Since overall sales of anti-obesity drugs projected to at least triple by 2010 [56], development of effective drugs has become a research priority and an area of intense clinical interest.

The new anti-obesity drugs under clinical development include agents affecting peripheral and central mechanisms. In the first group, we can include gastrointestinal lipase inhibitor (cetilistat), amylin and leptin analogs, thermogenic agents, that is, selective $\beta 3$ receptor agonists, agonist of the glucagon-like peptide 1 (GLP-1) (Exendin-4, Liraglutide). In the second group, we can consider agents that modulate the central activity of neuropeptides influencing food intake and including antiepilepsy drugs (topiramate, zonisamide), noradrenaline and dopamine reuptake inhibitors (bupropion, tesofensine) that were studied in monotherapy or in combination (bupropion-naltrexone and phentermine-topiramate); human ciliary neurotrophic factor (axokine), human GH fragment (AOD9604, AOD9401), melanocortin receptor selective agonists (MC4R), melanin-concentrating hormone antagonists, serotoninergic drugs including selective 5-HT2C receptor agonists (lorcaserin), components of neuropeptide Y (NPY) signaling pathway (CAMKK2).

We briefly cite these new drugs, although there are not yet studies in pediatric population, because these promising, more efficacious, and better-tolerated treatments for obesity may become available in the coming future also in pediatric population.

3.3.1. Drugs Affecting Peripheral Mechanisms

Cetilistat. Cetilistat (ATL-962) is a novel gastrointestinal lipase inhibitor. A phase II, multicentre, randomized, placebo-controlled, parallel group study demonstrated that treatment with cetilistat reduced mean body weight to similar extents at all doses, which were statistically significant compared with placebo. Adverse events were generally mild to moderate in intensity, occurred only on one occasion and mostly GIT in nature (such as flatus with discharge and oily spotting). Other lipase inhibitors are under investigation for obesity treatment [57].

Pramlintide. Pramlintide (Pro25, Pro28, Pro29-amylin), a soluble synthetic analogue of amylin, is approved in US as an adjunct to insulin for subcutaneous use in patients with type 1 or type 2 diabetes who are not able to achieve glucose control through optimal insulin therapy. Despite the well-known association between insulin and weight gain, insulin-treated type 1 or type 2 diabetic patients receiving adjunctive treatment with pramlintide demonstrated lower hemoglobin glycosylated levels as well as a significant and sustained reduction in body weight and BMI [4]. Pramlintide seems to mediate its anorectic effects (at least in part) through delayed GIT motility.

Recently, an association with metreleptin showed interesting results [58]. In fact, metreleptin, a leptin agonist, leads to weight loss that resulted significantly greater if associated with pramlintide. The greater reduction in body weight was significant as early as week 4, and weight loss continued throughout the study, without evidence of a plateau.

The most common adverse effects with pramlintide/metreleptin were injection site events and nausea, which were mostly mild to moderate and decreased over time.

Beta3-Adrenoceptor Agonists. $\beta 3$-adrenoceptor is the predominant subtype of adrenoceptor expressed in adipose tissue. It mediates the major effects of adrenaline and noradrenaline in adipose tissues, such as lipolysis in white adipose tissue and thermogenesis in brown adipose tissue. $\beta 3$-adrenoceptor agonist CL.316243 increases fat oxidation and decreases carbohydrate oxidation in humans [59]. Many pharmaceutical companies have attempted to develop $\beta 3$-adrenoceptor agonists for the treatment of human obesity, but there is no report on a compound that has progressed beyond phase II clinical trials [60]. Even though many companies have lost interest in the potential of $\beta 3$-adrenoceptor agonists for the treatment of obesity and diabetes, they can offer useful perspectives on pharmacology, physiology, and obesity drug discovery [4, 60].

Exendin-4, Liraglutide. The combination of anorectic and incretin effects of GLP-1 and analogues has attracted much attention as potential treatment for type 2 diabetes.

Exendin-4 (exenatide) is a natural agonist of the GLP-1 receptor isolated from the lizard *Heloderma suspectum* and has longer biological activity than GLP-1. It was recently approved for the treatment of type 2 diabetes. Twice daily subcutaneous administration of exenatide in patients with type 2 diabetes led to a dose-dependent weight loss of 1.8 $\pm$ 0.3 kg over 28 days, 2.8 $\pm$ 0.5 kg over 30 weeks, and 4.7 $\pm$ 0.3 kg over 2 years [61].

Liraglutide is a GLP-1 analogue with 97% primary amino acid homology to native GLP-1, recently approved in Europe and under evaluation by the FDA in the US. Like exenatide, liraglutide has been associated with dose-dependent weight loss in addition to metabolic and cardiovascular benefits: hemoglobin glycosylated decrease, improvements in triglyceride levels, a mean reduction in systolic and diastolic blood pressure [62].

Although actual interest in GLP-1 agonists has focused on their incretin effects for the development of therapeutics treatment of diabetes [63, 64], it seems likely that obesity indications will also be reached in the near future, although these might be limited to obese diabetic patient populations [4].

3.4. Drugs Affecting Central Mechanisms

3.4.1. Topiramate, Zonisamide, Bupropion, and Tesofensine. Two antiepileptic drugs (topiramate and zonisamide) and the antidepressant bupropion have been studied for their weight loss effects.

The mechanisms by which antiepileptic drugs produce weight loss are unclear, but they may be due to the antagonism of the glutamate kinase receptor by topiramate and to the serotonergic and dopaminergic activities of zonisamide [65]. Topiramate therapy was demonstrated to bring on a high frequency of adverse events due to the central and peripheral nervous system, such as paresthesias, somnolence, and difficulty with memory; for this reason, trials have been stopped in phase III while the manufacturers were going to develop an extended release formulation. Zonisamide's

adverse events occurring in the treatment group were fatigue and a small but significant increase in serum creatinine [65].

In an 8-week-study of weight loss in overweight and obese women, bupropion, currently marketed for treatment of depression and as a smoking cessation aid, produced a 6.2% loss of body weight compared with 1.6% weight loss in the placebo group [65, 66]. Clinicians must be aware of the 0.4% risk of seizure when the drug dose is 400 mg/day. The mechanism through which the drug leads to weight loss may be the inhibition of nor-epinephrine and dopamine uptake [65]. The pooled random-effects estimate of weight loss in bupropion-treated patients compared with placebo recipients was 2.77 kg (95% CI, 1.1 to 4.5 kg) [66], and the same authors indicate that the total weight loss in the bupropion-treated patients was 4.44 kg. In the adverse events analysis, there was an increase in dry mouth (pooled odds ratio:3.26 ; relative risk: 2.99) and no significant increases in diarrhea and constipation. In addition to dry mouth, insomnia is a commonly reported side effect in these studies [66].

Tesofensine is a new drug producing weight loss in obese individuals. It inhibits the presynaptic re-uptake of the neurotransmitters noradrenaline, dopamine, and serotonin. In phase II clinical trials with tesofensine in obese individuals, dose-related reductions in body weight, body fat, and waist circumference, as well as improvements in other obesity-related endocrine factors, were observed, while it was associated with minor adverse events, such as elevations in heart rate and blood pressure at the highest dose tested [67].

There are no recent data available on the use of these drugs in pediatric populations.

3.4.2. Bupropion-Naltrexone (Contrave). Contrave is an oral, sustained release combination of the dopamine and norepinephrine re-uptake antagonist bupropion and the opioid antagonist naltrexone. The mechanism of action of the compound involves complementary stimulation of central melanocortin pathways, resulting in increased energy expenditure and reduced appetite. Preliminary data of phase III clinical trials in adults demonstrated placebo-subtracted weight loss of 3 to 7% and improvements in obesity-related comorbidities and cardiovascular risk factors [68, 69].

3.4.3. Phentermine-Topiramate. We have cited before the specific characteristic of phentermine and topiramate, but phentermine HCI/topiramate controlled-release (PHEN/TPM CR) is a combination agent containing immediate-release phentermine and controlled-release topiramate. Clinical trials involving thousands of patients demonstrate PHEN/TPM CR to be effective in improving the weight and also the adiposopathy-associated metabolic diseases [70].

3.4.4. Endocannabinoid Receptor Blockers (Rimonabant, Taranabant). Endocannabinoid (ECB) receptor blockers, such as rimonabant, were initially intended as an anti-obesity and smoking cessation dual-purpose drug; however, the latter development program has been discontinued [20, 71]. ECB produces a dose-dependent reduction in food intake in various rodent models, and these effects seem to be both centrally and peripherally mediated [72]. Potential peripheral mechanisms include enhanced thermogenesis increasing oxygen consumption in skeletal muscle [73], reducing hepatic [74] and adipocyte lipogenesis [75], augmenting adiponectin concentrations [76], promoting vagus-mediated cholecystokinin-induced satiety [77, 78], inhibiting preadipocyte proliferation, and increasing adipocyte maturation without lipid accumulation [79].

Rimonabant in the United Kingdom was indicated for patients whose BMI exceeds 30 kg/m^2 or who had associated factors such as type 2 diabetes and/or dyslipidemia, while in other countries obesity alone was not an indication for its use and abnormal blood lipid levels are also required for the prescription [80]. Recently European Medicines Agency decided the withdrawal of rimonabant from the European market because of its important side effects.

Taranabant is a novel cannabinoid CB1 receptor inverse agonist that is in clinical development for the treatment of obesity. Mechanism-of-action studies suggest that engagement of the CB1 receptor by taranabant leads to weight loss by reducing food intake and increasing energy expenditure and fat oxidation [81]. Recent studies have demonstrated a weight loss after a 12-week therapy in obese adults, inducing statistically significant weight loss compared to placebo in obese subjects over the entire range of evaluated doses (0.5, 2, 4, and 6 mg once per day) ($P < .001$). Taranabant treatment was associated with dose-related increased incidence of clinical adverse events, including mild to moderate GIT and psychiatric effects.

3.4.5. Ciliary Neurotrophic Factor. Ciliary neurotrophic factor (CNTF) was originally characterized as a trophic factor supporting the survival of embryonic chick ciliary ganglion neurons in vitro [82, 83]. Initially, CNTF was described as being predominantly distributed within neural tissues [84] but it was also reported in skeletal muscle, adrenal gland, sciatic nerve, skin, kidney, and testes [85]. Although CNTF was firstly identified as a trophic factor in the ciliary ganglion, it was later found to act on other motor neuron populations [86]. Hence, it was evaluated as a therapeutic tool in patients suffering from motor neuron diseases [87]. Interestingly, during these trials, CNTF administration resulted in unexpected weight loss. CNTF can also cross the blood-brain barrier in a manner similar to leptin [88]. CNTF and its synthetic analogue, axokine, have also been found to suppress NPY gene expression [89]. It is believed to occur by resetting the hypothalamic weight set point, such that cessation of CNTF treatment does not result in overeating and rebound weight gain [90]. The results of recent studies have suggested that CNTF and axokine may play a role in the regulation of adipocyte metabolism and, perhaps, the control of adipose tissue mass, supporting the hypothesis that weight loss due to CNTF is not solely mediated by the CNS [91].

CNTF or axokine [92] failed first phase III trial because two-thirds of the patients developed antibodies against this factor. Thus, further studies have to be made to understand the effectiveness of the drug and its possible side effects.

3.4.6. Analogue (AOD9604-AOD9401) of Human Growth Hormone. It has generally been recognized that human GH (hGH) exerts a profound effect on body composition and systemic hGH administration reduces body fat mass as well as fat distribution. The use of hGH for the treatment of human obesity has not been advocated due to serious side effects, including glucose intolerance, insulin resistance, sodium retention, hypertension, edema, and carpal tunnel syndrome [93].

The aminoterminus of the hGH molecule is the functional domain for the insulin-like action of the hormone. Some studies have identified the specific metabolic domain responsible for the lipolytic/antilipogenic activity of the hGH molecule, referred as AOD9604 and studied for its metabolic actions in obese rats. Daily treatment with an oral dose of AOD9604 (500 μg/kg) for 19 days reduced over 50% of body weight gain of the animals in comparison with the control. The adipose tissues of the AOD9604-treated animals were found to present an increase in lipolytic activity. In contrast to chronic treatment with intact hGH, the chronic treatment with AOD9604 showed no adverse effect on insulin sensitivity, as demonstrated with euglycemic clamp techniques [94]. Unfortunately, AOD9604 recently failed phase IIb trials for obesity.

Another lipolytic domain (referred as AOD9401) of hGH, has been synthesized using solid-phase peptide synthesis techniques. This analogue of the hGH lipolytic domain may have the potential to be developed into an orally usable and safe therapeutic agent for obesity. Up to now, no human studies have been yet performed [94].

3.4.7. Melanocortin Receptor Selective (MC4R) Agonists. Much effort is currently underway to develop melanocortin receptor selective compounds directed for clinical use, with a focus on the development of MC4R selective agonists to treat obesity. There is, on the other hand, the possibility of treating anorectic and cachectic conditions with MC4R antagonists [95].

The most reported side effects of this drug appear to be mild and include flushing, somnolence, nausea, vomiting, headache, and taste disturbances [54, 96]. The only available clinical trial data on the effect of an MC4R agonist was conducted with adrenocorticotropic hormone (an agonist of MC4R) to assess its potential to cause weight reduction in humans. Therefore, clinical trials with proper drug candidates are needed.

3.4.8. Melanin-Concentrating Hormone (MCH) Antagonists. MCHR1 antagonists (recently patented) [97, 98] are one class of agents that shows particularly good promise for treating obesity that has now been well validated in animal models but remains to be validated in human clinical trials. MCHR1 antagonists reduce food intake, particularly highly palatable food, and subsequently decreases body weight, primarily due to decreased fat mass. Depending upon the animal model and paradigm utilized in the studies, MCHR1 antagonists also were shown to increase energy expenditure resulting in weight loss. Together, these data suggest that MCHR1 antagonists may be a key component in a program of diet, exercise, and therapeutic intervention in reducing the energy "gap" to allow people better regulation of their weight. A number of significant safety, selectivity, and metabolic stability hurdles were presented that continue to be challenges for pharmaceutical companies in their effort/research to be the first to deliver a new therapeutic agent targeting this receptor. Since, to date, only three companies have initiated clinical trials, and only one has reported conclusion of a first-in-human trial there is still a long road and many years ahead before a compound targeting MCHR1 receptors could ultimately be marketed for the treatment of obesity [98].

3.4.9. Serotonergic Drugs. M-chlorophenylpiperazine (mCPP) is a 5-HT2C receptor agonist studied in insulin resistant mouse models showing beneficial effects on glucose homeostasis and weight gain [40]. Diet-induced obese mice and leptin-deficient (ob/ob) mice treated for 2 weeks exhibited a dramatic 50% reduction in plasma insulin levels in both models. This was without effects on blood glucose, food intake, activity, or body mass at the doses used in this study. Pharmacological blockage of 5-HT2C receptors abolished effects on glucose tolerance, verifying that these effects were directly mediated by 5-HT2C receptors [97]. mCPP has been shown to modify appetite in both lean and obese humans.

A new generation of 5-HT2C receptor agonist is developing [97]. Recent studies show APD-356 (lorcaserin) to be a potent, selective, and efficacious agonist of the 5-HTC2 receptor, with potential for the treatment of obesity [99].

3.4.10. Ca^{2+}/Calmodulin-Dependent Protein Kinase Kinase 2 (CaMKK2). Recently, the adenosine monophosphate-dependent protein kinase (AMPK) was identified as one component of NPY signaling pathway. Acute intracerebroventricular (icv) administration of a CaMKK2 null mice resulted in decreased food intake that was correlated with decreased hypothalamic NPY and AgRP mRNAs. The absence of CaMKK2 was also demonstrated to protect mice from diet-induced weight gain, hyperglycemia, and insulin resistance. Furthermore, icv infusion of the selective CaMKK2 inhibitor STO-609 in adult WT mice resulted in the acute suppression of NPY expression and food intake. The resistance of CaMKK2 null mice to suppression of food intake by STO-609 infusion provides compelling evidence that CaMKK2 is the direct target of the drug and that the specific inhibition of CaMKK2 in NPY neurons is likely responsible for the reduced level of NPY and AgRP mRNAs and feeding behavior [100].

Thus, the behavior of CaMKK2 null mice on the high-fat diet underscores the value of targeting CaMKK2 as a possible therapeutic locus in the treatment of obesity and diabetes [100].

4. Conclusions

Childhood obesity is not only a cosmetic problem. Many adverse health effects associated with adult obesity are

already being seen in obese adolescents in which a significant increase in the cardiovascular risk has been observed, probably due to obesity-metabolic disarrangement (most incidence of hypertension, type 2 diabetes, and dyslipidemia), but also other co-morbidities such as nonalcoholic fatty liver disease, idiopathic intracranial hypertension, sleep apnea, and orthopedic abnormalities. In summary, there is insufficient evidence to conclude that any one-treatment approaches is superior in the management of adolescent obesity. However, the use of psychological interventions such us behavioral therapy and cognitive behavioral therapy, combined with strategies to improve diet and physical activity, shows promise.

The neurobiology of obesity is extremely complex with many overlapping and redundant pathways. This complexity decreases the probability that targeting any single pathway will result in dramatic weight loss and suggests that multiple drugs with different mechanisms will be needed to determine significant and persistent weight loss [20]. Although newer drugs are years away from clinical use, the hope for research investments made to date is translation into safe and effective anti-obesity drugs in the future. The search for novel drug treatments for obesity in childhood and adolescents is, therefore, both legitimate and necessary.

However, in our efforts to fill the therapeutic void that characterizes contemporary obesity management, the benefits of obesity pharmacotherapy must outweigh the risks and costs.

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Review Article

Pharmacological Treatment of Obesity in Patients with Polycystic Ovary Syndrome

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Polycystic ovary syndrome (PCOS) is a common disorder affecting women of reproductive age and it is associated with increased cardiovascular risk. Obesity plays an important role in the pathogenesis of PCOS, and the majority of patients with PCOS are obese. Over the last 20 years, the prevalence of obesity has dramatically increased, with probable associated increase in PCOS. Weight reduction plays an integral part in the management of women with PCOS. In this paper, current available weight reduction therapies in the management of PCOS are discussed.

1. Introduction

Polycystic ovary syndrome (PCOS) is a common disorder with a prevalence of 6-7% of women of reproductive age [1–4]. The clinical picture commonly includes obesity, hirsutism, oligomenorrhoea, and subfertility. Around 30–75% of PCOS women are obese [1, 5, 6], and many PCOS women show evidence of insulin resistance and hyperandrogenism. In 2003, the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) cosponsored the Rotterdam polycystic ovary syndrome consensus workshop that revised and broadened the National Institute of Health (NIH) 1990 diagnostic criteria [7].

While chronic anovulation with biochemical and/or clinical hyperandrogensim is essential for making the diagnosis under the NIH 1990, classic PCOS, [8] the revised Rotterdam criteria [7] (box 1) included women with hyperandrogenism but regular menses and those with menstrual disturbance without overt androgen excess, nonclassic PCOS. The revised Rotterdam criteria, although commonly, used are still controversial and reflect the heterogeneous presentation of the syndrome and the advances in understanding its uncertain aetiology [9].

2. The Role of Obesity in the Development of PCOS

The exact role that obesity plays in the development of PCOS remains to be determined. The theories that have been put forward to explain the metabolic abnormalities associated with obesity may also be applicable to explain the role of obesity in the development of PCOS.

The adipokine theory suggests that the adipose tissue is an endocrine organ that secretes several hormones (adipokines). Insulin resistance and hyperandrogenism are common features of PCOS [7, 10]. Alteration in adipokines levels may lead to the development of PCOS. Adiponectin is exclusively produced by the adipose tissue [11]. Several studies have shown adiponectin to be lower in PCOS women when compared to weight-matched controls [12, 13]. Adiponectin is inversely related to insulin resistance [14]. While some studies suggest a strong correlation between adiponectin and androgen levels in PCOS [13], others did not show similar association [12]. Leptin is not produced exclusively by adipocytes but its circulating levels are strongly correlated to adipose mass and are higher in obese people [15]. In most studies, leptin levels in PCOS were similar to those of controls of similar body weight [16]. Whether leptin

Two of the following three criteria must be fulfilled:
(i) A clinical diagnosis of oligomenorrhoea or amenorrhoea
(ii) Clinical or biochemical evidence of hyperandrogenism
(iii) Polycystic ovaries on ultrasound examination (either 12 or more follicles measuring 2–9 mm in diameter, or an ovarian volume of >10 cm^3)

Late onset congenital adrenal hyperplasia, androgen secreting tumours, and Cushing's syndrome must be excluded in women with raised androgens; thyroid disorders and raised prolactin should be excluded in women with menstrual disturbances.

Algorithm 1: Revised 2003 criteria for diagnosing polycystic ovary syndrome (PCOS) [7].

levels in PCOS are associated with androgen levels remains controversial [17, 18]. Hyperleptinaemia has been associated with insulin resistance [17] and is inversely related to fertility in PCOS women [19]. Resistin is another adipokine and its relationship to PCOS remains debatable. Increased levels of resistin have been linked to the development of insulin resistance [20] and may play a role in the development of cardiovascular disease [21]. However, while some studies suggest increased levels in PCOS [16], others showed PCOS women to have similar levels when compared to weight-matched controls [13].

On the other hand, the adipose tissue expandability theory [22] proposes that at a set point of positive energy imbalance, which is determined on an individual basis by genetics and environmental factors, the subcutaneous adipose tissue fails to expand to store more fat subcutaneously. This results in a state of lipotoxicity and fat starts to deposit in other tissues like the liver, muscles, and pancreas. Lipotoxicity drives insulin resistance and subsequently hyperandrogenism commonly seen in PCOS women [23].

One of the difficulties that face any theory trying to explain the origin of PCOS is that it is not one entity. Women with PCOS are not all obese or insulin resistant [24], and the presence of hyperandrogenism and oligo/amenorrhoea is not necessary for making the diagnosis.

3. Obesity and Cardiovascular Disease in PCOS

Many cardiovascular (CV) risk factors are increased in PCOS even after adjusting for weight. For example, women with PCOS have impaired glucose tolerance [25], dyslipidaemia [26], endothelial [27] and platelets dysfunction [28], lower adiponectin [12], and higher homocysteine levels [18]. Despite the lack of long-term CV outcome data, it has been suggested that PCOS women have a sevenfold increase in relative risk for myocardial infarction [29], higher prevalence of cerebrovascular disease [30], and worsening CV event-free survival [31].

Obesity contributes to the 43% prevalence of the metabolic syndrome in PCOS patients [32]. Central obesity is often associated with PCOS [33] and carries increased risk for developing cardiovascular disease and type 2 diabetes [34].

Over the past 2 decades, the rate of obesity has raised threefold [25]. Whether there is an increase in the prevalence of PCOS to parallel the increase in obesity is still controversial [35, 36].

4. Treatment of Obesity in the Setting of PCOS

Even modest weight loss of less than 10% of initial body weight has been shown to increase the frequency of ovulation, improve conception, and reduce testosterone, free androgen index, hyperlipidaemia, hyperglycaemia, and insulin resistance in women with PCOS [37, 38].

4.1. Life Style Changes. Lifestyle modification is regarded as the first line treatment for women with PCOS. Exercise and weight loss improve insulin sensitivity. 44–57% of PCOS women had improvement in either menstrual cycle or ovulation after lifestyle changes and subsequent weight loss [37, 39, 40]. One of the main challenges of lifestyle changes is the low participants' compliance rate over time [39]. Therefore, pharmaceutical intervention is an additional essential therapeutic tool to lifestyle changes in many patients.

4.2. Medical Therapy. Few safe and effective drugs are currently available for the treatment of obesity. Although Sibutramine [41] and rimonabant [42] have been shown to be effective in inducing weight loss in PCOS women, they have both been withdrawn from the UK. Rimonabant increased the risk of psychiatric disorders and sibutramine has been associated with hypertension and cardiovascular disease.

4.2.1. Orlistat. Orlistat is a lipase inhibitor that reduces fat absorption in the gut by approximately 30% [43]. In a meta-analysis, it is estimated that orlistat treatment led to an average placebo-subtracted weight loss of 2.7 kg at 1 year [44]. In a 4-year randomised controlled trial of 3305 obese, nondiabetic patients, orlistat treatment was associated with a 3.6-kg weight loss compared with 1.4 kg for placebo at 4 years [45].

In women with PCOS, a 4.69% weight reduction was reported with orlistat therapy with an associated improvement in total testosterone levels [46]. In a 12-week randomised open labelled study [47], orlistat reduced insulin resistance by around 20% and variability of insulin resistance by 40% in obese PCOS women. In a 6-month clinical trial, therapeutic intervention with orlistat and a low-calorie diet

resulted in a beneficial effect in PCOS in reducing advanced glycation end-products (AGE) levels and testosterone concentrations independently of BMI changes [48].

The use of orlistat is limited by its gastrointestinal side effects. Approximately, 15–30% of those taking orlistat experience oily stool, faecal urgency, or oily spotting, and 7% report faecal incontinence, particularly at the initiation of treatment [44]. Despite its relatively high rate of side effects, orlistat is a useful treatment tool in the management of obese PCOS women.

4.2.2. Metformin. Metformin is a biguanide commonly used for the treatment of type 2 diabetes. Metformin's primary action is on the liver, where it reduces gluconeogenesis. Extrahepatic sites of action include the skeletal muscles, adipose tissue, endothelium, and the ovary [49, 50]. Metformin is commonly used in women with PCOS and is reported to improve insulin resistance, sex hormone binding globulin (SHBG), hyperandrogenaemia, and ovulation [51, 52]. Metformin's effect on weight management remains controversial. In a small ($n = 56$) randomized, double-blinded, placebo controlled cross-over study, 6-month treatment with Metformin resulted in mean weight reduction of 2.3 kg ($P < .009$) [53]. However, in a systematic review and meta-analysis [54], metformin was found to have no effect on body weight and body mass index (BMI) in women with PCOS. Metformin may be more effective in subgroups of PCOS women and at higher doses. Nonobese women with PCOS responded better to metformin than obese women with regards to insulin sensitivity, free testosterone, and androstendione concentrations [55]. Metformin was also found to cause a dose-related weight loss in obese women with PCOS [56], (1.5 and 3.6 kg weight loss in 1500- and 2550-mg/day metformin treated groups, resp.). Metformin when given to obese PCOS women after rimonabant, an endocannabinoid blocker, maintained the weight reduction and decrease in waist circumference achieved by rimonabant and augmented the initial improvements in testosterone levels and insulin resistance [57]. It is possible that the insulin sensitisation action of metformin was complementary to the weight loss caused by rimonabant.

Most frequent side effects of metformin are gastrointestinal symptoms including nausea, anorexia, vomiting, abdominal discomfort, and diarrhoea and occur in up to 20% of patients. In 3–5% patients, therapy may have to be discontinued because of these adverse effects [50, 58]. Slow release forms of metformin are available and are believed to be better tolerated.

Despite the lack of strong evidence, the use of metformin in obese PCOS women is likely to be beneficial especially when taken at higher doses.

4.2.3. Incretin Mimetics Therapy. The gastrointestinal tract produces several peptide hormones that participate in regulation of food intake. Ingested nutrients, especially fats and carbohydrates, stimulate Glucagon-like peptide-1 (GLP-1) secretion from L cells in the distal small intestine [59]. GLP-1 accentuates glucose-dependent insulin release, inhibits glucagon secretion, increases pancreatic β cell growth, suppresses appetite and energy intake, and delays gastric emptying [60–63]. Glucagon-like peptide-1 receptor (GLP-1R) is expressed by the gut, pancreas, brainstem, hypothalamus, and vagal-afferent nerves [60]. Activation of the hypothalamic GLP-1R decreases food intake [64].

In a 24-week randomised controlled trial in women with PCOS [65], a combination treatment with exenatide (GLP1 mimetic) and metformin was found to be superior to exenatide or metformin monotherapy in reducing weight (mean weight loss of 6 ± 0.5 kg) and improving menstrual cycles, ovulation rate, free androgen index, and insulin sensitivity.

In another 20-week randomised open labelled study involving obese people without PCOS [66], comparing liraglutide (GLP1 analogue) to orlistat, it was shown that liraglutide treatment is well tolerated and induced significant dose-related weight loss (mean weight loss 4.8–7.2 kg) compared to placebo or orlistat. All the patients were obese and had a 500 kcal per day energy deficit diet and increased their physical activity throughout the study.

In a head-to-head comparison study in people with type 2 diabetes, 1·8 mg liraglutide daily and 10 μg exenatide twice daily produced similar weight loss (3·2 kg with liraglutide versus 2·9 kg with exenatide) [67]. However, liraglutide achieved better glycaemic control and was better tolerated than exenatide [67].

Most commonly reported side effects are nausea and vomiting, but the main safety concern remains a possible increased risk of pancreatitis attributable to drugs that act through the GLP-1 pathway [68]. One of the disadvantages of using GLP-1R agonists is that they require injection. Although, not yet licensed for obesity management, GLP-1R agonists offer a potential obesity treatment for women with PCOS.

4.2.4. Bariatric Surgery. In subjects with morbid obesity, bariatric surgery may be considered as an effective therapy. Few studies have shown bariatric surgery to be associated with significant improvement in weight, hirsutism, insulin resistance, and fertility in women with PCOS [69, 70].

5. Conclusions

PCOS is a common disorder of uncertain aetiology. It is associated with increased cardiovascular morbidity. Obesity plays an important part in the pathogenesis of PCOS. Many PCOS women are obese, and weight reduction is an essential part in their management.

In the management of obese women with PCOS, lifestyle modification is the first step although low long-term compliance rate may reduce benefit. Orlistat, with dietary changes, is beneficial despite its common gastrointestinal side effects. Metformin, at higher doses, may promote weight loss. Although not currently licensed, newer pharmacotherapeutic agents like incretin mimetics hold promise in managing obese women with PCOS.

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Review Article

The Use of *Garcinia* Extract (Hydroxycitric Acid) as a Weight loss Supplement: A Systematic Review and Meta-Analysis of Randomised Clinical Trials

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The aim of this systematic review is to examine the efficacy of *Garcinia* extract, hydroxycitric acid (HCA) as a weight reduction agent, using data from randomised clinical trials (RCTs). Electronic and nonelectronic searches were conducted to identify relevant articles, with no restrictions in language or time. Two independent reviewers extracted the data and assessed the methodological quality of included studies. Twenty-three eligible trials were identified and twelve were included. Nine trials provided data suitable for statistical pooling. The meta-analysis revealed a small, statistically significant difference in weight loss favouring HCA over placebo (MD: −0.88 kg; 95% CI: −1.75, −0.00). Gastrointestinal adverse events were twice as common in the HCA group compared with placebo in one included study. It is concluded that the RCTs suggest that *Garcinia* extracts/HCA can cause short-term weight loss. The magnitude of the effect is small, and the clinical relevance is uncertain. Future trials should be more rigorous and better reported.

1. Introduction

The prevalence of overweight and obesity has increased over the last decade [1], and current measures have not been able to stem the tide. A wide variety of weight management strategies are presently available, and some involve the use of dietary supplements marketed as slimming aids. One such slimming aid is *Garcinia* extract, (-)-hydroxycitric acid (HCA).

HCA is a derivative of citric acid and can be found in plant species native to South Asia such as *Garcinia cambogia, Garcinia indica,* and *Garcinia atroviridis* [2]. HCA is usually marketed as a weight loss supplement either alone or in combination with other supplements [2, 3]. Some authors have suggested that HCA causes weight loss by competitively inhibiting the enzyme adenosine triphosphatase-citrate-lyase [3–6]. HCA has also been reported to increase the release or availability of serotonin in the brain, thereby leading to appetite suppression [7]. Other postulated weight loss mechanisms include inhibition of pancreatic alpha amylase and intestinal alpha glucosidase, thereby leading to a reduction in carbohydrate metabolism [8].

Animal studies have suggested that HCA causes weight loss [3, 9], and human trials involving the use of HCA as a weight loss supplement have been carried out [3].

The primary objective of this systematic review was to examine the efficacy of HCA in reducing body weight in humans, using data from randomised clinical trials.

2. Methods

Electronic searches of the literature were conducted in the following databases: Medline, Embase, *The Cochrane Library*, Amed, and Cinahl. The search terms used included dietary supplements, antiobesity agents, body weight, hydroxycitrate, *garcinia*, and derivatives of these. Each database was searched from inception until March, 2010. We also searched the Internet for relevant conference proceedings and hand searched relevant medical journals, and our own files. The bibliographies of all located articles were also searched.

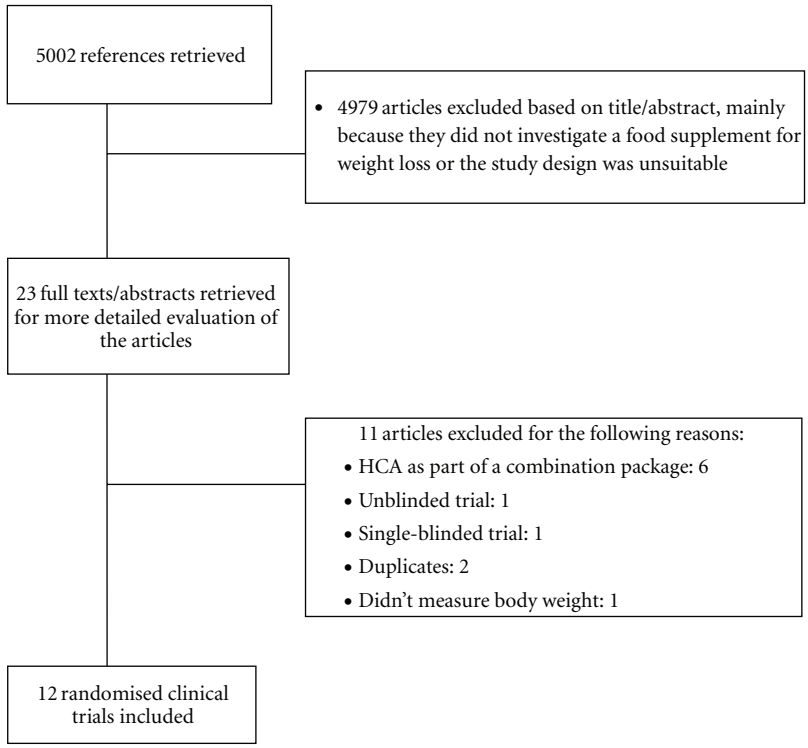

Figure 1: Flow chart showing the process for the inclusion of randomised controlled trials.

Only randomised, double-blind, placebo-controlled studies were included in this paper. To be considered for inclusion, studies had to test the efficacy of oral HCA or any of its salts for weight reduction in obese or overweight humans. Included studies also had to report body weight as an outcome. No age, time, or language restrictions were imposed for inclusion of studies. Studies which involved the use of HCA as part of a combination treatment (dietary interventions containing other supplements in addition to HCA), or not involving obese or overweight subjects based on body mass index (BMI) values, were excluded from this paper.

Two independent reviewers assessed the eligibility of studies to be included in the paper. Data were extracted systematically by two independent reviewers according to the patient characteristics, interventions, and results. The methodological quality of all included studies was assessed by the use of a quality assessment checklist adapted from the Consolidated Standard of Reporting Trials (CONSORT) guidelines [10, 11]. In addition, the Jadad score [12] was also used to assess the quality of included studies. Disagreements were resolved through discussion with the other authors.

Data are presented as means with standard deviations. Mean changes in body weight were used as common endpoints to assess the differences between HCA and placebo groups. Using the standard meta-analysis software [13], we calculated mean differences (MDs) and 95% confidence intervals (CIs). Studies included in the meta-analysis were weighted by SD (a proxy for study size). If a trial had 3 arms, only the HCA and placebo arms were included in the meta-analysis. The I^2 statistic was used to assess for statistical heterogeneity amongst studies. A funnel plot was used to test for publication bias.

3. Results

Our searches produced 5002 "hits" of which 23 potentially relevant articles were identified (Figure 1). Six trials were excluded because they involved the use of HCA in combination with other therapies [7, 14–18]. One trial was excluded because it was not blinded [19], and another because it was single blinded [20]. Two articles were excluded because they were duplicates. One of these articles [21] was the same trial published in another journal which had been earlier excluded, while the other article [22] was a report of two individual trials which were included in this systematic review. One trial was excluded because the investigators did not measure weight as an outcome [23]. Thus 12 randomised clinical trials (RCTs) including a total of 706 participants met our inclusion criteria, and were included in this systematic review [2, 4–6, 24–31]. Their key details are summarized in Tables 1, 2, and 3.

All of the studies had one or more methodological weaknesses (Table 1). None of the included studies reported on how double blinding was carried out, and all studies were also unclear about how the allocation was concealed. The randomization procedure was clear in only a third of included studies [4, 6, 25, 29].

Three RCTs [4, 28, 31] did not provide actual values to enable statistical pooling (Table 3). One of these RCTs

Table 1: Characteristics of included studies.[a]

Authors Year	Main outcome (s)	Main diagnoses of participants	Randomisation appropriate?	Allocation concealed?	Groups similar at baseline?	Similar follow-up of groups?	Outcome assessor blinded?	Care provider blinded?	Patients blinded?	Attrition bias?	ITT analysis?	Jadad Score
Hayamizu et al. 2001 [24]	Visceral fat, BW indices	Overweight subjects	?	?	+	+	?	?	?	–	–	2
Hayamizu et al. 2003 [4]	Visceral fat, BW indices	Overweight subjects	+	?	+	+	?	?	?	?	–	3
Heymsfield et al. 1998 [25]	BW, fat mass	Overweight subjects	+	?	+	+	?	?	?	?	+	5
Kovacs et al. 2001 [26]	Satiety, food intake, BW	Normal to moderately obese subjects	?	?	?	+	?	?	?	–	–	3
Kovacs et al. 2001 [27]	Satiety, food intake, BW	Normal to moderately obese subjects	?	?	?	+	?	?	?	–	–	3
Mattes and Bormann 2000 [5]	Satiety, body composition	Overweight subjects	?	?	+	+	?	?	?	?	–	2
*Preuss et al. 2002 [28]	BW, BMI, appetite	Moderately obese subjects	?	?	?	?	?	?	?	?	?	2
Preuss et al. 2004 [29]	BW, BMI, lipid profile, appetite	Healthy, obese volunteers	+	?	+	+	?	?	?	?	–	3
Preuss et al. 2004 [6]	BW, BMI, lipid profile, appetite	Healthy, obese volunteers	+	?	?	+	?	?	?	?	–	4
Ramos et al. 1995 [30]	BW, BMI, lipids	Obese subjects	?	?	?	?	?	?	?	?	?	2
Roongpisu-thipong et al. 2007 [2]	BW, BMI, BP, waist-hip ratio	Healthy, overweight volunteers	?	?	+	+	?	?	?	?	–	2
Thom 1996 [31]	BW, BP, total cho-sterol, appetite	Obese subjects	?	?	?	?	?	?	?	?	?	2

[a] Quality assessment checklist adapted from The CONSORT Statement and Jadad criteria [10–12].

Table 2: Results table for studies with adequate data for meta-analysis.[b]

Author Year Country	HCA formulation	Randomised/ Analysed	Age in yrs	HCA Dosage	Treatment Duration	Baseline weight indices for HCA/placebo groups	Mean change in weight indices for HCA/placebo groups	Adverse events (AE)	Control for lifestyle factors
Hayamizu et al. 2001 Japan [24]	Tablets	40/40	37.1 ± 12.5 (HCA) 36.5 ± 10.7 (PLA)	1 g daily	8 weeks	BW: 75.6 ± 10.3/73.3 ± 10.7 BMI: 27.9 ± 1.8/27.8 ± 1.8	BW: 0 ± 11.5/0.5 ± 11.7 BMI: 0 ± 1.97/0.3 ± 2.3	No serious AE reported	Dietary control
Heymsfield et al. 1998 U.S.A. [25]	Capsules	135/135	38.6 ± 7.7 (HCA) 39.4 ± 7.2 (PLA)	1.5 g daily	12 weeks	BW: 83.8 ± 10.7/88.2 ± 13.0 BMI: 31.2 ± 2.8/31.9 ± 3.1	BW: −3.2 ± 3.3/ − 4.1 ± 3.9	Headache, URTI & GI symptoms	High fibre diet, stable physical activity levels
Kovacs et al. 2001 Netherlands [26]	Unspecified	21/21	43 ± 10 for both HCA&placebo groups	1.5 g daily	2 weeks	Mean BW: 79.3 ± 9.0 Mean BMI: 27.6 ± 2	BW: −0.4 ± 0.9/−0.5 ± 1.4	Not reported	No restriction on food intake; 1 glass of alcohol maximum daily
∗§Kovacs et al. 2001 Netherlands [27]	Unspecified	11/11	47 ± 16 for both HCA&placebo groups	1.5 g daily	2 weeks	Mean BW: 85.4 ± 25.8 Mean BMI: 27.4 ± 8.2	BW: −1.5 ± 1.66/ −1.0 ± 1.34	Not reported	No restriction on food intake; 1 glass of alcohol maximum daily
Mattes and Bormann 2000 U.S.A. [5]	Caplets	167/89	40.97 ± 10 (HCA) 44.0 ± 9.5 (PLA)	1.2 g daily	12 weeks	BW: 75.5 ± 10.2/75.8 ± 12.6 BMI: 28.3 ± 0.6/28.8 ± 0.7	BW: −3.7 ± 3.1/−2.4 ± 2.9	Not reported	Dietary control, exercise encouraged, but no formal regimen prescribed
§Preuss et al. 2004 India [29]	Unspecified	60/53	Range: 21–50	2.8 g daily	8 weeks	BW: 91.7 ± 15.7/80.4 ± 36.9 BMI: 34.7 ± 5.5/32.5 ± 2.6	BW: −4.5 ± 16.6/ −1.6 ± 34.1 BMI: −1.7 ± 5.8/ − 0.7 ± 2.74	Gas, stomach burn, headache, skin rash	Dietary control, walking exercise programme
§Preuss et al. 2004 India [6]	Unspecified	30/29	Range: 21–50	2.8 g daily	8 weeks	BW: 88.5 ± 21.8/87.4 ± 15.9 BMI: 33.6 ± 6.2/34.0 ± 4.5	BW: −5.5 ± 23.7/ −1.4 ± 17.3 BMI: −2.1 ± 6.85/ − 0.5 ± 4.8	No serious AE reported	Dietary control, walking exercise programme
Ramos et al. 1995 Mexico [30]	Capsules	40/ 35	35.3 ± 11.8 (HCA) 38.7 ± 12.3 (PLA)	1.5 g daily	8 weeks	BMI: 32.6 ± 4.3/33.2 ± 4.4	BW: −4.1 ± 1.8/−1.3 ± 0.9	Nausea, headache	Dietary control
Roongpisu-thipong et al. 2007 Thailand [2]	Sachets	50/42	40.0 ± 10.0 (HCA) 36.0 ± 10.0 (PLA)	Unclear	8 weeks	BW: 69.0 ± 5.0/65.0 ± 5.0 BMI: 27.5 ± 1.0/26.7 ± 2.5	BW: −2.8 ± 0.5/ − 1.4 ± 0.5 BMI: −0.9 ± 1.0/ − 0.6 ± 1.0	Not reported	Dietary control

Abbreviations: HCA: Hydroxycitric acid; PLA: Placebo; BW: Body Weight; BMI: Body Mass Index.

[b] Unless otherwise specified, values for age, baseline weight and mean change in weight indices have been reported as means with standard deviations.

∗ Studies included as crossover design, otherwise all included trials had parallel-study design.

§ Studies with 3 intervention groups.

Table 3: Results of included studies without suitable data for meta-analysis.[ρ]

Author Year Country	HCA formulation	Randomised/ Analysed	Age in yrs	HCA Dosage	Treatment Duration	Baseline weight indices for HCA/placebo groups	Main Results	Adverse events (AE)	Control for lifestyle factors
Hayamizu et al. 2003 Japan [4]	Tablets	44/39	43.7 ± 11.9 (HCA) 45.2 ± 13.0 (PLA)	1 g daily	12 weeks	BW: 75.1 ± 12.3/ 75.9 ± 11.5 BMI: 28.9 ± 4.7/ 28.5 ± 4.6	No significant differences in BMI or body weight at week 12	Common cold, toothache, diarrhea	Dietary control
Preuss et al. 2002 (abstract) India [28]	Unspecified	48/unclear	Not reported	2.8 g daily	8 weeks	Not reported	4.8% loss in body weight, and 6.8% decrease in BMI for HCA group	Not reported	Diet control, exercise
Thom 1996 (abstract) Norway [31]	Capsule	60/unclear	Not reported	1.32 g daily	8 weeks	Not reported	Significant decrease in body weight in HCA group compared with placebo ($P < .001$)	Stomach ache	Low fat diet, exercise

Abbreviations: HCA: Hydroxycitric acid; PLA: Placebo; BW: Body Weight; BMI: Body Mass Index.
[ρ] Unless otherwise stated, all trials are parallel-study designs.

reported a nonsignificant difference in BMI or body weight between groups [4], another reported a significant difference ($P < .001$) in the HCA group compared with placebo [31]. The third RCT [28] reported a decrease in body weight and (BMI) from baseline for the HCA group, without providing results of intergroup differences.

A forest plot (random effect model) for studies with data suitable for statistical pooling is shown in Figure 2. The meta-analysis reveals a statistically significant difference in body weight between the HCA and placebo groups. The average effect size was, however, small (MD: −0.88 kg; 95% CI: −1.75, −0.00), with a P value of .05. This translates to about 1% in body weight loss in HCA group compared with placebo. The I^2 statistic suggests that there was considerable heterogeneity amongst the trials, the duration of treatment, and the dosages of HCA used in the different trials varied widely. A funnel plot of mean difference plotted against trial sample size (Figure 3) indicated that most of the studies (which had small sample sizes) were distributed around the mean difference of all the trials.

Sensitivity analyses were performed to test the robustness of the overall analysis. The first included 7 trials [2, 5, 6, 24, 25, 29, 30] with parallel-group design, excluding two studies which were crossover [26, 27]. Meta-analysis of these trials revealed MD of −1.22 kg (95% CI: −2.29, −0.14). Heterogeneity was substantial. A second meta-analysis for studies with parallel group designs and dosage ranges of HCA between 1 and 1.5 g per day [5, 24, 25, 30] did not reveal a significant difference between HCA and placebo; heterogeneity was also substantial in this analysis. A third meta-analysis excluding three studies with outlying data for MD [6, 29, 30] did not reveal a significant difference in weight loss between HCA and placebo, but heterogeneity was considerable. A further meta-analysis of the two trials with good methodological quality [6, 25] revealed a non-significant difference in weight loss (MD: 0.88 kg; 95% CI: −0.33, 2.10) between HCA and placebo, with I^2 value of 0, suggesting that heterogeneity might not be important. Finally, a meta-analysis of the change in BMI for four studies [6, 24, 29, 31] did not reveal any significant difference between HCA and placebo (MD: −0.34 kg; 95% CI: −0.88, 0.20), with I^2 value of 0.

One study [2] reported a significant decrease in fat mass in the HCA group compared with placebo ($P < .05$), while two studies [4, 24] reported a significant decrease in visceral, subcutaneous, and total fat areas in the HCA group compared with placebo ($P < .001$). In contrast two other studies [5, 25] found no significant difference in body fat loss between HCA and placebo.

Adverse events reported in the RCTs included headache, skin rash, common cold, and gastrointestinal (GI) symptoms. In most of the studies, there were no major differences in adverse events between the HCA and placebo groups. However, in one trial, GI adverse events were twice as frequent in the HCA group compared with the placebo group [25]. In total, there were 88 drop outs. A further 45 participants were reported to have been excluded from the analysis in one trial [5] because they either took a mixture of HCA and placebo (28), or were males (17).

4. Discussion

The objective of this systematic review was to assess the efficacy and effectiveness of HCA as a weight reduction agent. The overall meta-analysis revealed a small difference in change in body weight between the HCA and placebo groups. The effect is of borderline statistical significance and is no longer significant on the basis of a sensitivity analysis

Study or subgroup	HCA Mean	HCA SD	HCA Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Mean difference IV, random, 95% CI
Hayamizu 2001	0	11.5	20	0.5	11.7	20	1.4%	−0.5 [−7.69, 6.69]
Heymsfield 1998	−3.2	3.3	66	−4.1	3.9	69	14.7%	0.9 [−0.32, 2.12]
Kovacs 2001a	−0.4	0.9	21	−0.5	1.4	21	18%	0.1 [−0.61, 0.81]
Kovacs 2001b	−1.5	1.66	11	−1	1.34	11	14.4%	−0.5 [−1.76, 0.76]
Mattes 2000	−3.7	3.1	42	−2.4	2.9	47	14.4%	−1.3 [−2.55, −0.05]
Preuss 2004a	−4.5	16.6	19	−1.6	34.1	16	0.2%	−2.9 [−21.2, 15.4]
Preuss 2004b	−5.5	23.7	9	−1.4	17.3	10	0.2%	−4.1 [−22.93, 14.73]
Ramos 1995	−4.1	1.8	18	−1.3	0.9	17	16.6%	−2.8 [−3.74, −1.86]
Roongpisuthipong 2007	−2.8	0.5	23	−1.4	0.5	19	20%	−1.4 [−1.7, −1.1]
Total (95% CI)			229			230	100%	−0.88 [−1.75, −0]

Heterogeneity: $\tau^2 = 0.98$; $\chi^2 = 38.5$, df = 8 ($P < .00001$); $I^2 = 79\%$

Test for overall effect: $Z = 1.96$ ($P = .05$)

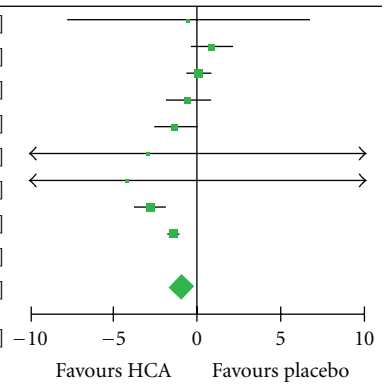

Figure 2: Forest plot of comparison showing the effect of hydroxycitrate on body weight. The *vertical line* represents no difference in weight loss between HCA and placebo.

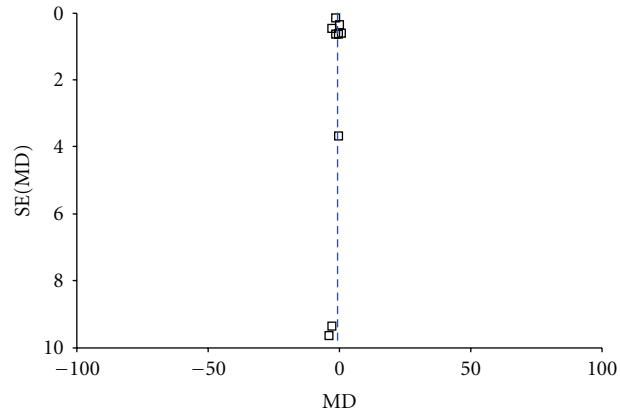

Figure 3: Funnel plot of the mean difference in body weight reduction trials of HCA, plotted against sample size. The *vertical line* depicts the weighted mean difference of all trials.

of rigorous RCTs. Arguably the overall effect size is also too small to be of clinical relevance. The overall meta-analytic result corroborates the findings from one of the studies without suitable data for statistical pooling [31], but is at variance with another study [4].

The overall result should be interpreted with caution. The pooled data from some of the studies were adjusted values. Three studies with small sample sizes [6, 29, 30] seemed to have influenced the overall meta-analytic result in favour of HCA over placebo. If these three trials are excluded, the meta-analysis result is no longer significant. The largest and most rigorous RCT [25] found no significant difference in weight loss between HCA and placebo.

The result of our systematic review corroborates the findings from a previous systematic review of weight loss supplements, which reported that the weight reducing effects of most dietary supplements is not convincing [32]. HCA is a commonly marketed as a complementary weight loss supplement. The meta-analysis from this systematic review suggests that HCA is not as effective as conventional weight loss pills, for example, orlistat. In a meta-analysis report of 16 studies including over 10 000 participants [33], overweight and obese patients taking orlistat had a clinically significant reduction in body weight compared to placebo (MD: 2.9 kg; 95% CI: 2.5, 3.2). Participants taking orlistat achieved a 5% and 10% weight loss compared to placebo in the results from pooled data. This contrasts quite sharply with the results from the meta-analysis of HCA clinical trials.

All of the studies included in this review had methodological issues, which are likely to have affected the outcomes in these trials. This is supported by the I^2 values from the overall meta-analysis result which suggested substantial heterogeneity. Most of the studies included in this systematic review had small sample sizes. Only one included study [25] reported that they performed a power calculation. Larger study sizes with *a priori* sample size calculation will help eliminate a type II error (i.e., failure to reject the null hypothesis when it should have been rejected). Only one study [25] performed an intention to treat (ITT) analysis, while all the participants in three other studies [24, 26, 27] were reported to have completed the trial. The failure of about 66% of the included studies to report ITT analyses casts a doubt as to the validity of their results. In several of the RCTs, drop-outs/attrition was unclear. In one study [5], participants were excluded due to mixed-pill ingestion (an error in coding of pill bottles resulted in some participants receiving a mixture of HCA and placebo). Male participants were also excluded from the analysis of this RCT because they were too few in number compared with females in the trial. It was also unclear to which intervention group the excluded participants belonged to in this study.

The dosage of HCA, and the duration of study also varied amongst the RCTs. The dosage of HCA used ranged from 1 g to 2.8 g daily. The optimal dose of HCA is currently unknown. Two included studies which differed widely in results [25, 29] also differed widely in dosage of HCA.

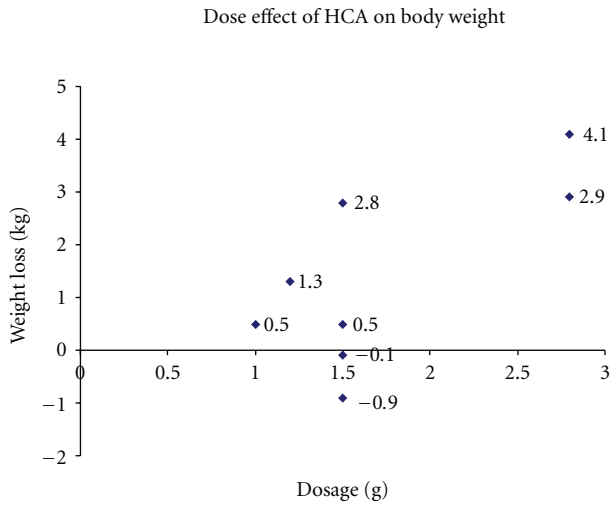

Figure 4: Effect of dosage of HCA on body weight. The dosages from included RCTs did not produce a linear effect on body weight.

Though one of these studies claimed the bioavailability of the HCA used in their trial was high [25], the dosage of HCA used was almost twice that used in the other trial [29]. It is not clear if the higher HCA dosage ensures a higher bioavailability of HCA. A nonlinear, significant ($P < .05$) correlation between the dosage of HCA and body weight loss seems to exist (Figure 4). *Garcinia cambogia* was the main source of HCA in most studies, with *Garcinia atroviridis* being the source of HCA in one included study [2]. None of the trials used *Garcinia indica* as an intervention. It is unclear if the strain of *Garcinia* species influences the bioavailability of HCA. Furthermore HCA is also reported to be found in *Hibiscus subdariffa* [8], and none of the studies included in this review used HCA extracted from this plant species. The duration of the studies included in the review also differed, with a range of 2 to 12 weeks, and mode of 8 weeks. This is probably too short a time to assess the effects of HCA on body weight.

There was some variation in the design of the RCTs included in the review. All of the studies included had parallel-study designs except two which were crossover trials [26, 27]. Four included RCTs comprised three intervention groups [6, 26, 27, 29]. None of the included studies indicated whether or not outcome assessors were blinded, and seven studies did not specify the source of funding [2, 4, 6, 24, 28, 29, 31]. The failure of study investigators to adhere strictly to the CONSORT guidelines [10, 11] may have contributed to the variation in methodology (and heterogeneity) of the trials included in the review.

Most (7/12) RCTs reported adverse events, with headache, nausea, upper respiratory, and gastrointestinal tract symptoms being the most frequent ones. In most of the trials, there were no significant differences in adverse events between HCA and placebo. This seems to corroborate the report in another article [34] which suggested that HCA is safe for human consumption. A few of the studies reported a positive effect of HCA on the blood lipid profile [6, 24, 29–31], while one did not find any significant difference between HCA and placebo on this blood parameter [2]. However, given the short duration of the studies involving the use of HCA, it is unclear how safe this dietary supplement is on the intermediate and long term. In 2009, the Food and Drug Administration (FDA) warned consumers about the potential for serious adverse effects associated with the consumption of hydroxycut, a popular HCA-containing slimming pill. This resulted in the withdrawal of this supplement from the market [35].

All of the studies included in this review except two [26, 27] incorporated some form of dietary control into their trials, with participants in one study receiving high fibre diets [25]. The daily caloric intake for participants in the trials included in this review ranged from as low as 1,000 kcal [2, 30], to as high as 3,009 kcal [27]. Half the number of studies in this review did not institute any form of exercise. The extent to which the variation in these lifestyle adjustment factors could have influenced study results is uncertain. Two studies [28, 31] reported a significant reduction in appetite in the HCA group ($P < .001$), but not with placebo. Three other studies did not find any significant difference between HCA and placebo groups in terms of satiety effect [5, 26, 27].

All of the studies described their participants as overweight, obese, or both. However, in one RCT [2], the definition of the participants as obese individuals is questionable, because they had a BMI between 25–30 kg/m^2. Based on the World Health Organisation definition [36], a BMI between 25–29 kg/m^2 is considered overweight, while a BMI $\geq$ 30 kg/m^2 is termed obese.

This systematic review has several limitations. Though our search strategy involved both electronic and non-electronic studies, we may not have identified all the available trials involving the use of HCA as a weight loss supplement. Furthermore, the methodological quality of most of the studies identified from our searches is poor, and most studies are of short duration. These factors prevent us from drawing firm conclusions about the effects of HCA on body weight.

5. Conclusion

The evidence from RCTs suggests that *Garcinia* extracts/HCA generate weight loss on the short term. However, the magnitude of this effect is small, is no longer statistically significant when only rigorous RCTs are considered, and its clinical relevance seems questionable. Future trials should be more rigorous, longer in duration, and better reported.

Conflict of Interests

I. Onakpoya was funded by a grant from GlaxoSmithKline. The funder had no role in the preparation of the paper. S. K. Hung, R. Perry, B. Wider and E. Ernest declare no potential competing interests.

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Review Article

Pharmacotherapies for Obesity: Past, Current, and Future Therapies

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Past therapies for the treatment of obesity have typically involved pharmacological agents usually in combination with a calorie-controlled diet. This paper reviews the efficacy and safety of pharmacotherapies for obesity focusing on drugs approved for long-term therapy (orlistat), drugs approved for short-term use (amfepramone [diethylpropion], phentermine), recently withdrawn therapies (rimonabant, sibutamine) and drugs evaluated in Phase III studies (taranabant, pramlintide, lorcaserin and tesofensine and combination therapies of topiramate plus phentermine, bupropion plus naltrexone, and bupropion plus zonisamide). No current pharmacotherapy possesses the efficacy needed to produce substantial weight loss in morbidly obese patients. Meta-analyses support a significant though modest loss in bodyweight with a mean weight difference of 4.7 kg (95% CI 4.1 to 5.3 kg) for rimonabant, 4.2 kg (95% CI 3.6 to 4.8 kg) for sibutramine and 2.9 kg (95% CI 2.5 to 3.2 kg) for orlistat compared to placebo at ≥12 months. Of the Phase III pharmacotherapies, lorcaserin, taranabant, topiramate and bupropion with naltrexone have demonstrated significant weight loss compared to placebo at ≥12 months. Some pharmacotherapies have also demonstrated clinical benefits. Further studies are required in some populations such as younger and older people whilst the long term safety continues to be a major consideration and has led to the withdrawal of several drugs.

1. Introduction

Management strategies for weight reduction in obese individuals include physical interventions such as exercise, diet, and surgery, behavioural therapies, and pharmacological treatments. These strategies may be used alone or in combination for greater efficacy. Most randomized controlled trials (RCTs) evaluating pharmacotherapies include a calorie-controlled diet, and some also encourage participants to increase their physical activity.

Drugs used to induce weight loss may reduce appetite or increase satiety, reduce the absorption of nutrients, or increase energy expenditure. Weight loss with pharmacotherapies is generally modest, that is, usually 2 to 7.9 kg more than that achieved with placebo treatment [1]. In the past drug therapies available have included thyroid hormone, dinitrophenol and amphetamines, followed by amphetamine analogues, aminorex, and the fenfluramines [1]. More recently a number of newer agents have been trialed though only orlistat and sibutramine were approved for long-term use (≥24 weeks). Following the recent withdrawal of sibutramine this leaves only orlistat (Table 1).

Amongst the drugs marketed for weight loss there have been several instances of market withdrawal due to serious adverse events. The agents involved include dinitrophenol, aminorex, the fenfluramines, phenylpropanolamine and most recently rimonabant. Other drugs such as the amphetamines are severely restricted due to their abuse potential. Fenfluramine and dexfenfluramine were recalled from the world market in 1997 due to concerns of an increased prevalence of valvular heart disease, and the possible association with primary pulmonary hypertension [2–6]. In April 2000, the European Medicines Agency (EMEA) recommended the withdrawal of several weight loss drugs from the market including phentermine, amfepramone (diethylpropion) and mazindol due to an unfavourable risk

Table 1: Drugs used for weight loss in obesity.

Drug	Introduced	Mechanism of action	Status
Dinitrophenol	1930s	Increases metabolic rate	Withdrawn—risk of neuropathy and cataracts
Amphetamines: dexamphetamine, methamphetamine	1936	Appetite suppression	Banned, restricted or discouraged—dependency and abuse potential, cardiovascular adverse effects
Amphetamine-like analogues: Phentermine, diethylpropion, phenylpropanolamine	1959-US	Appetite suppression	Diethylpropion—available for short-term use (≤12 weeks) Phentermine—available for short-term use (≤12 weeks) in some countries, withdrawn 2000 (UK) Phenylpropanolamine-withdrawn 2000—increased risk haemorrhagic stroke
Aminorex	1965	Appetite suppression	Withdrawn 1968—pulmonary hypertension
Mazindol	1970s	Appetite suppression	Discontinued 1993—Australia
Fenfluramine	1963-Europe 1973-US	Appetite suppression	Withdrawn 1997—valvular heart disease, pulmonary hypertension
Dexfenfluramine	1985-Europe 1996-US	Appetite suppression	Withdrawn 1997—valvular heart disease, pulmonary hypertension
Orlistat	1998-Europe and US	Decreased fat absorption	Also available *over-the-counter* in several countries
Sibutramine	1997-US 2001-Europe	Appetite suppression	Temporarily withdrawn 2002 Italy-concerns of raised risk of heart attacks and strokes Increase in contraindications 2010-US, Australia Suspension of market authorization 2010
Rimonabant	2006-Europe		Withdrawn 2009—potential of serious psychiatric disorders

to benefits ratio [7]. This was followed by the voluntary withdrawal of medications containing phenylpropanolamine due to reports of haemorrhagic stroke in women [8] (Table 1).

Rimonabant was approved as an adjunct to diet and exercise for the treatment of obese or overweight patients by the EMEA in 2006. However the FDA never approved its use in the US due to serious safety concerns. Then in January 2009, the EMEA withdrew market authorisation for rimonabant in all countries of the European Union due to an increased risk of psychiatric adverse events, including depressed mood disorders, anxiety, and suicidal ideation [9–11]. Concern was recently raised regarding the safety of sibutramine, following earlier reports of increased systolic and diastolic blood pressure and heart rate [10]. With this concern in mind, the safety was investigated in patients with a history of cardiovascular disease in the Sibutramine Cardiovascular Outcomes Trial (SCOUT). The release of preliminary results from SCOUT led to the compulsory inclusion of contraindications and precautions in the US and Australian product information, whilst the EMEA recommended total suspension of market authorisation for the drug in Europe [12–16]. Following the subsequent publication of the SCOUT study [17] the FDA considered whether to severely restrict access to the sibutramine or remove it from the market. Sibutramine was subsequently withdrawn by the manufacturer.

Some drugs which had demonstrated positive weight loss potential such as taranabant have been abandoned during late phase clinical trials due to unacceptable adverse events. Whilst axokine, a ciliary neurotrophic factor that was administered as a daily subcutaneous injection, was abandoned due to the low percentage of responders as a result of the development of antibodies in the majority of patients taking the drug [18].

The efficacy and safety of long-term drug therapy is a very important consideration in the management obesity which often requires ongoing therapy to achieve and maintain the weight loss. This paper provides a review of the efficacy and safety of drug therapies for weight loss with at least six months of patient follow-up focusing on randomised controlled trials (RCTs) published over the last 4 years of recent past and current pharmacotherapies, as well as those in late phase clinical trials.

2. Measuring Effectiveness of Drug Therapy

There are some challenges in establishing the medium and longer-term efficacy of pharmacotherapies designed to induce weight loss. These include the continuance of patients throughout the entire study duration and the likelihood that patients who report more weight loss will be more likely to complete the study. In an effort to control bias from this source the use of last observation carried forward is commonly used to approximate weight loss for the patients withdrawing from a study [7, 19, 20].

There is also some controversy as to which primary outcome measures are best to evaluate the efficacy of drug therapies, that is, absolute weight loss (in excess of placebo), percentage weight loss, percentage of patients achieving ≥5% or ≥10% weight loss of initial weight, BMI, or waist circumference (WC). The length of time over which weight loss is sustained is also important which implies prolonged follow-up, at least twelve months or if possible longer. In studies involving children, the BMI appears to be the most appropriate measure of effectiveness [21]. Secondary efficacy endpoints are increasingly reported especially in more recent studies, and these include clinical measures such as blood pressure, glycaemic control (blood glucose or HbA1C levels) and cholesterol levels [14, 22, 23].

3. Past Drug Therapies and Current Approved Drugs

Drugs that have been prescribed or evaluated for obesity may reduce fat absorption or regulate satiety via their action on serotonin, noradrenergic or dopaminergic or the cannabinoid receptor systems in the brain (Table 2) [1, 3, 24–26].

3.1. Amphetamines and Amphetamine-Like Analogues. Amphetamines and amphetamine-like analogues (phentermine, diethylpropion, phenylpropanolamine) are indirect-acting sympathomimetic agents that act by releasing noradrenaline (NA) from presynaptic vesicles in the lateral hypothalamus [1]. Mazindol, a related but discontinued drug, blocks the reuptake of NA by presynaptic neurons (Table 2) [1]. The increase in NA concentration within the synaptic cleft results in the stimulation of β2-adrenergic receptors and a resultant inhibition of appetite.

There is little data from large randomized controlled trials (RCTs) relating to the long-term efficacy or safety of amphetamines and amphetamine-like analogues, especially when used as monotherapy. These drugs have limited use in the routine management of obesity and are not currently approved for long-term use. *Phentermine* has been available since the late 1950s and is approved for short-term use in the US and Australia (Table 2). It has been evaluated as both monotherapy and as combination therapy though not in large-scale studies [27, 28]. A 36-week RCT in 108 overweight women demonstrated a mean weight loss of 12.2 kg (13%) with phentermine (30 mg daily) compared to 4.8 kg (5.2%) with placebo ($P < .001$). Phentermine has been used in combination with fenfluramine and with fluoxetine [29]. Combination therapy with phentermine (15 mg) and fenfluramine (60 mg), demonstrated significantly more weight loss than placebo in a 28-week RCT (15.5% versus 4.9%, $P < .001$) [28]. Phentermine is currently under evaluation in combination with topiramate and with pramlintide *(see Drug Monotherapies and Combination Therapies in Clinical Development)*.

3.1.1. Diethylpropion (amfepramone). another amphetamine-like analogue has been available for weight loss since the early 1960s; however there are few if any RCTs of its long-term use especially with large sample sizes [30, 31]. Diethylpropion (75 mg daily) demonstrated significantly greater weight loss in a small 24-week study of 20 patients than placebo (11.6 kg versus 2.5 kg, $P < .01$) [31]. Recently, diethylpropion (50 mg twice a day) was shown to be more effective than placebo in a small 6-month RCT with 69 obese adult patients (9.3 kg [95% CI 7–11.5 kg] versus 3.1 kg [95% CI 1.8–4.3 kg], $P < .0001$) [32]. Greater than 5% weight loss was achieved in 67.6% of diethylpropion patients and 25.0% of those receiving placebo ($P = .0005$). After further 6 months during an open label period of the study patients who were originally in the diethylpropion group lost a mean of 10.1 kg (95% CI 7.5–12.8). The most common side effects were dry mouth and insomnia ($P = .02$ and $P = .009$, respectively). These were experienced in the first 3 months but become less apparent with continuing treatment [32].

3.2. Fenfluramines. Fenfluramine and dexfenfluramine elevate serum levels of serotonin (5HT) in the central nervous system by stimulating 5HT release and inhibiting its reuptake (Figure 1). Increased levels of 5HT appear to stimulate the hypothalamus, which controls satiation as well as mood, sleep, body temperature and other vital functions. These agents also activate melanocortin 4 receptors that in turn stimulate activation of 5-HT2C receptors, producing an increased release of 5HT within the hypothalamic-pituitary-adrenal axis which is claimed to lead to hypophagia and anorexia [33–36].

A meta-analysis of RCTs with fenfluramine and dexfenfluramine demonstrated higher weight loss than placebo following up to 12 months of treatment. The greatest efficacy was shown following 3 months treatment, 3.7 kg weight loss [37].

Although RCTs with fenfluramines (fenfluramine and dexfenfluramine), either alone [38, 39] or with phentermine [40], demonstrated significant weight-loss, they were withdrawn from the market due to increased reports of valvular heart disease and primary pulmonary hypertension [2, 3, 29, 41–43]). The prevalence rates of both valvular heart disease and primary pulmonary hypertension were higher following longer exposure to the fenfluramines [3].

3.3. Antidepressants

3.3.1. Fluoxetine, Bupropion. Fluoxetine, a selective serotonin reuptake inhibitor (SSRI) that augments 5HT within the central nervous system has been prescribed *off-label* for weight loss. Although significant weight loss was reported

Table 2: Central mechanisms of action of anti-obesity pharmacotherapies.

Central Subsystem	Drugs targets	Possible receptor subtypes involved
Monoamine system (indirect agonists and subtype selective receptor antagonists)	Single therapies	
	(i) Dex/fenfluramine (WD), fluoxetine	(i) 5HT
	(ii) Phentermine/Diethylpropion (ST)	(ii) DA, NA
	(iii) Sibutramine	(iii) α_1, β_1, $\beta_3$adrenergic and $5HT_{2B/C}$
	(iv) Bupropion	(iv) DA, NA
	(v) Tesofensine	(v) DA, NA, 5HT
	(vi) Lorcaserin	(vi) $5HT_{2C}$
Opioid system (μ-opioid receptor antagonist)	(i) Naltrexone	(i) μ-opioid
	(ii) Topiramate	(ii) AMPA/kainite glutamate*
	(iii) Zonisamide	(iii) 5HT, DA*
Cannabinoid system	Single therapies:	
	(i) Rimonabant (WD)	(i) CB_1
	(ii) Taranabant (DC)	(ii) CB_1
Monoamine/Opioid system	Bupropion/naltrexone	(i) DA, NA/μ-opioid
	Bupropion/zonisamide	(ii) DA, NA/5HT, DA*
Neuropeptide Y/Agouti-related peptide system	Pramlintide/metreleptin	(i) Calcitonin receptor*/Leptin receptor

5HT: serotonergic, DA: dopaminergic, NA: noradrenergic, WD:withdrawn; DC: phase III trials discontinued; ST: short term;*: unknown; AMPA: α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate.

with 60 mg of this agent in short-term studies of 6–8 weeks, with maximum weight loss achieved at 12–20-weeks, this is followed by a regain in bodyweight [44–47]. Most RCTs have not shown a significant difference when fluoxetine was compared to placebo at 52 weeks [46, 48]. Significantly greater weight loss has however been demonstrated at 8 months when fluoxetine was used in combination with dexfenfluramine (13.4 versus 6.2 kg with placebo) [49]. In clinical practice fluoxetine 10–20 mg has been used with phentermine (i.e., *phen-pro or phen-flu*) but there are no RCTs of either the long-term efficacy or safety of this combination [50]. A retrospective chart review suggested this combination is not as effective as fenfluramine with phentermine [51]. Fluoxetine generally has a tolerable safety profile with reported adverse events of headache, asthenia, nausea, diarrhoea, somnolence, insomnia, nervousness, sweating, and tremor [47].

3.3.2. Bupropion. is another antidepressant which inhibits reuptake of dopamine (DA) and noradrenaline (NA) resulting in a loss of appetite and decreased food intake [52] and modest weight loss in obese people [53–56]. The efficacy of bupropion as a sustained release (SR) formulation was demonstrated at 48 weeks in obese patients [53]. Weight loss was dose dependent with 7.5% initial weight loss for subjects taking 300 mg bupropion-SR and 8.6% with 400 mg [53]. Bupropion-SR was generally well tolerated, and weight loss was maintained at 48 weeks. A meta-analysis of weight loss treatments which included 5 bupropion studies reported a mean weight loss of 2.8 kg (95% CI, 1.1 to 4.5 kg) at 6 to 12 months with bupropion compared to placebo [56] (Table 3). Although bupropion is not approved for weight loss, it has been used *off-label* and is currently under evaluation as combination therapy with naltrexone, a μ-opioid receptor antagonist and zonisamide, a GABA receptor activator (*see Drug Monotherapies and Combination Therapies under Investigation*).

3.4. Orlistat. Orlistat (a gastrointestinal lipase inhibitor) is a synthetic drug derived from a naturally occurring lipase inhibitor. It does not directly act on appetite as other obesity pharmacotherapies, rather it decreases fat absorption by binding to pancreatic lipase, the principle enzyme that hydrolyses triglyceride (Table 2) (Figure 1) [26]. A detailed review of the efficacy of orlistat treatment in obesity has previously been described [1]. The long-term efficacy of orlistat (120 mg three times daily) for weight loss has been demonstrated in several RCTs of 2- to 4- year therapy compared to placebo [61–64], as well as improvements in blood pressure, insulin resistance, and serum lipid levels [57, 64–66]. Several systematic reviews in adults [56, 57, 67–70] and a systematic review with 2 short-term studies in adolescents [71] demonstrated significantly more weight loss with orlistat than placebo, 6.2 kg (95% CI, 1.7 to 14.0 kg).

The most commonly experienced side effects of orlistat are gastrointestinal and include diarrhoea, flatulence, bloating, abdominal pain, and dyspepsia [25, 66, 70]. Recently, severe liver injury has been reported. The FDA received 32 reports of serious liver injury in patients using orlistat between 1999 and October 2008, including 6 cases of liver failure [72]. This prompted the FDA to undertake a review of the safety of orlistat treatment. The review identified a

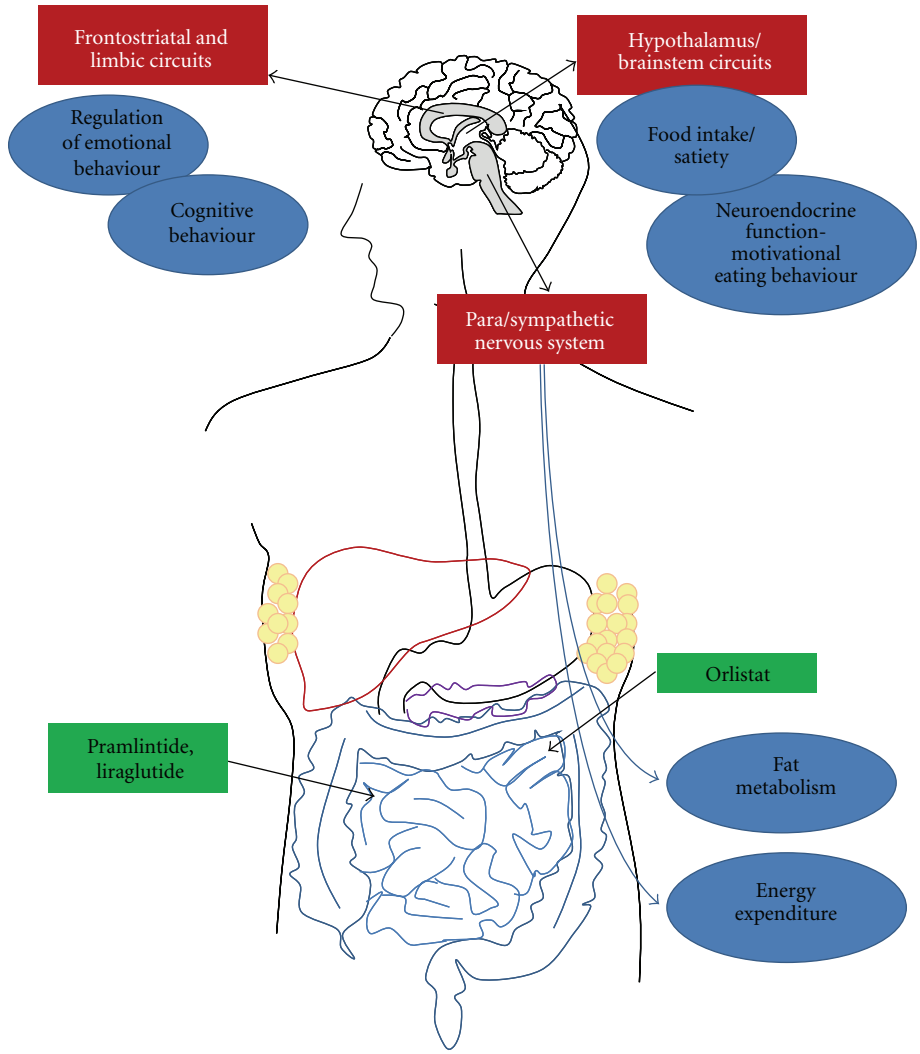

Figure 1: Overview of central and peripheral functions associated with anti-obesity pharmacotherapies.

total of 13 cases of severe liver injury (12 foreign reports with orlistat 120 mg and a US report with the lower dose *over-the-counter* product [orlistat 60 mg]) and in May 2010 led to a label revision and the addition of a warning of severe liver injury.

3.5. Sibutramine. Sibutramine, a 5HT and NA uptake inhibitor, was originally developed as an antidepressant and subsequently found to reduce appetite [26]. It has 2 active metabolites, which inhibit NA and 5HT uptake (and to a lesser extent DA) without any direct effect on neuronal NA, DA and 5HT release. It has been suggested that sibutramine has a dual action to facilitate weight loss, an anorectic effect suggested to be mediated through the central $\alpha_1$and β_1 adrenergic receptors and thermogenic effects through β_3 adrenergic receptors peripherally [73].

Maximal weight loss occurs by 6 months with sibutramine treatment [74, 75] and was dose related [74, 76, 77]. Sibutramine has consistently demonstrated significantly more weight loss than placebo in several RCTs with ≥1 year of therapy [1, 74, 75, 78–80]. Systematic reviews which included 7 sibutramine RCTs reported 4.3 kg (95% CI: 3.6 kg to 4.9 kg) or 4.6% (95% CI: 3.8% to 5.4%) greater weight loss than placebo [58, 70] (Table 3). There was ≥10% weight loss in 18% (95% CI: 11% to 25%) more sibutramine patients than placebo [58, 70]. Attrition rates in sibutramine studies were approximately 30%–40% [58]. In RCTs of 3 to 12 months that compared sibutramine and orlistat, the weighted mean difference in weight loss was 2.2 kg (95% CI 0.5–3.9) in favour of sibutramine [59]. A systematic review in adolescents which included 5 short-term studies has demonstrated significantly more weight loss with sibutramine than placebo, 5.3 kg (95% CI, 3.5 to 7.2 kg) [71].

Although treatment with sibutramine has resulted in lowered concentrations of cholesterol and triglycerides, blood pressure and pulse rate may be increased [57]. Increases in diastolic blood pressure (DBP) with sibutramine were reported in 2 meta-analyses, one in hypertensive patients which included 2 studies where the weighted mean difference was +3.2 mm Hg (95%CI +1.4 to +4.9 mm Hg)

Table 3: Comparative efficacy of pharmacotherapy from recent meta-analyses of long-term studies in adults (12 months or more).

Drug	No. of studies	Total subjects	Mean weight difference (kg) (95% CI)	Reference
Rimonabant	4	Placebo: ~1600 Rimonabant: ~2500	4.7 (4.1, 5.3)	[9, 57, 58]
Orlistat	14	Placebo: 4509 Orlistat: 4948	2.9 (2.5, 3.2)	[57, 58]
Sibutramine	7	Placebo: 699 Sibutramine: 837	4.2 (3.6, 4.8)	[57, 58]
Sibutramine Orlistat	5	Sibutramine: 229 Orlistat: 249	3.4 (2.3, 4.6)	[59]
Bupropion*	5	Bupropion: 618 Placebo: 344	2.8 kg (1.1 to 4.5 kg)	[56]

CI: confidence interval; * 6 to 12 month studies.
Note: another meta-analysis of 5 studies with rimonabant compared to placebo, did not provide mean weight difference in kg, however the odds ratio was 1.07 (95% CI 0.9, 1.3) [60].

[66], whilst another reported a placebo-controlled change in DBP of +1.7 (95% CI 0.7, 2.6) and a small nonsignificant change in systolic BP (+0.5 mm Hg, 95% CI −1.1, 2.1) [81]. Although sibutramine may reduce body weight by a similar amount as orlistat in hypertensive patients, it does not have the same beneficial effects on BP [65].

Weight loss was significantly greater at 1 year when sibutramine was combined with lifestyle modification (10.8% ± 10.2%, mean ± SD, $P < .05$) and diet (16.5% ± 8.0%, $P < .05$) than when sibutramine was used alone (4.1% ± 6.3%) [82]. Although the addition of orlistat to sibutramine therapy does not appear to enhance weight loss [83, 84], combination therapy with the amylin analogue pramlintide is producing promising results [85] *(see* Titled *Drug Monotherapies and Combination Therapies under Investigation).*

Apart from increases in BP and heart rate the most common side-effects reported with sibutramine are dry mouth, constipation, and headache [57, 66].

Following the report of two sibutramine-related deaths in Britain and serious side effects in France, the EMEA demanded a long-term trial in patients at high risk of cardiovascular disease hence the Sibutramine Cardiovascular Outcome trial (SCOUT) was initiated [86, 87]. SCOUT is a double-blind, randomized, placebo-controlled outcome trial in 10,742 overweight or obese patients at high-risk for cardiovascular disease that commenced recruitment in December 2002. Of the total patients 97% had cardiovascular disease, 88% had hypertension, and 84% had type 2 diabetes [88]. Until recently the only published results from SCOUT were from the 4–6-week lead in period [13–15, 87, 89]. At 6 weeks there was a significant reduction in body weight (2.2 kg), waist circumference (2.0 cm), systolic (3.0 mm Hg) and diastolic blood pressure (1.0 mm Hg) with sibutramine treatment, however pulse rate was increased by 1.5 bpm (all $P < .001$) [89]. Results were similar for the diabetic patients in the study, that is, a 2.1 kg decrease in weight and decrease in blood pressure by 3.5/1.0 mm Hg with sibutramine compared to placebo [14]. A total of 9,800 patients were followed up for six years. The preliminary data released in late 2009, suggested that sibutramine was associated with a higher rate of CV events than placebo [90], whilst data from a FDA early communication indicated that there was an increased rate of CV events (heart attacks, strokes, resuscitated cardiac arrest, CV death) in patients with cardiovascular disease and diabetes (11.9% placebo, 13.9% sibutramine, hazard ratio 1.18, 95% CI 1.02–1.35, $P = .023$) [91]. The EMEA concluded that the benefits of sibutramine did not outweigh the risks and recommended that all marketing authorisations for medicines containing sibutramine should be suspended throughout Europe [10]. The FDA initially allowed sibutramine to be available, but asked for stronger warnings on the product labels [92]. The warning recommended that sibutramine should not be used by people who have a history of stroke or heart attacks and uncontrolled high blood pressure. The recent publication of the SCOUT study which had a mean follow-up period of 3.4 years reported a large number of patients that discontinued treatment (40.2% sibutramine, 42.3% placebo), a higher risk of cardiovascular outcome with sibutramine (11.4% versus 10%, hazard ratio 1.16 95% CI 1.03–1.31, $P = .02$). [17] In particular there was a higher rate of nonfatal MI and nonfatal stroke for sibutramine (4.1% and 2.6%, resp.) than placebo (3.2% and 1.9%).

A 3-year prospective observational study of 15,686 patients prescribed sibutramine in New Zealand has not demonstrated a higher risk of death from a cardiovascular event [93]. The FDA is currently reviewing the potential benefits and risks of sibutramine [94].

3.6. Rimonabant. Rimonabant, an endocannabinoid receptor (subtype 1) blocker, was developed as a result of observations on the appetite stimulation associated with recreational cannabis use (Table 2). The drug has a range of both central and metabolic peripheral effects and had also been investigated for smoking cessation [26, 95].

Attrition rates in a pooled study of 5,580 patients without diabetes and 1,047 patients with diabetes taking rimonabant 20 mg daily for one year and a hypocaloric diet were approximately 40% [96]. In the nondiabetic patient subgroup, rimonabant reduced body weight by 6.5 kg compared to

placebo ($P < .001$). Weight-loss of ≥5% was achieved in 50.8% of the treatment group, and waist circumference was reduced by 6.4 cm compared to placebo ($P < .001$) (Table 4) [96]. There was an improvement in glycaemic control in diabetic patients with a reduction in mean HbA1C levels of 0.6% ($P < .001$) [96]. Discontinuation due to side-effects occurred in 13.8% of rimonabant patients and in 7.2% of placebo patients. The most commonly experienced adverse events were gastrointestinal disorders, mood alterations with depressive symptoms, anxiety, dizziness, nausea, and upper respiratory tract infections.

Four large Rimonabant in Obesity and Related Metabolic Disorders (RIO) Phase III studies (*RIO-Europe, RIO-North America, RIO-Diabetes, RIO-Lipids*) were included in two meta-analyses and a systematic review to investigate the efficacy and safety of rimonabant in improving cardiovascular and metabolic risk factors in overweight patients [9, 60] (Table 3). Compared with placebo, rimonabant (20 mg) produced a 4.9 kg (95% CI 4.3, 5.0) greater reduction in body weight as well as improvements in waist circumference (−3.84 cm, 95% CI −4.26, −3.42), high-density lipoprotein cholesterol, triglyceride levels, and systolic and diastolic BP [60]. A subsequent meta-analysis which included the 4 RIO studies provided evidence of the likelihood of experiencing serious side effects with rimonabant [9]. The odds ratio (OR) for depression was 2.51 (95% CI, 1.23–5.12) and 3.03 (95%, 1.09–8.42) for anxiety [9]. A systematic review and meta-analysis reported that the 20 mg rimonabant dose was associated with an increased risk of adverse events (RR 1.35; 95% CI 1.17–1.56), increased discontinuation rate (RR 1.79; 95% CI 1.35–2.38), and psychiatric (RR 2.35; 95% CI 1.66–3.34), and nervous system adverse events (RR 2.35; 95% CI 1.49–3.70) [100]. The number needed to harm (NNH) for psychiatric adverse events was 30 [100]. In a comparison with other pharmacotherapies the risk ratios for discontinuation in RCTs due to adverse events were significantly elevated for rimonabant (2.00; 95% CI 1.66–2.41) and orlistat (1.59; 95% CI 1.21–2.08), but not sibutramine (0.98, 95% CI 0.68–1.41) [20]. The risk difference was largest for rimonabant (7%, 95% CI: 5%–9%; NNH 14, 95% CI: 11–19) compared with placebo, followed by orlistat (3%, 1%–4%; NNH 39, 95% CI: 25–83), while no significant difference was seen for sibutramine (0.2%, 95% CI: −3% to 4%; NNH 500).

In late 2008, the manufacturers of rimonabant announced that all clinical research studies would be stopped permanently. This announcement followed a decision by the EMEA to withdraw marketing of the drug as the risks especially of psychiatric side effects were considered to outweigh the drug's benefits [101].

3.7. Systematic Reviews Comparing Several Drug Therapies. Several meta-analyses and systematic reviews have demonstrated that pharmacotherapy in combination with a low calorie diet and in some cases exercise generally results in a maximum weight reduction at six months of 1–9.6 kg, maintenance of weight loss with continued therapy, and a regain in weight after drug therapy is discontinued [7, 30]. The largest mean effect sizes were demonstrated with amphetamines, fenfluramines and sibutramine, though no drug demonstrated clear superiority [30, 58] and most of the drugs have been prescribed for a limited duration. A systematic review which included 14 RCTs with orlistat, 7 RCTs of sibutramine and 4 RCTs with rimonabant compared to placebo, reported 2.9 kg greater weight loss with orlistat than placebo, 4.2 kg for sibutramine and 4.7 kg for rimonabant (Table 3). Patients on active drug therapy were significantly more likely to achieve ≥5% and ≥10% weight loss [57]. Continuation on treatment was a problem with attrition rates averaging 30%–40% within 12 months [57].

In adolescents a meta-analyses of RCTs with orlistat and sibutramine demonstrated a mean decrease in weight between the intervention and control groups of 5.25 kg (95% CI: 3.03–7.48) after a minimum follow-up of 6 months [71]. Systemic reviews of pharmacotherapy for overweight and obese children, adolescents, and older adults only include a limited number of mainly short-term studies [21, 102–104] hence, there is a lack of high-quality evidence to support the efficacy and safety of drug therapy in these populations.

4. Drug Monotherapies and Combination Therapies under Investigation

Some already marketed drugs (that are approved for other indications) and several new agents are currently being evaluated for the management of obesity [25, 105, 106]. These include tesofensine, a pharmacological agent that targets the inhibition of NA, DA, and 5HT reuptake and, liraglutide a glucagon-like peptide-1 analog and lorcaserin the selective serotonin 2C (5-HT2C) receptor agonist (Table 2). There are also several combination drug therapies in Phase III trials including bupropion and naltrexone, bupropion and zonisamide, phentermine and topiramate, and pramlintide and metreleptin. Some drugs that were in late phase trials such as axokine, a naturally occurring re-engineered human protein known as cilary neurotrophic factor, taranabant a CB1R inverse agonist, and ecopipam a selective dopamine D1/D5 antagonist have been abandoned, the latter two due to an increase in psychiatric adverse events. There are also some weight loss medications that have previously been used in the management of diabetes that are being evaluated for weight loss, that is, pramlintide, liraglutide, and exenatide.

4.1. Pramlintide. Pramlintide, a synthetic analog of the pancreatic hormone amylin, was originally used for the treatment of type 1 and 2 diabetes. It has been associated with reduced, appetite, food intake and enhanced satiety through delayed gastrointestinal motility and is currently under investigation as a potential treatment for obesity [25, 105]. In a 16-week dose escalation RCT 3.7% mean weight loss was demonstrated with pramlintide 240 μg given as a subcutaneous (SC) injection compared to placebo ($P < .001$) and ≥5% weight loss was achieved in 31% of patients ($P < .001$) [107]. In obese patients participating in a 4-month RCT of pramlintide at doses of 120, 240, and 360 μg administered two or three times a day, followed by a single blind extension to 1 year, weight loss was regained in the placebo group but maintained or continued in all but the

Table 4: Recent randomised controlled trials of weight loss therapies with 6-month followup.

Drug	No. subjects	Outcomes				Serious adverse events	Reference
		Absolute weight loss (kg) (95% CI)	≥5% weight loss	≥10% weight loss	Change in WC (cm)		
Bupropion (400 mg) plus Naltrexone	Placebo-85	0.9 ± 0.5	15%	2%	−0.9 ± 0.9		[22]
	N 48 mg-56 B-60	1.1 ± 0.7	10%	2%	−3.8 ± 1.2	Nausea: 3.5% P, 28.1% BN16, 39.7% BN32, 41% BN48	
	BN16 mg-64	2.6 ± 0.6	26%	7%	−2.9 ± 1.1		
		5.1 ± 0.6$P < .05$	52%, $P < .05$	17%, $P < .05$	−3.7 ± 1.1, $P < .05$		
	BN 32 mg-63	5.1 ± 0.6$P < .05$	51%, $P < .05$	19%, $P < .05$	−4.6 ± 1.0, $P < .05$		
	BN 48 mg-61	4.0 ± 0.6$P < .05$	39%, $P < .05$	15%, $P < .05$	−4.7 ± 1.2, $P < .05$		
Bupropion (120 mg/360 mg) + Zonisamide (360 mg/360 mg)	729 (total)	1.4^ P	15%^ P	4%^ P	NA	NA	[97]
		3.2^ Z120 5.3^ Z360	27%^ Z120 44%^ Z360	9%^ Z120 18%^ Z360			
		2.3^ B360	21%^ B360	11%^ B360			
		6.1^ ZB120 $P < .001$ 7.5^ ZB360 $P < .001$	47%^ ZB120 ($P < .05$) 60%^ ZB360 ($P < .05$)	25%^ ZB120 $P < .05$ 32%^ ZB360 $P < .05$			
Rimonabant	P-417	0.5 (0.3, 1.3)	NR	NR	1.0 (−0.2, 1.9)	Psychiatric: 28.4% P, 43.4% R, $P < .001$	[98]
	R-422	4.3 (3.5, 5.1) $P < .001$			4.5 (−3.7, −5.4), $P < .001$	Severe psychiatric: 3.5% P, 4.8% R, $P = .52$	
Rimonabant*	3165 (total)	Placebo 1.6 R 6.5	19.7% 50.8%, $P < .001$	7.8% 27%, $P < .001$	6.4 2.5	Depression: 0.8% P, 1.9% R, NS Anxiety: 0.3 P, 1.0% R, NS	[96]
Tesofensine	Placebo-52	2.2 (0.9, 3.5)	13 (29%)	3 (7%)	−2.4 (0.7, 4.2)	Anger and hostility: 4% P, 14.3% T 1 mg, $P = .018$	[99]
	T 0.25 mg-52	6.7 (5.4, 8.0), $P < .0001$	29 (59%)	17 (35%)	−5.9 (4.0, 7.8), $P = .007$	Increased confusion: P-NA,	
	T 0.5 mg-50	11.3 (9.9, 12.7), $P < .0001$	41 (87%)	25 (53%)	–9·4 (7.7, 11.0), $P < .0001$	T 0.5 mg $P = .015$,	
	T 1.0 mg-49	12·8 (11.6, 14.1), $P < .0001$	42 (91%)	34 (74%)	–9·3 (7.6, 11.0), $P < .0001$	T 1 mg $P = .0003$	

Absolute weight loss: weight loss from baseline; NS: not significant; NR: not recorded; WC: waist circumference; P: placebo; B: bupropion; N: naltrexone; Z: zonisamide; R: rimonabant; T: tesofensine; NA: not available; *pooled non-diabetic patients; #mean ± standard deviation; ^estimated absolute weight based on mean baseline weight of 100 kg.

pramlintide 120 μg twice daily arm [108]. Nausea was the most common adverse event.

4.2. Glucagon-Like Peptide-1 (GLP1) Analogues: Liraglutide, Exenatide. Liraglutide and exenatide are glucagon-like peptide-1 (GLP1) analogues developed and approved for the treatment of type 2 diabetes (Table 2) [109]. Phase III trials of liraglutide have demonstrated beneficial weight loss in obese patients. These analogues have a dual mechanism of action, that is, on the gastrointestinal (GI) tract and the brain. Signals from the GI tract are sent to the brain to increase the secretion of leptin, resulting in suppressed appetite, energy intake and a delay in gastric emptying. A key benefit with long-term use of liraglutide and exenatide is a decrease in HbA1c levels and systolic BP [110, 111]. A recent 20 week dose-ranging RCT of liraglutide (1.2, 1.8 mg, 2.4 mg, 3.0 mg) in comparison with orlistat (120 mg) treatment in 564 nondiabetic obese patients demonstrated a mean weight loss of 4-8 kg, 5.5 kg, 6.3 kg, and 7.2 kg, resp. compared with 2·8 kg with placebo and 4·1 kg with orlistat ($P = .003$ for 1.2 mg, $P < .0001$ for 1.8–3.0 mg liraglutide) [112]. Higher doses of liraglutide (2.4 and 3.0 mg) demonstrated significantly greater mean weight loss than orlistat. The most common adverse events with liraglutide were nausea and vomiting, but these were not significantly different to the placebo group. Patients treated with liraglutide also showed a significant reduction in blood pressure and the prevalence of prediabetes (84%–96%).

Exenatide is currently only in Phase II trials [113] for obesity but early results from an open-label study have demonstrated weight loss as well as an improvement in glycemic control [114].

4.3. Taranabant. Taranabant a cannabinoid CB-1 receptor (CB1R) inverse agonist which reduces appetite and increases energy expenditure has been evaluated for the treatment of obesity [115]. It demonstrated greater weight loss with higher doses in a 12 week RCT that assessed its safety and efficacy. Four Phase III trials have been published, two assessed the risk/benefit profile of low and high doses and one included patients with type 2 diabetes [23, 116–118]. Mean weight loss after 1 year of taranabant was 5.0 kg with the 0.5 mg dose, 5.2 kg with the 1mg, 6.4 kg with the 2 mg compared to 1.4 kg for placebo (all $P < .001$) [118] Significantly more patients achieved ≥5% and ≥10% loss of baseline body weight with taranabant than placebo ($P < .001$ for all doses) (Table 5). Approximately 80% of patients from each taranabant dose group experienced one or more adverse events [118].

A study using higher doses (2 mg, 4 mg, and 6 mg) achieved greater mean weight loss at 1 year of treatment which persisted to 2 years (Table 5) [23]. Although weight loss with the highest dose of 6 mg proved to be the most efficacious after 1 year of treatment, the adverse events were significantly increased with increasing doses particularly serious psychiatric events which included depression, depressive mood, anxiety, anger, and aggression [23]. The odds ratios for suicidality with increasing doses of taranabant after 1 year treatment were 1.74 (95% CI 0.87–3.51) with the 2 mg dose, 2.16 (95% CI 1.10–4.25) for 4 mg, and 2.34 (95% CI 1.11–4.96) with the 6 mg. Hence, only the lower doses (2 mg and 4 mg) were used for the remainder of the study.

The overall safety and efficacy profile of taranabant from the Phase III trials did not support its further development in the treatment of obesity, and clinical trials were ceased [23, 117, 119].

4.4. Lorcaserin. Lorcaserin is a selective serotonin 2C receptor agonist (5-HT2C), sharing characteristics similar to fenfluramines, which acts through another serotonin receptor (5-HT2B) that has been associated with cardiac valvular disease [124] (Table 2).

Recent clinical trials with lorcaserin have demonstrated effective weight loss compared to placebo along with a good safety profile [125, 126]. Results from two recently presented pivotal Phase III trials, BLOOM (Behavioral modification and Lorcaserin for Overweight and Obesity Management) and BLOSSOM (Behavioral modification and Lorcaserin Second Study for Obesity Management) indicated greater weight loss with lorcaserin than with placebo (Table 5) [120, 127, 128]. In these RCTs, 6380 non-diabetic patients aged 18–66 years with a BMI 27–45 kg/m^2 were treated for 52 weeks with lorcaserin 10 mg twice daily or with placebo. Using the pooled data from these two trials, weight loss at 52 weeks decreased by 5.8% in the lorcaserin group and 2.5% in the placebo group ($P < .0001$) [127]. Weight loss was similar amongst males and females but was higher in Caucasian patients than African American patients or Hispanic patients and patients >50 years lost more weight than younger patients. Average weight loss at 1 year in the BLOOM study was 5.8 ± 0.2 kg with lorcaserin and 2.2 ± 0.1 kg with placebo ones ($P < .001$) with 47.5% and 20.3% loosing ≥5% of their body weight (Table 5) [120]. Weight loss was maintained in 67.9% of lorcaserin patients in year 2 and 50.3% of placebo ($P < .001$) (Table 6) [120]. After 52 weeks of lorcaserin treatment, changes in lipid and glucose values were more favourable in responders than nonresponders, and twice as many patients responded to lorcaserin as placebo (i.e., ≥5% body weight loss in 47.1% lorcaserin patients and 22.6% placebo) [128]. The most frequent adverse events reported were headache, dizziness and nausea, but these were not significantly different between treatment groups (Table 5). There was no increase in the rate of cardiac valvulopathy after 2-year treatment with lorcaserin [120].

Although the recently published study indicated lorcaserin was safe and moderately effective, there was a high dropout rate [120]. Lorcaserin was submitted for FDA approval however in September 2010 the advisors recommended against approval as they did not consider that the potential benefits of the drug outweighed the risks. In particular they claimed that patients on lorcaserin did not achieve the percentage point criterion set by the FDA. The FDA which usually takes the advice of its committees is expected to decide in October whether to approve lorcaserin.

4.5. Tesofensine. Tesofensine is another novel pharmacological agent which inhibits the uptake of presynaptic NA, DA, and 5HT (Table 2, Figure 1). Tesofensine was discovered

Table 5: Recent randomised controlled trials of weight loss therapies with 12-month followup.

Drug	No. subjects	Outcomes				Serious adverse events	Reference
		Absolute weight loss (kg) (95% CI)	≥5% weight loss	≥10% weight loss	Change in WC (cm)		
Lorcaserin	Placebo-716	2.16 ± 0.14^	20.3%	7.7%	3.9 ± 0.2	Headache 2% P, 0.8% L	[120]
	L-883	5.81 ± 0.16 $P < .001$	47.5% $P < .001$	22.6% $P < .001$	6.8 ± 0.2 $P < .001$	Dizziness 0.8% P, 0.1% L	
Taranabant	Placebo-417	2.6 (1.8, 3.3)	27.2%	8.4%	−3.1 (2.3, 3.9)	Nausea 6.5% P, 16.7% TB 2 mg, 21.4% TB 4 mg, $P < .001$	[31]
	TB 2 mg-415	6.6 (5.9, 7.4), $P < .001$	56.5%, $P < .001$	27.9%, $P < .001$	−7.0 (6.1, 8.1), $P < .001$	Vomiting 3.4% P, 8.4 TB 4 mg, $P < .01$	
	TB 4 mg-414	8.1 (7.4, 8.9), $P < .001$	64.2%, $P < .001$	35.8%, $P < .001$	−7.5 (6.7, 8.3), $P < .001$	Diarrhoea 7.2% P, 12.3 TB 2 mg, $P < .05$, 13.7% TB 4 mg, $P < .01$	
						Anxiety 3.4 P, 9.9 TB 4 mg, $P < .01$	
Taranabant	Placebo-196	+1.7 (0.8, 2.7)	62.2%	NR	−0.7 (−0.3, 1.8)	Irritability TB 1 mg and 2 mg, $P \leq .038$	[116]
	TB 0.5 mg-196	0.1 (−1.0, 0.8), $P < .007$	71.8%	NR	−1.5 (−2.6, −0.5)		
	TB 1 mg-196	0.6 (−1.5, 0.4), $P < .007$	78%, $P < .05$	NR	−2.3 (−3.4, −1.3)		
	TB 2 mg-196	1.2 (−2.1, −0.3), $P < .007$	83.3%, $P < .001$	NR	−2.4 (−3.4, −1.3)		
Taranabant	Placebo-137	1.4 (−0.5, −2.4)	24.3%	7.3%	−3.0 (−1.9, −4.1)	Psychiatric-17.7% P, 28.8% TB1mg, 29%TB 2 mg, $P \leq .038$	[118]
	TB 0.5 mg-141	5.0 (−4.0, −5.9), $P < .001$	44.2, $P < .001$	21.3, $P < .001$	−5.6 (−4.5, −6.6), $P < .001$		
	TB 1 mg-138	5.2 (−4.2, −6.2), $P < .001$	45.3, $P < .001$	18.2, $P < .001$	−5.7 (−4.6, −6.7), $P < .001$		
	TB 2 mg-277	6.4 (−5.7, −7.2), $P < .001$	53, $P < .001$	28, $P < .001$	−6.9 (−6.1, −7.7), $P < .001$		
Bupropion/naltrexone*	Placebo-202	7.3% ± 0.9%	60.4%	30.2%	−6.8 (5.3, 8.3)	Nausea 10.5% P, 34.1% BN $P < .001$	[121]
	BN 360/32–591	11.5% ± 0.6%, $P < .001$	80.4%, $P < .001$	55.2%, $P < .001$	−10.0 (9.0, 10.9), $P < .001$	Dizziness 4.5% P, 14.6% BN, $P < .001$	
Bupropion/naltrexone	B-60	2.7 ± 0.9	33%	12%	NA	NA	[22]
	BN 16 mg-64	5.0 ± 0.9	50%	22%			
	BN 32 mg-63	6.1 ± 0.8, $P < .05$	51%	25%			
	BN 48 mg-61	4.6 ± 0.9	39%	20%			
Pramlintide	Placebo-63Pramlintide-61	2.1 ± 0.9	3%	11%	NR	Nausea 0% P, 30% Pramlintide	[85]
		3.6 ± 0.7	28%	36%			
Topiramate	Placebo-55	2.5 ± 3.1	19%	2%	−2.3 ± 4.7	Paraesthesia −0% P, 28% TPsychiatric −11% P, 33% T	[122]
	T−54	6.0 ± 5.2, $P < .001$	50%, $P < .001$	20%, $P < .001$	−4.2 ± 5.7, $P = .078$		
Topiramate/phentermine	P-498	1.6	17%	NA	NA	NA	[123]
	TP 3.75/23 mg-234	5.1, $P < .0001$	45%, $P < .0001$				
	TP15/92 mg-498	11, $P < .0001$	67%, $P < .0001$				
Topiramate/phentermine	P-979	1.8	21%	NA	NA	NA	[123]
	TP 7.5/46 mg-488	8.4, $P < .0001$	62%, $P < .0001$				
	TP15/92 mg-981	10.4, $P < .0001$	70%, $P < .0001$				

Absolute weight loss: weight loss from baseline; NS: not significant, NA: not available; L: lorcaserin; NB: naltrexone/bupropion, TB: taranabant; T: topiramate controlled release; TP: topiramate/phentermine; ^mean ± standard error; *both groups also received intensive behavior modification.

Table 6: Recent randomised controlled trials of weight loss therapies with 2-years followup.

Drug	No subjects	Outcomes				Serious adverse events	Reference
		Absolute weight loss (kg) (95% CI)	≥5% weight loss	≥10% weight loss	Change in WC (cm)		
Lorcaserin	P-684	3.0% ± 0.2%	50.3%	7.7%	4.3 ± 0.2	NS	[120]
	L-564	7.0% ± 0.2%	67.9%	22.6%	8.1 ± 0.2		
		$P < .001$	$P < .001$	$P < .001$	$P < .001$		
Taranabant	P-244	1.4 (0.3, 2.5)	30.3%	13.4	−2.7 (1.5, 3.8)	NS	[23]
	TB 2 mg-264	6.4 (5.3, 7.4), $P < .001$	59.6, $P < .001$	33, $P < .001$	−6.3 (5.2, 7.4) $P < .05$		
	TB 4 mg-260	7.6 (6.5, 8.7), $P < .001$	64.8, $P < .001$	37.9, $P < .001$	−7.0 (5.9, 8.1), $P < .01$		

Absolute weight loss = weight loss from baseline; WC: waist circumference, NR: not recorded, TB: taranabant, NS: not significant; P: placebo; L: lorcaserin.

to decrease weight in patients receiving the drug for the treatment of Alzheimer's and Parkinson's disease [129]. Investigators performed a dose-dependent analysis in obese patients for 14 weeks, demonstrating a mean change in weight loss for tesofensine doses of 0.125 mg, 0.25 mg, 0.5 mg and 1 mg of 2.1%, 8.2%, 14.1%, and 20.9%, resp. [129]. Of the total obese patients in the study, 32.1% achieved a ≥5% weight loss with tesofensine, ($P < .001$ for 0.25, 0.5, and 1.0 mg versus placebo). No effect on blood pressure was observed, but there were increases in heart rate with increasing dose.

Further evidence was demonstrated in another 24-week Phase IIb randomised dose-dependent tesofensine trial in 203 obese individuals, with 79% of participants completing the study [99]. Weight loss was dose dependant with 4.5% weight loss (0.25 mg), 9.2% (0.5 mg), and 10.6% (1.0 mg) and was greater than that achieved with diet and placebo ($P < .0001$) (Table 4). The drug was well tolerated with no significant increases in systolic or diastolic blood pressure however, heart rate was increased by 7.4 beats/min in the middose group ($P = .0001$).

4.6. Naltrexone. Naltrexone, a high affinity and long-acting opioid receptor antagonist which was originally produced for the treatment of opioid and alcohol dependence, decreased food intake and led to weight loss in former narcotic addicts. The role of opioid receptors in eating behaviour was initially demonstrated following the administration of naloxone to rats resulting in a significant reduction in short-term food intake by blocking β-endorphin (Table 2) [130]. In RCTs naltrexone (an analogue of naloxone) has not consistently demonstrated statistically significant weight loss in obese and lean subjects [131–134].

4.7. Bupropion Plus Naltrexone (Contrave). Bupropion was combined with the naltrexone following the recognition that naltrexone blocks β-endorphin mediated pro-opiomelanocortin (POMC) autoinhibition to sustain α-MSH release, whilst bupropion (through DA receptors) activates POMC neurons and enhances the release of the anorexiant neuropeptide α-MSH in the hypothalamus [22, 135, 136]. The bupropion-naltrexone combination is said to tackle the motivation/reinforcement that food brings (DA effect) and the pleasure/palatability of eating (opioid effect) [137].

A 24 week dose ranging study of naltrexone/bupropion-SR did not demonstrate increased weight loss with increasing doses of naltrexone (weight loss for 16 mg dose was 4.62% [95% CI: −6.24 to −2.99, $P < .001$], for 32 mg dose 4.65% [95% CI: −6.20 to −3.09, $P < .001$], and for the 48 mg dose 3.53% [95% CI: −5.15 to −1.90, $P < .001$]) (Table 4) [22]. Nevertheless, weight-loss was maintained in a 24-week extended period.

An open-label 24-week study demonstrated that naltrexone 32 mg SR/bupropion-SR 360 mg resulted in significant improvements in depressive symptoms in addition to weight loss and improved control of eating in overweight and obese women with major depression [138]. Depression scores as measured with the Montgomery-Asberg Depression Rating Scale decreased from an average of 23.7 at baseline to 10.5 (consistent with mild depression) at week 12 ($P < .001$) and 8.4 (consistent with remission) at week 24 ($P < .001$).

Several Phase III trials have been conducted in both diabetic and non-diabetic patients including COR-I, COR-II, COR-BMOD and COR-Diabetes [105, 121, 139–141]. COR-Diabetes was a 56-week RCT of 505 overweight or obese patients with type 2 diabetes (Hb A1C levels 7% to 10%, mean 8.0%) randomized to naltrexone 32 mg SR/bupropion 360 mg SR or placebo [140]. The naltrexone/bupropion patients lost significantly more weight (5.0% versus 1.8%, $P < .001$) at 56 weeks [140] with 44.5% of patients achieving ≥5% loss of body weight compared to 18.9% on placebo. Greater improvement in glycemic control was achieved in the treatment group with average baseline HbA1C reduced by 0.6% compared to 0.1% for placebo. The investigators noted that over 44% of treated patients achieved the American Diabetes Association treatment target of <7% for HbA1C compared to 26% of placebo patients ($P < .001$).

This drug combination has generally been welltolerated in most patients (Table 5). Nausea was the most frequent adverse event, and this occurred more frequently with higher naltrexone doses. A new drug application has been submitted

for review by the FDA with the outcome expected in December 2010.

4.8. Bupropion Plus Zonisamide. The combination of bupropion with the epilepsy agent, zonisamide has been evaluated in three Phase II trials [97, 142–144]. The mechanism of action for zonisamide has not been fully characterised, however it has demonstrated biphasic DA and 5HT activity [142, 145]. The potential of zonisamide in the management of obesity was demonstrated in a small RCT where zonisamide patients experienced significantly more weight lost than those on placebo [145]. A 24-week RCT of bupropion 300 mg combined with zonisamide 400 mg achieved greater weight loss (9.2%) than either drugs alone (bupropion 6.6%, zonisamide 3.6%) or placebo (0.4%) [143]. Similar results were observed in a randomised open-label study [142]. Weight loss in a 24 week multicentre RCT with either drug alone and different combinations of zonisamide SR with bupropion SR were 1.4% with placebo, 3.2% with zonisamide SR 120 mg, 5.3% with zonisamide SR 360 mg, 2.3% with bupropion SR 360 mg, 6.1% with zonisamide SR 120 mg/bupropion SR 360 mg, and 7.5% for zonisamide SR 360 mg/bupropion SR 360 mg with ≥5% weight loss in 15%, 27%, 44%, 21%, 47%, 60%, respectively, [97]. The most frequent adverse events reported were headache, nausea and insomnia.

Weight loss with zonisamide and bupropion appears to be greater than that observed with the bupropion/naltrexone combination over the same period of treatment [22].

4.9. Topiramate Plus Phentermine (Qnexa). Topiramate is a GABA agonist and an approved antiepileptic drug which has been trialed as monotherapy for weight loss [1]. It acts as an appetite suppressant that has been suggested to influence kainate/α-amino-3-hydroxy-5-methylisoxozole-4-propionicacid glutamate receptors, voltage-gated sodium channels, and γ-aminobutyric acid-A activity [146], however the exact mechanism of action for weight loss is not known (Table 2). Several RCTs demonstrated greater weight loss with topiramate monotherapy than placebo with continued weight loss throughout the duration of the study [1]. However concerns regarding central and peripheral nervous system adverse effects led to Phase III trials of topiramate being halted and topiramate being reformulated. As the sustained release formulation did not have better tolerability trials were discontinued in December 2004.

The combination of controlled release low dose topiramate with low dose phentermine has recently been shown to be effective for weight loss treatment [147]. A 28-week RCT using phentermine with topiramate (92 mg/15 mg and 46 mg/7.5 mg doses) demonstrated a 9.2% weight loss compared to a 6.4% weight loss with topiramate alone, 6.1% for phentermine alone and 1.7% for placebo [123]. The tolerance and safety of this drug combination are being evaluated in several Phase III trials (EQUATE, EQUIP, CONQUER). In July 2010 an FDA advisory committee agreed that the phentermine/topiramate combination was effective in reducing weight loss however it refused to endorse a recommendation for the treatment of obesity due to safety concerns which included increased heart rate, possible birth defects, and psychiatric problems (depression, suicidal thoughts, impaired memory and concentration) [148]. The final FDA determination on the drug combination is expected in late October 2010.

4.10. Pramlintide Combination Therapies. Pramlintide has been combined with recombinant methyl human leptin (metreleptin), an adipocyte-derived hormone involved in long-term signalling of adiposity and energy intake [149]. In early trials this combination of an amylin and a leptin agonist has demonstrated greater weight loss than either drug alone [148, 149]. Weight loss with pramlintide/metreleptin was 12.7% ± 0.9% (mean ± SE) to week 20 compared with 8.4% ± 0.9% for pramlintide ($P < .001$) and 8.2% ± 1.3% for metreleptin ($P < .01$) [149]. Pramlintide is also being evaluated in combination with sibutramine and phentermine [85]. In a 24-week open-label study weight loss was in subjects taking pramlintide and sibutramine was 11.1% ± 1.1% (mean ± SE), 11.3% ± 0.9% for those taking pramlintide plus phentermine, 3.7% ± 0.7% with pramlintide alone, and 2.2% ± 0.7% with placebo ($P < .001$) [85]. Common side effects experienced with combination treatments were nausea and increased heart rate [85]. There was a significant increase in heart rate and blood pressure with the combination of pramlintide and sibutramine (3.1 ± 1.2 beats/min, $P < .05$; 2.7 ± 0.9 mm Hg, $P < .01$) and pramlintide with phentermine (4.5 ± 1.3 beats/min, $P < .01$; 3.5 ± 1.2 mm Hg, $P < .001$). Pramlintide is also being investigated with exenatide, the GLP-1 agonist used for the treatment of obesity in diabetic and non-diabetic patients [113].

5. Conclusion

Pharmacological interventions in addition to lifestyle changes (diet and physical activity) and in some cases behavioural modifications are used to promote weight loss. At present, only two drugs are currently approved and available for the long-term treatment of obesity—orlistat and sibutramine. However, there are several drugs and combination drug therapies undergoing Phase III trials that may be approved in the next few years. Pharmacotherapies have demonstrated a significant though modest decrease in weight compared to placebo over 1-2 years. Unfortunately weight loss following pharmacological intervention is not sustained when therapy is discontinued with individuals regaining some or all of the weight that was originally lost.

Obesity is often considered a chronic disease, hence it requires long-term therapy. Currently, there is a lack of high quality evidence from long-term studies of both the efficacy and safety of pharmacological interventions for obesity. Serious safety concerns have resulted in the withdrawal of some drugs that had originally received market approval whilst other drugs have been abandoned during Phase III evaluation. An increase in psychiatric disorders following Phase III studies (RIO-Europe, RIO-North America, RIO-Diabetes and RIO-Lipids) with rimonabant treatment resulted in its withdrawal from the European market two

years after its approval. Orlistat treatment is associated with troublesome side effects such as diarrhoea, flatulence, bloating, abdominal pain, and dyspepsia which may not be acceptable to some patients on long-term treatment whilst the recent concerns of severe liver disease have led to a review of its safety. Long-term treatment with sibutramine is associated with a positive though modest efficacy profile and a low risk profile for neuropsychiatric adverse events; however we will need to wait for the publication of the full results of the SCOUT study to determine if there is an increase rate of CV events in patients with cardiovascular disease and diabetes.

Among the drugs in late phase trials, lorcaserin appears to be a potential candidate for long-term treatment in obesity due to its demonstrated efficacy and tolerable safety profile. Treatment with topiramate and taranabant result in significant weight loss in long-term studies, however both of these drugs have serious adverse effects. In the case of taranabant the psychiatric adverse events have led to the discontinuation of Phase III trials. Amongst the combination therapies both bupropion with naltrexone and bupropion with zonisamide have demonstrated effective weight loss and appear to be generally well tolerated based on published results from RCTs whereas there appears to be concerns regarding the safety of combination therapy using topiramate with phentermine.

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Research Article

Canadian Physicians' Use of Antiobesity Drugs and Their Referral Patterns to Weight Management Programs or Providers: The SOCCER Study

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Antiobesity pharmacotherapy and programs/providers that possess weight management expertise are not commonly used by physicians. The underlying reasons for this are not known. We performed a cross-sectional study in 33 Canadian medical practices (36 physicians) examining 1788 overweight/obese adult patients. The frequency of pharmacotherapy use and referral for further diet, exercise, behavioral management and/or bariatric surgery was documented. If drug treatment or referral was not made, reasons were documented by choosing amongst preselected categories. Logistic regression models were used to identify predictors of antiobesity drug use. No single antiobesity management strategy was recommended by physicians in more than 50% of patients. Referral was most common for exercise (49% of cases) followed by dietary advice (46%), and only 5% of eligible patients were referred for bariatric surgery. Significant predictors of initiating/continuing pharmacotherapy were male sex (OR 0.70; 95% CI 0.52–0.94), increasing BMI (1.02; 95% CI 1.01–1.03), and private drug coverage (1.78; 95% CI 1.39–2.29). "Not considered" and "patient refusal" were the main reasons for not initiating further weight management. We conclude that both physician and patient factors act as barriers to the use of weight management strategies and both need to be addressed to increase uptake of these interventions.

1. Introduction

Excess body weight affects 1.6 billion individuals globally [1], is associated with substantial premature morbidity and mortality [2, 3], impairs quality of life [4], and accounts for 2%–7% of direct healthcare spending in developed nations [5]. Sixty-six % and 60% of the adult population in the US and Canada, respectively, are overweight (body mass index (BMI) $\geq 25\,kg/m^2$) or obese (BMI $\geq 30\,kg/m^2$) [6, 7].

Despite the increasing recognition of obesity as a public health problem and the well-documented benefits of even modest weight loss on comorbidities [7], there is concern that obesity is underreported and undertreated by healthcare professionals. Although weight loss counseling increases the likelihood of attempted weight loss by 3-fold [8], only 43% of obese participants in a nationally representative survey of US adults reported receiving this intervention during their annual checkup [9]. At odds with patient perceptions is the finding that 75% of physicians report "always" or "nearly always" administering weight management counseling to their overweight or obese patients [10].

It also appears that, even among physicians who are providing weight management counseling to their patients, additional treatment options such as pharmacotherapy or referral to an additional program or provider are often not used [10, 11]. Weight management strategies such as

expert-led diet and exercise counseling, commercial weight loss programs, pharmacotherapy, and behavioural therapy are recommended by current guidelines [7], and each can reduce weight by a clinically significant amount (at least 3%–5% of initial weight), which is associated with improvements in cardiovascular risk factors [12–15]. A recent study reported the following rates of referral/use: dietician (67%), commercial weight loss program (59%), exercise specialist (34%), and pharmacotherapy (29%) [10]. In a survey of 18 primary care practices in the US, only 14% of overweight or obese patients received a referral for further weight management [16].

Prior studies in this area have been retrospective in nature and thus potentially subject to recall bias [10, 16]. They have also not attempted to examine underlying reasons why physicians are not using these antiobesity management strategies. This report details the major findings of the State of Obesity Care in Canada Evaluation Registry (SOCCER) study, which was designed to examine in closer detail Canadian physicians' use of antiobesity pharmacotherapy and referral patterns to additional obesity management programs and providers.

2. Methods

2.1. Participating Practices and Patient Recruitment. Registration in a provincial registry is a mandatory requirement for all practicing physicians in Canada. Accordingly, provincial medical registries were used to identify all primary care and specialist physicians across Canada in 2005–2007. Approximately 2000 physicians were contacted by phone, fax and/or E-mail, and the 50 physicians across 45 medical practices that agreed to participate were sent study materials, including the study protocol, case report forms, and enrolment logs. Physicians received telephone instructions describing the proper procedure for recruiting patients and populating study forms. This included reading through the entire study form with the physician to ensure that accurate information was collected for each question. No specific instructions or education regarding weight management practices were provided to the physicians. Thirty-six physicians from 33 of the 45 sites (27 primary care and 6 specialist practices) recruited participants. The specialist practices consisted of endocrinologists, cardiologists, and/or general internists (who provide consultative specialty care within the Canadian health care system rather than primary care).

2.2. Inclusion and Exclusion Criteria. Consecutive patients ≥18 years with BMI levels ≥27 kg/m^2 who were able to provide informed consent were eligible for inclusion. A BMI threshold of 27 kg/m^2 was chosen instead of 25 kg/m^2 to increase the likelihood that patients were truly overweight (because BMI is an indirect measure of body fat) and because this cutoff is congruent with current recommendations for initiating drug therapy [7]. Patients were also required to have at least one indication for weight management, as judged by their physician.

Consecutive subjects seen during routine clinic operation and identified on predefined recruitment days were asked to participate. A patient could only be enrolled once in the study. Patients that were already participating in a clinical trial, hospitalized, pregnant, nursing, or unable to attend followup visits were excluded.

2.3. Assessment of Obesity Management Strategies. Because each patient enrolled was deemed by the physician to require weight management, it was assumed that the physician would provide some counseling at the encounter. However, no prior instructions were given to standardize the weight management advice given. The focus of SOCCER was to identify whether or not at this visit the physician initiated or continued obesity pharmacotherapy for a given patient and whether or not the physician referred the patient for further weight management. If referral was made, the type of weight management strategy or strategies involved was recorded. The weight management strategies examined included dietary counseling, commercial weight loss program/popular diet, exercise program (e.g., a trainer or gym membership), behavioral therapy (e.g., psychologist), and bariatric surgery. Depending on the strategy, referral could take the form of explicit written communication to another provider (e.g., surgery) or simply consist of verbal instructions to the patient to seek a specific type of treatment (e.g., commercial weight loss program). In the case of bariatric surgery, data collection was limited to those individuals considered potentially eligible for surgery (BMI ≥ 35 kg/m^2) [7].

Patients did not fill out any forms; forms were populated solely by physicians, and each physician was instructed to consider each weight management strategy in sequence and perform data entry in real time during the actual visit. For pharmacotherapy, physicians were asked to indicate if the patient will "start or continue pharmacotherapy TODAY". For the other weight management strategies, such as dietary counseling, instructions to the physician read as follows: "please indicate if you referred the patient for *Dietary Counseling* as a weight management strategy TODAY." Demographic information, employment status, medical history, physical examination, and current medications were also recorded at the time of the visit. In addition, physicians were asked to document if the reason for the referral was patient initiated and to provide the primary reason if referral was not made for a given strategy, choosing from the following categories (using check boxes): "patient refused", "not affordable", "not feasible", "past treatment failed", "contraindicated", and "not considered."

2.4. Predictors of Antiobesity Drug Use. An additional goal of SOCCER was to identify predictors of antiobesity drug use. Covariate-adjusted, binary logistic regression models were created to identify these predictors. Age, sex, BMI (per unit increase), ethnicity, type of practice (primary care versus specialist), supplemental health insurance, employment status (employed versus unemployed), gender concordance between patients and physicians (concordant versus discordant), and additional covariates that achieved a Wald Chi-square P-value significance level of .1 univariately were also considered. The final model was created using a backwards

selection method to determine which of these additional covariates contributed to the model at a Wald Chi-square *P*-value of .1. Supplemental health insurance indicates the presences of private health coverage, which is primarily used to cover drug expenses. Sibutramine and orlistat are not covered by Canadian provincial health care plans and therefore patients typically pay out of pocket or through private insurance for these medications.

2.5. Data Collection and Statistical Analysis. Paper-based case report forms were populated at point-of-care, faxed to the project management centre (Population Health Research Institute, McMaster University) and optically scanned using DataFax (Clinical DataFax Systems Inc., Hamilton, Ontario). The forms were reviewed for missing, illegible, or contradictory data input. All of the data management processes followed written standard operating procedures (SOPs) and conformed to Good Clinical Practice (GCP) standards for the conduct and data management of clinical studies.

Statistical analysis was performed using SAS, version 9.1 (SAS Institute, Cary, NC). For all statistical tests, two-tailed *P* values less than .05 were considered statistically significant. Survey methods (PROC SURVEYLOGISTIC) account for the clustering of patients within individual physician practices [17]. With the exception of one practice that contained three physicians, only one physician per practice participated in the study. Furthermore, in the multi-physician practice, there were no differences in the use of pharmacotherapy or referral practices among the three physicians. Therefore, it was not necessary to control for physician clustering within practices.

2.6. Ethics Approval. Ethics approval was obtained from both the Canadian Shield Research Ethics Board and the Research Ethics Board of McMaster University, Hamilton Health Sciences.

2.7. Funding. SOCCER was funded by an unrestricted Grant from Abbott Laboratories Canada. The design, conduct and analysis of the study were carried out independently of the study sponsor.

3. Results

3.1. Study Population and Comorbidities. Thirty-six physicians enrolled 1904 patients across 33 participating sites in 7 provinces. 108 (5.7%) did not have an indication for obesity management or meet age or BMI inclusion thresholds and were excluded. Eight patients were excluded because of missing BMI data, leaving 1788 patients included in the final analysis. Patients enrolled from specialty practices had higher mean BMI levels and greater comorbidity compared to patients seen in primary care practices (Table 1). The most common comorbidities were hypertension (48%), dyslipidemia (43%), osteoarthritis (27%), back pain (29%), and type-2 diabetes (24%).

The percentage of patients with 0, 1, 2, 3, or >3 comorbidities was 14.2%, 17.6%, 16.0%, 16.5%, and 35.7%, respectively.

3.2. Use of Pharmacotherapy and Referral for Other Obesity Management Strategies. Pharmacotherapy was initiated or continued in only 21% of cases (39% were by patient request). The frequencies of referral for 0, 1, 2, or >2 weight management strategies (including use of pharmacotherapy) were 29.0%, 22.8%, 26.2%, and 22.0%, respectively. Overall, referral was most common for exercise, in 49% of cases, followed by dietary advice, in 46% of cases. Only 5% of eligible patients were referred for bariatric surgery (Table 2).

Predictors of initiating/continuing pharmacotherapy are summarized in Table 3. In the multivariable adjusted model, male sex (OR 0.70; 95% CI 0.52–0.94) was associated with a lower likelihood whereas increasing BMI (1.02; 95% CI 1.01–1.03) and private drug coverage (1.78; 95% CI 1.39–2.29) were associated with a greater likelihood of initiating or continuing antiobesity drug therapy.

3.3. Reasons for Lack of Referral. In all cases, physicians listed "not considered" as the main reason for not using pharmacotherapy (44% of cases) or not referring a patient additional weight management (32%–65% of cases and highest for bariatric surgery, Table 2). The second most common reason overall for lack of referral was patient refusal and in the cases of referral for diet, exercise and behavioral therapy, "not feasible" was also cited as a reason in substantial minority of cases (Table 2). "Past treatment failed", "not affordable", and "contraindicated" were cited in only a minority of cases.

4. Discussion

In summary, in this study of 36 physicians seeing nearly 1800 patients specifically identified as requiring weight management, antiobesity pharmacotherapy was used in only 21% of cases. Furthermore, referral to additional weight management provider or programs was recommended less than 50% of the time. Physicians recorded "not considered" and "patient refused" as the primary reasons for not using these strategies.

The relatively low rates of use of pharmacotherapy or referral for additional weight management have been demonstrated in previous studies [8, 10], although three major differences in the design of SOCCER compared to previous studies are notable. First, only patients that, in the mind of the physician, unequivocally required weight management were enrolled. Second, physicians were instructed to record data in real time to avoid recall bias and were aware that they were being studied. Given these design factors, one perhaps might have expected the prevalence of drug therapy or use of additional weight management strategies to be higher than that observed. Third, SOCCER also focused on identifying the reasons for not using pharmacotherapy or referring patients, and it is clear that the decision not to proceed with these weight management strategies is related to both patient and physician decisions. Only in a minority of cases were these decisions based upon the presence of specific barriers such as cost. We also found that pharmacotherapy was more likely to be initiated or continued in women,

Table 1: Baseline characteristics.

	Overall $n = 1788$	Primary care $n = 1300$	Specialist $n = 488$	P-value for specialist versus primary care
Age, mean (SD), y	52.7 (14.3)	52.6 (14.6)	52.7 (13.6)	.94
Weight, mean (SD), kg	100.6 (25.1)	95.8 (20.1)	113.6 (31.7)	<.01
BMI, mean (SD), kg/m^2	36.1 (7.9)	34.8 (6.3)	39.8 (10.2)	<.01
Male	663 (37)	460 (35)	203 (41)	.015
Caucasian	1614 (90)	1153 (89)	461 (95)	<.01
Employed	987 (55)	737 (57)	250 (51)	.04
Supplemental health insurance	996 (56)	715 (55)	281 (58)	.33
Current smoker	245 (14)	189 (15)	56 (12)	.09
Gout	75 (4)	45 (4)	30 (6)	.02
Cancer	90 (5)	63 (5)	27 (6)	.56
Polycystic ovarian syndrome	43 (2)	24 (2)	19 (4)	.02
Type 2 diabetes	436 (24)	239 (18)	197 (40)	<.01
Impaired fasting glucose/impaired glucose tolerance	183 (10)	89 (7)	94 (19)	<.01
Coronary artery disease	166 (9)	79 (6)	87 (18)	<.01
Congestive heart failure	46 (3)	21 (2)	25 (5)	<.01
Peripheral arterial disease	38 (2)	10 (1)	28 (6)	<.01
Stroke	49 (3)	34 (3)	15 (3)	.60
Arrhythmia	73 (4)	45 (4)	28 (6)	.04
Dyslipidemia	760 (43)	518 (40)	242 (50)	<.01
Hypertension	853 (48)	582 (45)	271 (56)	<.01
Depression	395 (22)	261 (20)	134 (28)	<.01
Anxiety	284 (16)	213 (16)	71 (15)	.34
Eating Disorder	40 (2)	19 (2)	21 (4)	<.01
Osteoarthritis	478 (27)	289 (22)	189 (39)	<.01
Back Pain	526 (29)	347 (27)	179 (37)	<.01
Fibromyalgia/chronic fatigue	88 (5)	69 (5)	19 (4)	.21
Gall bladder disease	127 (7)	72 (6)	55 (11)	<.01
Abnormal liver enzymes	60 (3)	26 (2)	34 (7)	<.01
Gastrointestinal reflux	357 (20)	249 (19)	108 (22)	.16
Incontinence	111 (6)	69 (5)	42 (9)	.01
Sleep apnea	146 (8)	54 (4)	92 (19)	<.01
Thrombosis/embolism	25 (1)	13 (1)	12 (3)	.03

Data are expressed as no. (%) unless otherwise noted.
SD: standard deviation; BMI: body mass index.

heavier patients, and those with private drug coverage. This sex difference is consistent with previous studies (both from RCTs and population-based analyses of prescription fills) [13, 18]. Furthermore, no antiobesity drug is covered under a provincial drug plan in Canada; therefore, the drugs may be unaffordable to many who do not have private coverage.

One limitation of SOCCER is that followup probes examining why the physician failed to consider pharmacotherapy or referral or why the patient refused these actions were not performed. It is certainly possible that physicians may view such interventions as ineffective, may not be familiar with their availability, or may expect that the patient can be successful without further help. Physicians may be aware of data demonstrating poor long-term persistence rates with pharmacotherapy or may not view the benefit/risk ratio of current drugs to be favorable. A recent physician survey reported that physicians might have unrealistic expectations regarding how successfully patients can lose weight [10]. Weight losses of 38% were categorized as "a dream outcome", and 10% losses were viewed as "disappointing" despite data demonstrating that such relatively small amounts of weight loss can lead to clinically significant benefits including a reduction in the incidence of type 2 diabetes in high-risk patients [7, 19]. Conversely, patients may refuse help from healthcare providers because they are more confident that a self-directed weight management plan will be successful [20]. Patients may also not view a physician's office as appropriate venue for weight management and may instead

Table 2: Frequency of use of pharmacotherapy or referral for antiobesity management expertise.

	Pharmacotherapy used or referral recommended *n* (%)		Pharmacotherapy not used or referral not recommended *n* (%)	Reason not recommended *n* (%)							
Strategy	All	By patient request	All	Patient-related reason	Patient refused	Past treatment failed	Not affordable	Contra-indicated	Not feasible	Not considered	Missing
Pharmacotherapy	375 (21)	146 (39)	1413 (79)	792 (56)	491 (35)	47 (3)	167 (12)	63 (5)	24 (2)	618 (44)	3 (0.02)
Dietary counseling	813 (46)	257 (32)	974 (55)	614 (63)	300 (31)	86 (9)	39 (4)	9 (1)	180 (19)	343 (35)	17 (2)
Exercise training	866 (49)	291 (34)	920 (52)	573 (62)	216 (24)	37 (4)	57 (6)	18 (2)	245 (27)	297 (32)	50 (5)
Behavioral therapy	277 (16)	49 (18)	1508 (85)	713 (47)	404 (27)	28 (2)	49 (3)	2 (0.1)	230 (15)	720 (48)	75 (5)
Commercial programs/popular diets	261 (15)	97 (37)	1527 (85)	626 (41)	332 (22)	103 (7)	116 (8)	11 (1)	64 (4)	853 (56)	48 (3)
Obesity surgery (BMI $\geq 35\,kg/m^2$)	41 (5)	24 (59)	742 (95)	227 (31)	134 (18)	4 (1)	16 (2)	12 (2)	61 (8)	482 (65)	33 (4)

Table 3: Predictors of initiating pharmacotherapy*.

Variable	Odds ratio (95% CI)
Age	0.98 (0.97–0.99)
Male	0.70 (0.40–1.23)
Body mass index	1.06 (1.03–1.08)
Caucasian ethnicity	1.52 (0.76–3.02)
Specialist physician	0.65 (0.21–2.0)
Private drug coverage	2.36 (1.52–3.66)
Currently employed	1.16 (0.82–1.66)
Patient-physician gender concordance	0.71 (0.34–1.48)
Current smoker	1.61 (1.13–2.30)
Gastroesophageal reflux disease	1.41 (0.97–2.05)

*Multivariable final adjusted model.

seek alternate methods. In addition, barriers to weight management may limit uptake of weight management strategies. Many patient-related barriers have been identified, including a lack of motivation, failure to recognize obesity as a major health condition, time constraints, low socioeconomic status, intimate saboteurs, and comorbid health conditions (particularly psychological dysfunction and sleep disorders) [21].

Bariatric surgery was recommended in only 5% of eligible patients in SOCCER. Surgery is the only intervention that consistently leads to substantial weight reduction, and surgery also has been associated with reductions in mortality; improvements in quality of life, and has been shown to be cost effective at commonly cited thresholds. We theorize that physicians may simply fail to consider surgery as a viable treatment option or may be unaware of recent evidence demonstrating that surgery substantially reduces morbidity and mortality in severely obese patients [22]. Conversely, physicians may fail to consider surgery because of the absence of a surgical program in their vicinity or because of the extended (several years) wait times that exist in Canadian surgical programs, although one would have expected physicians to categorize this scenario as "not feasible" rather than "not considered" [23].

Because volunteer physicians practices (only 2% of the total number of practices contacted) within Canada were enrolled in SOCCER, results may be subject to selection bias and may not be generalizable to all physician practices within and outside this country. Compared to practices that were not interested in participating, practices volunteering to take part likely had higher levels of interest and expertise in weight management and may have been more likely to initiate weight management strategies. Because the study specifically entailed detailing antiobesity management strategies, participants may also have been more likely to use management strategies because they knew these were being measured (i.e., the Hawthorne effect [24]). Therefore, it is probable that the frequency of physician-initiated drug treatment or weight management referral was overestimated compared to "usual care" practices, and, therefore, our results may be considered conservative.

In conclusion, we have demonstrated in this analysis of Canadian physician practice patterns that rates of antiobesity drug use and referral for additional weight management strategies are low. In the majority of cases, either physicians fail to consider these management strategies or patients refuse them. If increased uptake of these guideline-concordant strategies is to be achieved, both patients and physician-related barriers to weight management will need to be examined and addressed.

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Research Article

Taking Orlistat: Predicting Weight Loss over 6 Months

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This study explored the predictors of weight loss following orlistat with a focus on both baseline variables and changes in beliefs and behaviours occurring over the course of taking the drug. Patients ($n = 566$) prescribed orlistat completed a questionnaire at baseline and after 6 months concerning their weight, beliefs and behaviours. By 6 months the majority had lost some weight and showed improvements in diet. Many had also stopped taking the drug and a large minority reported using it flexibly as a lifestyle drug. Those who lost most weight showed a decrease in beliefs in a medical solution, a decrease in unhealthy eating, an increased belief in treatment control and an increased belief that the unpleasant consequences are both due to their eating behaviour and just part of the drug. When taken with fatty food orlistat causes symptoms such as anal leakage and oily stools. These may encourage some patients to focus on the behavioural aspects of their weight problem thus promoting the dietary changes needed for both short and longer term weight loss. When prescribing orlistat, clinicians should encourage patients to see the consequences as an education as a means to promote the effectiveness of this form of medical management.

1. Introduction

Orlistat (Xenical) is currently the only available form of prescribed obesity medication which acts on the gastrointestinal system and works by reducing fat absorption in the gut which is eliminated in bowel movements. It also blocks the availability of fat-soluble vitamins (vitamins A, D, E, and K), so patients may also take a vitamin supplement [1, 2]. Current recommendations suggest that it is used for patients who have a history of failed weight-loss attempts using behavioural methods and who can demonstrate at least 2.5 kg weight loss by diet and exercise in the month prior to their first prescription [1]. It is suggested that patients reduce their daily calorie intake by 500 to 1000 calories to promote weight loss, and the Dietary Guidelines for Americans recommend that dietary fat is limited to about 30% of daily calories. As a result of its impact upon fat absorption, orlistat has unpleasant side effects including liquid stools, an urgency to go to the toilet, and anal leakage which are particularly apparent following a high-fat meal as the drug causes the fat consumed to be removed from the body. Between 1998 and 2005, orlistat prescriptions rose 36-fold from 17,880 to 646,700 and the total cost increased by over 35-fold. Recent years have seen an additional significant increase in the number and cost of prescriptions for orlistat [3].

Research has explored the effectiveness of orlistat compared to other drug treatments, placebo, or behaviour-focused interventions. For example, Padwal et al. [4] reported that patients taking orlistat lost 2.7 kg more than patients taking placebo, and Avenell et al. [5] carried out a systematic review of trials involving a combination of diets, drug therapy, exercise, and behaviour therapy and concluded that adding orlistat to a dietary intervention improved weight loss by 3.26 kg up to 24 months. Research also indicated that orlistat reduces cholesterol and blood pressure levels and improves glycemic control when compared to placebo [4]. Similarly, Phelan and Wadden [6] concluded from their review that adding orlistat to lifestyle modification interventions improves both weight loss and weight-loss maintenance. Furthermore, in a recent updated meta-analysis, Rucker et al. [7] synthesised the results of randomised placebo- controlled trials of approved antiobesity drugs in adults aged 18 and over for one to four years. They concluded that with the active drug treatments patients were more likely to reach 5% and 10% weight-loss thresholds

and that weight losses for three key drugs were as follows: sibutramine: 4.2 kg; rimonabant: 4.7 kg, and orlistat: 2.9 kg. Research therefore indicates that orlistat can improve weight loss if used alongside behavioural and lifestyle interventions.

There remain, however, two main problems with orlistat as a treatment for obesity. First, although evidence indicates that it can improve weight-loss outcomes, these improvements are not always substantial and there is much variability with many patients showing no improvements at all. Second, research also indicates high attrition rates with patients not adhering to their medication due to the unpleasant side effects and many stoppings taking the drug entirely or using it selectively according to the content of their diet. For example, Padwal et al. [4] concluded from their review of randomised control trials that the mean attrition rate for orlistat was 33% (11 studies, $n = 6021$), and Vray et al. [8] suggested that in clinical practice attrition rates are even higher at 64%–77%. Research has therefore addressed whether the effectiveness of orlistat can be improved, and some studies have explored whether specific patients benefit more than others and whether baseline variables predict outcomes. However, whereas research exploring alternative forms of medical management has explored a range of clinical, psychological, and behavioural variables as predictors of outcomes (e.g., [9–11]),research focusing on orlistat has mainly emphasised laboratory and clinical variables [12]. In general, however, such studies conclude that the best predictor of outcome following medical management is initial weight loss, but to date few studies have explored psychological and behavioural predictors of outcome following orlistat. An alternative approach has addressed the mechanisms of how orlistat works, and from a medical perspective the main consequence of orlistat is to reduce fat absorption in the gut. However, due to the unpleasant side effects, Finer has labelled it the "antabuse effect" [13] as it deters the intake of high-fat foods. Further, Ogden and Sidhu [14] carried out a qualitative study with patients who had taken orlistat to explore their beliefs about why it either did or did not facilitate weight loss. The results showed that inline with previous research some patients stopped taking their medication due to the unpleasant symptoms such as anal leakage or oily stools. However, the results also showed that these highly visual side effects encouraged some people to consider their behaviour as a cause of their obesity. Many obese people focus on medical causes of their problem such as hormones and genetics [15, 16]. The results from this qualitative study of orlistat users indicated that by, showing patients the fat they have consumed, orlistat can shift patient models of obesity towards a more behavioural perspective, thus encouraging them to adopt a healthier diet. Leventhal et al. [17] described the notion of coherence between beliefs about causes and solutions to any particular medical problem. Inline with this, Ogden and Sidhu [14] argued that orlistat functions by educating patients and creating coherence between behavioural causes and therefore behavioural solutions for obesity. To date, however, this process remains untested in a larger quantitative study.

In summary, although orlistat is currently the most commonly prescribed medication for the obese, there remains much variability in its effectiveness with only a minority of patients showing weight loss. Research has therefore explored the possible reasons for the effectiveness of orlistat, and whereas some studies have emphasised baseline characteristics, others have highlighted changes in beliefs and behaviour brought about by the mechanisms of the drug itself. To date, however, such studies have focused either on drugs other than orlistat, have been limited in their choice of variables, or have used small qualitative designs. The present study, therefore, aimed to explore weight loss following a 6-month course of orlistat and to explore the role of demographics, beliefs, and behaviour in predicting outcomes in a large sample of patients. Furthermore, inline with a focus on mechanisms, the study aimed to assess the role of both baseline variables and the changes occurring whilst orlistat was being taken.

2. Method

2.1. Design. The study used a longitudinal design with measures concerning BMI, experiences of taking orlistat, and beliefs and behaviour being completed at baseline and six-month followup.

2.2. Sample. Participants who had been prescribed orlistat by the GP and registered on the Xenical support system (MAP) funded by Roche were invited to take part in the study and sent the baseline questionnaire. Those who returned the baseline questionnaire were sent a further questionnaire at six months. Only those who completed the baseline questionnaire within the first three months of starting to take the medication and returned the 6 month followup questionnaire were included in this study. 566 participants returned both the baseline questionnaire within the first three months of starting to take orlistat and the 6 month followup questionnaire. This represented a response rate of 36% of total baseline responders. The University Ethics Committee approved the study. The data presented here reflect the short-term followup from baseline to six months.

2.3. Procedure. MAP gained initial consent from participants to pass on their contact details to take part in the study. An information sheet and questionnaire were then sent out to participants by post. Those who returned the baseline questionnaire were sent a similar followup questionnaire at six months.

2.4. Measures. Baseline and followup questionnaires examined demographics, beliefs about obesity, beliefs about side effects, and behaviour. All beliefs and behaviour were assessed using items which were rated using a 5-point Likert scale ranging from "not at all" (1) to "totally" (5). For each construct the individual items were summated and the reliability of each construct was assessed using Cronbach's alpha. Most alphas were above the established cutoff level illustrating acceptable reliability. Some were lower, but this is generally acceptable if there is diversity in the constructs being measured.

(1) *Demographics:* participants completed measures of weight, height, BMI, age, sex, and employment.

(2) *Beliefs about obesity:* this included measures relating to (i) behavioural causes of obesity (α = .671) (e.g., "eating too much," "not enough exercise"); (ii) medical causes of obesity (α = .659) (e.g., "genetics/inheritance," "slow metabolism"); (iii) behavioural solutions to obesity (α = .850) (e.g., "eating fewer calories," "being more active"); (iv) medical solutions to obesity (α = .630) (e.g., "medication," "surgery"); (v) personal control over weight (e.g., "how much control do you think you have over your weight"); (vi) treatment control over weight (e.g., "how much do you think Xenical can help your weight?").

(3) *Beliefs about side effects:* participants rated (i) the extent to which they had experienced side effects (α = .761) (e.g., "liquid stools," "bloating"), (ii) the extent to which they believed these side effects were part of the drug (α = .671) (e.g., "they are a necessary part of taking the drug"), and (iii) whether the side effects were caused by eating behaviour (α = .826) (e.g., "they have made me realise what is in different foods").

(4) *Behaviour:* participants rated their behaviour in terms of (i) adherence to medication (α = .577) (e.g., "I take it religiously," "I stop taking it before a fatty meal" (which was reverse scored)), (ii) healthy eating (α = .580) (e.g., healthy snacks (e.g., rice cakes, crackers, and fruit), healthy cooking (e.g., boil, steam, and cooked meals at home), and healthy food choices (e.g., skimmed milk, low-fat cheese, and high intake of fruit and vegetables) (iii) unhealthy eating (α = .547) (e.g., unhealthy snacks (e.g., crisps, cakes, and chocolate), unhealthy cooking (e.g., shallow fry, deep fry, processed foods), and unhealthy food choices (e.g., full-cream milk, full-fat butter/margarine, and low intake of fruit and vegetables).

All measures related to the past month were based on previous qualitative research which has explored people's beliefs and experiences of taking obesity medication and successful weight loss and maintenance [14, 18]. Participants also completed the personal and treatment control items of the brief Illness Perception Questionnaire that assesses participants' beliefs about their illness [19]. In addition the healthy and unhealthy eating measures were taken from the World Health Organisation 2001/02 protocol [20], the food frequency questionnaires found in the study by Inchley et al. [21], the seven-day food diary [22], and consumer market-research report data [23].

2.5. Inclusion/Exclusion Criteria. Participants were included if they registered with the MAP program within a four-month period, were 18 years or over, and had been prescribed orlistat by their GP and if they had completed the baseline questionnaire within the first three months of starting to take orlistat and also returned the followup questionnaire at six months.

2.6. Data analysis. The data were analysed to describe the participants' demographics, differences between responders and nonresponders, and overall changes in BMI, weight, and behaviour. Further, the data were analysed to assess the role of baseline demographics, beliefs, and behaviour in predicting improvements in BMI and to assess the role of changes in beliefs and behaviour over the course of 6 months in predicting improvement in BMI by 6 months.

3. Results

3.1. Participants' Demographics and Responders versus Non-responders. Responders', to the questionnaire, versus nonresponders' (at 6 months) demographic variables are shown in Table 1.

The results showed that the mean age of the people who returned a completed questionnaire at both time points (responders) was 50 years and that the majority were white, female, not working, married, educated up until college, and with a mean BMI of 36. Further, the responders and nonresponders (those who returned the baseline questionnaire but not the followup questionnaire at 6 months) were comparable on all baseline demographics apart from age with the responders being older than the nonresponders.

3.2. Overall Changes in BMI, Weight, and Behaviour. Change scores for BMI, beliefs, and behaviour were calculated (T1-T2) and then classified into groups: weight loss versus no weight loss; decrease in BMI versus no decrease in BMI; improvement in healthy eating versus no improvement; increased unhealthy eating versus no increase, and for adherence those who had stopped taking it by 6 months were grouped as "nonadherers", those who rated their adherence as "totally" were rated as "adherers", and those who reported flexible adherence according to what they were eating were recoded as "lifestyle adherers." These results are shown in Table 2.

The results showed that by 6 months the majority of the responders had lost weight and decreased their BMI. The mean weight loss was 4.09 kg (SD: 6.21), the median weight loss was 3.63 kg, and percentage weight losses were as follows: 0–2 kg: 19.36%; 2.1–5 kg: 28.1%; 5.1–7 kg: 15.9%; 7.1–10 kg: 17.4%; 10.1 kg: 19.3%. In addition, the majority had increased their healthy eating and decreased their unhealthy eating and were no longer taking orlistat although a large minority reported either full adherence or being lifestyle users by 6 months.

3.3. Predictors of Improvement in BMI over 6 Months. The results were then analysed to assess the role of beliefs and behaviour in predicting an improvement in BMI by 6 months both in terms of baseline and change scores using Multiple Regression Analysis and using forced entry method.

Table 1: Responders versus nonresponders at baseline.

Variable	Responders ($n = 568$)	Nonresponders ($n = 1008$)	t/χ^2	P
Age	$x = 50.24$	$x = 47.60$	14.59	.0001*
	SD = 13.01	SD = 13.11		
	$n = 560$	$n = 993$		
Sex	Male = 98 (17.5%)	Male = 181 (18.1%)	.109	.741
	Female = 463 (82.5%)	Female = 817 (81.9%)		
Ethnicity	White = 543 (97.1%)	White = 950 (94.8%)	4.705	.319
	Black Caribbean = 4 (0.7%)	Black Caribbean = 14 (1.4%)		
	Black African = 2 (0.4%)	Black African = 7 (0.7%)		
	Asian = 5 (0.9%)	Asian = 16 (1.6%)		
	Other = 5 (0.9%)	Other = 15 (1.5%)		
Job	Full time = 174 (31.6%)	Full time = 324 (33.5%)	.935	.627
	Part time = 113 (20.5%)	Part time = 204 (21.1%)		
	Not Working = 264 (47.9%)	Not Working = 439 (45.4%)		
Marital Status	Married = 330 (59.6%)	Married = 598 (61.5%)	1.370	.849
	Divorced = 66 (11.9%)	Divorced = 122 (12.6%)		
	With Partner = 64 (11.6%)	With Partner = 98 (10.1%)		
	Single = 71 (12.8%)	Single = 118 (12.1%)		
	Widowed = 23 (4.2%)	Widowed = 36 (3.7%)		
Education	<secondary = 68 (12.4%)	<secondary = 125 (13.2%)	9.525	.146
	Sec Sch Grad = 178 (32.5%)	Sec Sch Grad = 332 (35%)		
	Some Coll = 133 (24.3%)	Some Coll = 239 (25.2%)		
	Coll Grad = 78 (14.3%)	Coll Grad = 140 (14.8%)		
	Graduate = 54 (9.9%)	Graduate = 54 (5.7%)		
	Postgraduate = 12 (2.2%)	Postgraduate = 21 (2.2%)		
	Doct/Prof = 24 (4.4%)	Doct/Prof = 37 (3.9%)		
BMI	$x = 36.20$	$x = 35.98$	.452	.501
	SD = 5.84	SD = 6.01		
	$n = 527$	$n = 948$		

Table 2: Descriptive analysis of baseline to 6 months data.

	No	Yes	Lifestyle
Lose weight	$n = 131$ 26.4%	$n = 365$ 73.6%	
	Range = −18 to 0.07	Range = 0.08 to 25	
Decrease in BMI	$n = 128$ 26%	$n = 364$ 74%	
	Range = −6.15 to 0.02	Range = 0.03 to 9	
Increased healthy eating	$n = 187$ 41.7%	$n = 261$ 58.3%	
	Range = −8 to 0.07	Range = 0.08 to 13	
Increased unhealthy eating	$n = 318$ 74.1%	$n = 111$ 25.9%	
	Range = −8 to 0.07	Range = 0.08 to 5	
Adherence	$n = 194$	$n = 124$	$n = 90$
	47.5%	30.4%	22.1%

3.3.1. Baseline Predictors of BMI Improvement. The role of baseline beliefs and behaviour in predicting improvements in BMI are shown in Table 3.

The results showed that an improvement in BMI was predicted by a greater endorsement of a medical solution to their weight problem at baseline, accounting for 7.9% of the variance ($F = 2.862$, $P = .001$). No other baseline variables were significant.

3.3.2. Changes in Beliefs and Behaviour as Predictors of BMI Improvement. Change scores in beliefs and behaviour were calculated (T1-T2). The role of these variables in predicting improvements in BMI is shown in Table 4.

The results showed that a decrease in BMI over 6 months was predicted by a decrease in endorsing a medical solution to their weight problem, a decrease in unhealthy eating, an increased belief in treatment control, and an increased belief

Table 3: Baseline predictors of a decrease in BMI over 6 months.

Variables	Standardised β coefficient	P
Age	.082	.194
Sex	.072	.234
Behavioural cause	−.100	.161
Medical cause	−.048	.430
Behavioural solution	.078	.267
Medical solution	.157	.012*
Treatment control	.044	.527
Personal control	.078	.217
Experience of side effects	−.134	.066
Side effects part of the drug	−.001	.984
Side effects due to eating behaviour	−.032	.621
Healthy diet	.018	.766
Unhealthy diet	−.125	.054
		Adjusted $R^2 = .079$

Table 4: Change scores as predictors of improvements in BMI over 6 months.

Variables	Standardised β coefficient	P
Change in behavioural cause	.123	.151
Change in medical cause	.013	.866
Change in behavioural solution	−.004	.963
Change in medical solution	.228	.004*
Change in treatment control	−.259	.001*
Change in personal control	−.052	.498
Change in experience of side effects	−.020	.810
Change in side effects part of the drug	−.167	.039*
Change in side effects due to eating behavior	−.218	.005*
Change in healthy eating	.115	.129
Change in unhealthy eating	.183	.017*
		Adjusted $R^2 = .173$

that the side effects are both due to their eating behaviour and just part of the drug, accounting for 17.3% of the variance ($F = 4.015$, $P = .0001$).

4. Discussion

The present study aimed to explore the predictors of weight loss following 6 months after being prescribed orlistat with a focus on both baseline variables and changes in beliefs and behaviour over the course of taking the drug.

The results showed that by the end of 6 months three quarters of patients reported both weight loss and a reduction in their BMI with the majority falling within the expected range inline with previous outcome studies [4, 5, 7]. The majority also reported an increase in healthy eating and a decrease in unhealthy eating which provides some support for the impact of orlistat on diet and its use as the "antabuse effect" [13]. Furthermore, just less than half had stopped taking their medication by 6 months, and a large minority reported using it flexibly in response to their dietary choices which is consistent with high attrition rates found in previous studies and the use of orlistat as a lifestyle drug [4, 8].

In terms of predictors of outcomes, only one baseline variable was related to a reduction in BMI by six months. In particular, the results showed that a greater endorsement of a medical solution to obesity predicts a greater reduction in BMI by followup indicating that those who have greater expectations of success for the drug show greater improvements. This finds reflection in the focus on baseline predictors reported for other forms of medical management (e.g., [9, 10]) but suggests that it would be difficult to profile those patients at baseline who would most benefit from taking the drug.

The data were also analysed, however, to explore the role of changes in beliefs and behaviour over the course of taking orlistat and produced more promising results. In particular, those who lost most weight showed a decrease in beliefs in a medical solution, a decrease in unhealthy eating, an increased belief in treatment control, and an increased belief that the side effects are both due to their eating behaviour and just part of how the drug works. Therefore, it would seem that taking orlistat may encourage patients to focus on their behaviour rather than medical factors as solutions to obesity and subsequently improve their diet and that if such changes in beliefs and behaviour occur, weight loss is greater. This provides quantitative support for previous smaller-scale qualitative research [14] and indicates that the highly visual side effects of orlistat, while being unpleasant and a deterrent for some users, for others may help educate them towards a more behavioural focus on their weight problem.

There are some issues with the study which need to be addressed. First, the study did not include a control group as the study aimed to explore the predictors of outcomes after taking orlistat rather than to assess the effectiveness of this drug. The effectiveness of the drug has been explored elsewhere [4]. Second, both diet and weight were assessed using self-report rather than an objective measurement tool. This means that there may well be inaccuracies in the data as research has shown that people tend to underestimate both their weight and what they eat. However, for the present study such self-report measures represented the best means of measuring these variables in a large-scale nationwide survey as it would not have been feasible to call all participants into the clinic to collect more objective data. Third, due to the recruitment procedure, participants were generally completing the questionnaires within the first 3 months of taking orlistat. The baseline data, therefore, reflects their beliefs and experiences at the start of their course of taking orlistat but does not reflect that, either before or at the very start of this process. Future research should aim to recruit participants just before they take their first prescription of orlistat in order to gain a true baseline. Finally, the data does not show exactly when the participants

stopped taking orlistat. However, by followup it is known whether the participants were currently taking the drug or if they had stopped within the last month. Therefore, although we do not have an exact marker of when the course of medication was stopped and therefore when its impact upon beliefs and behaviour ceased to occur, we do have an approximate measure which enables some assessment of the interrelationships between drug use and beliefs and behaviour change. Given these limitations, however, the study does provide some insights into the mechanisms of orlistat with a focus on the role of beliefs and behaviour in predicting weight loss.

5. Conclusions

To conclude, orlistat is currently the only prescribed obesity medication available for obese patients. Although research indicates that it can promote weight loss, there remains problems with adherence and much variability in patient outcomes. The present study aimed to explore predictors of outcomes as a means to improve its effectiveness. The results indicate that changes in beliefs and behaviour occurring throughout the course of taking orlistat are the best predictors of outcomes rather than baseline variables. Further, the results indicate that those patients, who show a shift away from a medical model of their problem towards a focus on their own behaviour and show improvements in their diet, lose more weight. These results have implications for patient management and the use of orlistat for weight loss. In particular, orlistat causes unpleasant side effects which may cause nonadherence. However, rather than conceptualising such side effects as unfortunate, they may be the very "active" ingredients necessary to bring about change in patients' behaviour. Therefore, when prescribing orlistat, clinicians should not only alert patients to the possibility of such consequences of eating high-fat foods, but also encourage them to focus and learn from them in terms of what they are eating, what this looks like when it is excluded from their bodies, and what this would do to their bodies if it had remained inside. Such an emphasis may encourage patients to see these consequences of the drug if they eat a diet high in fat, as an education, thus enabling them to take more ownership of their weight problem, in turn facilitating and promoting the changes in eating behaviour necessary for both short- and longer-term weight loss and maintenance.

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Review Article

Anti-Inflammatory Nutrition as a Pharmacological Approach to Treat Obesity

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Obesity is a multifactorial condition resulting from improper balances of hormones and gene expression induced by the diet. Obesity also has a strong inflammatory component that can be driven by diet-induced increases in arachidonic acid. The purpose of this paper is to discuss the molecular targets that can be addressed by anti-inflammatory nutrition. These molecular targets range from reduction of proinflammatory eicosanoids to the modulation of features of the innate immune system, such as toll-like receptors and gene transcription factors. From knowledge of the impact of these dietary nutrients on these various molecular targets, it becomes possible to develop a general outline of an anti-inflammatory diet that can offer a unique synergism with more traditional pharmacological approaches in treating obesity and its associated comorbidities.

1. Introduction

It is becoming more evident that inflammation plays an important role in the metabolic consequences of obesity, as well as other chronic degenerative conditions [1–3]. However, the understanding of the molecular mechanisms behind the control of the inflammatory process at the genetic level is only beginning to be understood. Pharmacology allows one to determine which parts of the inflammatory process are important in treatment of obesity. However, understanding how natural components of the diet can also affect the same molecular targets as pharmacological interventions could provide attractive and cost-effective alternatives to more traditional pharmacologic interventions.

The purpose of this paper is to begin to establish linkages between diet, hormones, and genetic factors affecting inflammation, and while proposing alternative approaches for the treatment of obesity and its metabolic consequences driven by chronic low-level inflammation.

2. A New Perspective on Obesity

The percentage of overweight and obese individuals appears to have stabilized in the United States at about 2/3 of the adult population [4]. Furthermore despite being exposed to the same obesogenic environment, about 30% of adults appear to be resistant to the development of excess body weight. This would suggest that a strong genetic component might be involved with the current obesity epidemic. This would also indicate that there may be genetic factors that when activated by the diet could be responsible for the rapid change. Therefore it would be highly desirable to determine how diet-induced chronic low-level inflammation could impact the expression of genes that can affect fat accumulation and the metabolic consequences after its accumulation.

3. Overview of Inflammation

We are in a constant struggle with microbes. The inflammatory responses that developed over millions of years of evolution allow us to coexist with them and to maintain a state of wellness. Most think of inflammation in terms of the pain associated with cellular destruction that comes as a result of the inflammatory response. This is why the ancient Greeks described inflammation as the internal fire. The ancient Roman physician Celsus described inflammation as pain, swelling, redness, and heat. Those are terms still used by

many physicians to describe the inflammatory process. Today we know the inflammatory process is a complex interaction of both the pro- and anti-inflammatory phases [5, 6]. The pro-inflammatory phase induces pain, swelling, redness and heat, which are indicators that cellular destruction is taking place. Yet there are equally important anti-inflammatory mechanisms of the inflammation process that are necessary for cellular repair and regeneration. Only when these two phases are continually balanced, and progenitor endothelial cells can effectively repair the microtissue injury that results from inflammatory events, that molecular wellness is re-established. However, if the pro-inflammatory phase continues at a low, but chronic level that is below the perception of pain, its presence can become a driver of many chronic diseases.

There are several events that can turn on inflammatory responses. The most obvious is microbial invasion. Injuries and burns (both chemical and radiation) can also induce the most basic components of the inflammatory response. However, we are now beginning to understand how diet can also activate the same inflammatory responses induced by microbes.

All of these different factors can turn on inflammation through the innate immune response. The primary cellular components of the innate immune system include toll-like receptors, cytokine receptors, and various transcription factors that work together to activate the expression of inflammatory genes that amplify the pro-inflammatory attack phase of inflammation.

4. Types of Inflammation

There are two distinct types of inflammation. The first type is inflammation resulting in acute pain. This can be considered classical inflammation. A second type of inflammation can be described as chronic low-level inflammation that is below the threshold of pain. This can be termed "silent inflammation" [7–9]. Since there is no pain associated with this type of inflammation, nothing is done to stop it, and thus it can linger for years, if not decades, causing continual organ damage. As long as appropriate reparative mechanisms and the regenerative/compensatory potential of organs and tissues are maintained, the development of chronic degenerative conditions could be prevented or delayed. However, eventually, exhaustion of the reparative/regeneration potential will occur, with subsequent organ damage, loss of function and the onset of overt chronic disease although the initiating pathogenetic events may have started decades earlier, triggered by the underlying silent inflammation process.

Both types of inflammation are primarily driven by the production of pro-inflammatory eicosanoids derived from arachidonic acid (AA). AA is an omega-6 fatty acid whose levels are entirely controlled by the diet. Anti-inflammatory drugs interact with molecular targets that are downstream from AA, primarily by either inhibiting the enzymes that convert AA into pro-inflammatory eicosanoids or inhibiting the release of AA from phospholipids in the membrane. Anti-inflammatory nutrition works upstream by reducing the levels of AA. The overall goal (reduction of pro-inflammatory eicosanoids) remains the same, but the mechanisms to reach that goal are very different.

5. Innate Immune System

The linkage of diet and inflammation lies within the innate immune system. The innate immune system is the most primitive part of our overall immunological response. As a result, it has been conserved for hundreds of millions of years of evolution and is sensitive to nutrients [10]. More importantly, its activation of the inflammatory response is based on primitive pattern recognition. This is why the diet remains intimately connected to the regulation of inflammation. Certain food components can activate the inflammatory process of the innate immune system, and others can inhibit it. When advances in molecular biology finally began to unravel the control mechanisms inherent in the innate immune system, a more detailed understanding of unexpected mechanisms for a variety of commonly used pharmacological drugs was achieved [11, 12]. Likewise, these same advances illustrate how the diet could affect inflammation induced by the innate immune system. Today the understanding of the linkage of toll-like receptors, signaling pathways, gene transcription factors, and silent inflammation allows nutrition to evolve to a new level of gene therapy, especially the silencing of genes involved in the generation of silent inflammation.

6. Clinical Markers of Silent Inflammation

It is very difficult to discuss a concept of silent inflammation if you cannot measure it, especially since there is no pain associated with it. It is only recently that new clinical markers of silent inflammation have emerged. The first of these clinical markers is high-sensitivity C-reactive protein (hs-CRP). It is not a very selective marker since simple infections can raise it [13–15]. A much more selective marker of silent inflammation is the ratio of two key fatty acids in the blood. The first is the omega-6 fatty acid arachidonic acid (AA), which is the precursor to pro-inflammatory eicosanoids. The other fatty acid is the omega-3 fatty acid eicosapentaenoic acid (EPA), which generates anti-inflammatory eicosanoids. The higher the AA/EPA ratio in the blood, the greater the level of the silent inflammation that is likely to be found in various organs [7–9].

7. Dietary Origin of Silent Inflammation: The Perfect Nutritional Storm

There has not been one dietary change alone in past 30 years that has increased the levels of silent inflammation. However, there has been a convergence of three distinct dietary changes that can be termed as "The Perfect Nutritional Storm" [9]. These dietary factors include

(i) increased consumption of refined carbohydrates,

(ii) increased consumption of refined vegetable oils rich in omega-6 fatty acids,

(iii) decreased consumption of long-chain omega-3 fatty acids.

The first of these dietary changes is the increased consumption of refined carbohydrates that has significantly increased the glycemic load of the diet. The glycemic load of a meal is defined as the amount of a particular carbohydrate that is consumed at a meal multiplied by its glycemic index [16, 17]. Today high glycemic-index carbohydrates are not only the major components in virtually all processed foods, but also in potato, rice, and white bread products. As the cost of production of refined carbohydrates has dramatically decreased in the past 25 years, the availability of products made from these ingredients has dramatically increased [18]. Increased consumption of refined food products generates meals with a high glycemic load. This results in the increased secretion of the insulin necessary to lower the resulting postprandial rise in blood glucose [17].

However, increased insulin production alone is not sufficient to explain the rapid increase in silent inflammation. This requires the presence of another recent dietary component: the increased consumption of refined vegetable oils rich in omega-6 fatty acids. The primary fatty acid in the most common vegetable oils is the omega-6 fatty acid known as linoleic acid. Until the last 50 years linoleic acid has been a relatively minor component of the human diet. As an example, traditional cooking fats such as butter, lard, and olive oil contain less than 10% linoleic acid. Common vegetable oils such as corn, soy, sunflower, and safflower contain 50%–75% linoleic acid. The usage of these vegetable oils has increased by more than 400% since 1980 [19]. Since refined carbohydrates and vegetable oils are now the cheapest source of calories [18, 20–22], it is not surprising that the combination of these two dietary trends has increased the production of AA thus leading to an epidemic increase in silent inflammation.

There is epidemiological evidence that suggests that high intake of omega-6 fatty acids may have a potential cardiovascular benefit [23, 24]. However, this epidemiological hypothesis was tested in a carefully controlled secondary prevention trial [25, 26]. This study, known as the Lyon Diet Heart Study, placed patients who already had a previous heart attack into one of two intervention groups. The first group followed a diet rich in omega-6 fatty acids following the American Heart Association dietary guidelines. The other group followed a diet that was low in omega-6 fatty acids. After 3.5 years the group with the low omega-6 fatty acid intake had 70% fewer fatal and nonfatal heart attacks and a complete elimination of sudden cardiac death compared to the group following the high omega-6 fatty acid diet.

The impact of a high omega-6 fatty acid diet can be understood from the metabolic pathway of linoleic acid conversion to AA as shown in Figure 1.

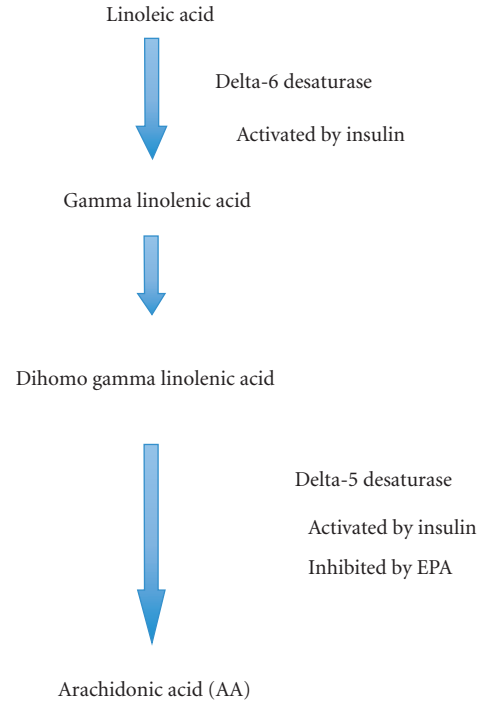

Figure 1: Metabolism of Omega-6 Fatty Acids.

The two rate-limiting steps in this metabolic cascade of linoleic acid to arachidonic acid are the enzymes delta-6 and delta-5 desaturase. These enzymes insert cis-double bonds into unique positions in the omega-6 fatty acid molecule. Normally, these steps are very slow, thus limiting the production of AA. However, insulin is a strong activator of each of these enzymes [27–30]. This means that a high glycemic-load diet coupled with increased intake of vegetable oils rich in linoleic acid will lead to increased production of AA and a corresponding increase in silent inflammation.

Finally, there is the role of the omega-3 fatty acid EPA in this metabolic cascade and its effect on silent inflammation. In high enough concentrations, EPA can partially inhibit the activity of the delta-5-desaturase enzyme thus reducing AA formation by acting as a weak feedback inhibitor as both fatty acids use the same enzyme for their production. More importantly, an increased EPA content in the membrane phospholipids decreases the release of AA that is necessary to make pro-inflammatory eicosanoids. In this regard, increased consumption of EPA dilutes out existing AA, thus decreasing the production of pro-inflammatory eicosanoids. Finally, EPA is the molecular building block to powerful anti-inflammatory eicosanoids known as resolvins [31–34].

Unfortunately, the consumption of long-chain omega-3 fatty acids, such as EPA, has dramatically decreased over the past century [35]. With that decrease in EPA intake coupled with the increased consumption of refined carbohydrates and vegetable oils, the dietary stage was set for a dramatic increase in silent inflammation.

8. How Obesity Induces Chronic Disease

Obesity can be defined as accumulation of excess body fat. However, it is the location of that excess body fat that determines whether or not obesity leads to acceleration of chronic diseases. If the extra body fat is constrained to the adipose tissue and there is no compromise of metabolic function, that obese individual would be considered metabolically healthy [36]. Apparently 1/3 of obese Americans fall into this category [37]. On the other hand, if increasing amounts of the excess fat become deposited in other organs (muscles, cardiac tissue, liver, pancreas), this is known as lipotoxicity [38–40]. With lipotoxicity comes an acceleration of those chronic diseases (diabetes, heart disease, cancer, etc.) associated with obesity. The extent of lipotoxicity will be determined by the health of the fat cells in the adipose tissue.

9. Adipose Tissue as a Staging Area for Systemic Silent Inflammation

The adipose tissue is the only organ in the body that can safely store triglycerides. As a consequence, it occupies the central position in controlling silent inflammation by acting as a fat-buffering system especially by controlling the blood levels of AA. Healthy fat cells have the ability to extract any excess fatty acid (including AA) in the blood and to safely store it as a triglyceride. In addition, the adipose tissue can readily induce the formation of new fat cells from internal stem cells to increase the storage of increasing levels of circulating fat in blood coming from either ingestion of dietary fat or metabolism of excess carbohydrates and protein that have been converted into circulating fat by the liver [41].

10. Fat Cell Life Cycle

The definition of a healthy fat cell is one that can easily expand to sequester incoming fats, in particular, for long-term storage, and also governs the controlled release of stored fat for ATP production in the peripheral tissues. The ability to sequester circulating fat into the fat cell depends upon the integrity of insulin signaling that brings adequate levels of glucose into the fat cell that can be converted to glycerol. This necessary step is required to convert incoming free fatty acids into triglycerides for long-term storage.

The problem begins to arise when AA levels become too great in a particular fat cell. As an initial defensive mechanism, the generation of new fat cells is induced by metabolites of AA [42, 43]. Although this is associated with greater adiposity [44], the creation of new healthy fat cells maintains the capacity of the adipose tissue to prevent potential lipotoxicity. However, as the AA levels continue to increase in any particular fat cell, the cell's response to insulin signaling becomes compromised due to internal silent inflammation that interrupts the flow of glucose into the fat cell to provide the necessary glycerol for fatty acid storage [45]. It appears this is a consequence of the generation of pro-inflammatory eicosanoids (leukotrienes) that are derived from AA [46, 47]. As a result, the fat cell has a more difficult time sequestering newly formed AA as well as other fatty acids circulating in the blood. At the same time, insulin inhibition of the hormone-sensitive lipase in that particular fat cell becomes compromised because of the same disruption in the insulin-signaling cascade. As a result, more free fatty acids are being released into circulation [48, 49]. These are the hallmarks of classical insulin resistance. It appears that insulin resistance due to increased AA levels may arise in the fat cell prior to developing in the muscle cells [50, 51]. As a result, greater amounts of AA remain in circulation to be taken up by other cells potentially leading to acceleration of insulin resistance in the muscle cells, which in turn causes increased hyperinsulinemia. To further compound the situation, a compromised fat cell is releasing greater amounts of previously sequestered AA in the fat cells into the circulation [52].

As the levels of AA further increase in any one particular fat cell beyond a critical threshold barrier, cell death can take place [53]. The necrosis of that particular fat cell causes a migration of macrophages into the adipose tissue [54, 55]. This increase in macrophage accumulation in the adipose tissue is clearly seen in both animal models of obesity as well as in humans [56]. These newly recruited macrophages cause the secretion of additional inflammatory mediators, such as IL-1, IL-6 and TNFα, which increase inflammation within the adipose tissue [57–69]. These newly released inflammatory cytokines can interact with their receptors at the surface of nearby fat cells to signal a further activation of NF-κB, the key gene transcription factor that drives the inflammatory responses of the innate immune system. Support for this hypothesis of AA-driven inflammation in the fat cells comes from the observations that the amount of macrophage accumulation can be significantly reduced upon supplementation with high-dose fish oil rich in EPA to reduce the inflammation in the adipose tissue [70–72].

As inflammation in the adipose tissue increases, inflammatory cytokines, such as IL-6 derived from the macrophages attracted to the inflamed fat cells, can exit into the circulatory system to cause an increase in CRP formation in the liver. Hence the correlation between obesity and CRP levels [73]. Likewise TNFα generated by the same macrophages causes further insulin resistance in the surrounding fat cells, thus decreasing their ability to sequester newly formed AA as well as causing the release of even more stored AA into the circulatory system. In many ways, the staging area for insulin resistance in other organs (muscles, liver, and eventually the pancreas) can be considered to start in the adipose tissue. As insulin resistance spreads to other organs, the end result is lipotoxicity in the muscle cells (both smooth muscle and cardiac muscle), liver, and the beta cells in the pancreas.

As long as the adipose tissue is composed of healthy fat cells, any increased production of dietary-induced AA can be safely handled by their continued expansion that can rapidly remove any excess AA from the blood and store it safely in the fat cells. In the absence of a large percentage of healthy fat cells in the adipose tissue, the combination of the growing lack of ability to sequester AA from the blood coupled with

the accelerated release of stored AA from the fat mass into the circulation is similar to the metastatic spread of a tumor; only now it is silent inflammation that is spreading. Depositions of lipid droplets that cause lipotoxicity characterize this metastasis of silent inflammation. If these accumulated lipid droplets are also enriched in AA, then the development of inflammatory diseases, such as type 2 diabetes, will be accelerated.

Understanding the role of healthy fat cells may explain why approximately one-third of obese individuals are actually quite healthy [37]. These individuals appear to have higher levels of the adipose-derived hormone adiponectin [74]. This is confirmed by studies of the overexpression of adiponectin in diabetic animals [75]. It should be noted that adiponectin is an adipose-derived hormone that can be increased by high levels of fish oil rich in EPA possibly acting through the PPARγ transcription factor [76–78].

One of the first indications that lipotoxicity is taking place is the appearance of metabolic syndrome. Metabolic syndrome can be considered to be prediabetes. It is characterized by a combination of clinical markers, such as a high TG/HDL ratio, increasing abdominal fat, and hyperinsulinemia. Recent data indicate that there is a strong correlation between metabolic syndrome and levels of AA in the adipose tissue [50].

Left untreated, metabolic syndrome will usually result in the development of type 2 diabetes within 8–10 years. During this time period, the insulin resistance of the individual is continually increasing. This will cause even more AA formation, especially if consumption of omega-6 fatty acids remains high. Since the fat cells are now compromised in their ability to sequester this increased AA production, the AA levels remain in the blood to be picked up by other organs.

The final development of type 2 diabetes only occurs when the lipotoxicity has metastasized to the pancreas, causing a decreased output of insulin [79]. With insulin secretion decreased, there is a rapid rise of blood sugar levels. The development of type 2 diabetes indicates that the metastasis of silent inflammation from the adipose tissue to the pancreas is now complete.

Ironically, even extreme lipotoxicity can be reversed by the creation of new healthy fat cells. This has been demonstrated in transgenic obese, diabetic mice that overexpress adiponectin, an adipocyte-derived hormone that reduces insulin resistance [75]. It is hypothesized that this increased production of adiponectin activates PPARγ, which causes the proliferation of adipose stem cells to produce new healthy adipocytes. These transgenetic obese mice become even more obese, but there is a normalization of blood glucose and lipid levels [75]. This is similar to the elevated levels of adiponectin found in metabolically healthy obese individuals [74]. One mechanism of protection against lipotoxicity might be that the new healthy fat cells in the adipose tissue can now sequester circulating fatty acids (including AA) more effectively to allow the resolution of the inflammatory lipid droplets in the muscle, liver and beta cells of the panaceas. Essentially this resolution process represents a reverse flow of the lipotoxic lipid droplets in other organs back to the adipose tissue and reverses insulin resistance in the muscle and liver cells as well as decreasing the inflammation in the beta cells of the pancreas.

Support of this hypothesis regarding the impact of AA on fat cell metabolism comes from studies on AA levels in fat cells on various chronic disease conditions. In particular, increased AA levels in the fat cells are significantly associated with increased body fat, development of metabolic syndrome, and incidence of nonfatal heart attacks [44, 50, 80].

11. Anti-Inflammatory Nutrition

The goal of anti-inflammatory nutrition is to understand how pharmacological targets of inflammation can also be impacted by dietary nutrients. This would include reduction of those dietary components (a) that directly activate the inflammatory responses of innate immune system that directly affecting gene transcription factors such as NF-κB or (b) that indirectly activate NF-κB by interacting with toll-like receptors or cytokine receptors. These dietary nutrients that induce an inflammatory response can disrupt hormonal signaling patterns between hormone receptors and their internal targets giving rise to insulin and leptin resistance. These dietary nutrients (AA and saturated fats) that induce inflammatory responses via NF-κB are ones that must be significantly reduced in the diet. Other dietary components (such as omega-3 fatty acids and polyphenols) that either inhibit toll-like receptors or activate anti-inflammatory gene transcription factors such as PPARα and PPARγ must be increased to therapeutic levels in the diet. The combination of these two dietary strategies should lead to a comprehensive inflammatory gene silencing technology.

Before discussing these molecular targets in detail in the context of anti-inflammatory nutrition, it is best to start with our knowledge of the molecular targets in terms of current anti-inflammatory pharmacological approaches used today especially in the treatment of obesity and diabetes.

12. Pharmacological Targets for Reducing Inflammation

12.1. Anti-Inflammatory Drug Targets. If the hypothesis is correct that obesity is caused by silent inflammation, then modulating molecular targets of anti-inflammatory drugs would hold promise. Anti-inflammatory drugs remain the foundation of medical treatment of pain caused by acute inflammation. The relief of acute pain requires immediate action with drugs. The more acute the pain, the more powerful the anti-inflammatory drug that has to be used. Unfortunately, the more powerful the anti-inflammatory drug, the greater the side effects. Our working hypothesis is that the obesity and the metabolic consequences of obesity are caused by chronic low-level inflammation or silent inflammation. Nonetheless the molecular targets of anti-inflammatory drugs are the same for anti-inflammatory nutrition.

The classical pathways of anti-inflammatory drugs have been focused on the COX and LOX pathways of eicosanoid production. Inhibitors of the COX enzymes, such as aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs), have different targets. Aspirin is a suicide inhibitor of the PGH_2 synthase enzyme that is the rate-limiting step of the formation of pro-inflammatory eicosanoids. NSAIDs, on the other hand, are competitive inhibitors of various enzymes involved in the generation of pro-inflammatory prostaglandins. However, neither of these drugs has much effect on the LOX pathways that generates leukotrienes. Corticosteroids inhibit both the COX and LOX pathways by inhibiting the release of AA from the phospholipids in the cell's membranes.

12.2. Gene Transcription Factor Targets. The key component of the inflammatory response is the activation of NF-κB, which acts as master genetic switch to cause the express of inflammatory proteins (such as the COX-2 enzyme and various inflammatory cytokines) that amplifies the inflammatory response. Recent evidence has indicated that aspirin, salicylates, and statins also inhibit the activation of NF-κB at high concentrations [11, 12]. It is now hypothesized that many of the cardiovascular benefits of statins may come from their anti-inflammatory actions as opposed to their cholesterol-lowering effects [81].

If NF-κB can be considered to be an inflammatory gene transcription factor, then the PPAR family of gene transcription factors can be considered to be anti-inflammatory. There are a number of drugs that activate the PPAR systems. PPARα is the transcription factor that induces the increased expression of fat oxidation enzymes. It is this transcription factor that is activated by drugs such as fibrates [82]. These drugs are effective in lowering triglyceride levels and are widely used in cardiovascular treatment. Once the PPARγ system is activated, it has profound anti-inflammatory effects including the generation of new healthy fat cells that reduce lipotoxicity. The thiazolidinediones, a mainstay of diabetes treatment, are examples of such drugs that activate the PPARγ gene transcription factor [83]. However unlike omega-3 fatty acids, thiazolidinediones do not have a protective effect against heart attack [84]. In fact, one of the more commercially successful thiazolidinediones (i.e., rosiglitazone) has been implicated in increased cardiovascular events [85].

12.3. Regulatory Enzyme Targets. Certain enzymes act as energy sensors and can regulate a great number of metabolic systems especially dealing with glucose and lipid metabolism, especially AMP kinase. The diabetic drug metformin is one such drug that activates this particular enzyme [86, 87]. This enzyme not only generates adequate levels of ATP, but also regulates a wide number of enzyme systems involved in glucose and lipid metabolism [88]. As a result, metformin is sometimes used as an off-label weight loss drug [89].

12.4. Hormone Targets. Obviously, the most important hormones in this category are the eicosanoids derived from AA described earlier. Another group of hormones derived from AA include endocannabinoids that are involved in generating hunger. The endocannabinoid receptor antagonist (rimonabant) was developed as an appetite-suppressant for the treatment of obesity [90]. This drug was never approved in the United States due to its various neurological problems, including an increase in suicidal tendencies. These problems far overshadowed any potential weight loss benefits.

Other hormones involved in the appetite control come from the gut, including the satiety hormones PYY and GLP-1. Currently the only long-term medical intervention to treat obesity is gastric bypass surgery. The most successful of these types of surgery is Roux-en-Y gastric bypass that reroutes much of the small intestine thus delivering most of the dietary nutrients to the distal part of the small intestine (i.e., ileum). Unlike other gastric bypass methods, this surgery also increases the release of gut hormones such as PYY and GLP-1 from the L-cells in the ileum, to give a profound degree of satiety [91]. This has led many drug companies to test various drugs to increase satiety hormones in order to achieve the same weight loss benefits of Rous-en-Y bypass surgery. Two diabetic drugs, exenatide (a GLP-1 receptor agonist) and sitagliptin (an inhibitor of the enzyme that degrades GLP-1) have demonstrated the potential for inducing weight loss. Combination products consisting of hormones such as pramlinitide and leptin are in various stages of testing, but have not yet been approved.

12.5. Neurotransmitter Targets. Although we have many successful examples of drugs for the treatment of acute inflammation, cardiovascular, and diabetes, there are relatively few examples of drugs that are very successful in the treatment of obesity. The most successful of these drugs are amphetamines that stimulate dopamine receptors in the brain leading to increased satiety. Unfortunately, they can also lead to addiction [92]. It was though a combination drug known as fen-phen that might circumvent this problem. Fen-phen was a combination of a weaker amphetamine (phentermine) and a powerful serotonin agonist (fenfluramine). This drug combination was dropped then, it was discovered that it increased the incidence of primary pulmonary hypertension and heart valve degeneration [93]. A new combination of phentermine and the antiepilepsy drug topiramate is under investigation, but has not yet been approved.

Phentermine and other ampthetamine derivatives are still in use today for the treatment of obesity but only for short-term use (approximately 12 weeks). In an effort to bypass these restrictions, various amphetamine-like drugs used to treat attention-deficient conditions are often used as off-label weight loss drugs.

The only currently approved drugs for long-term treatment of obesity are sibutramine (i.e., structurally similar to amphetamines) that acts as serotonin and norepinephrine reuptake inhibitor and orlistat that inhibits the breakdown of fat in the GI tract. Unfortunately, neither of these drugs has demonstrated any significant impact in reversing the obesity epidemic.

13. Molecular Targets for an Anti-Inflammatory Nutrition

With the above as a short review of the pharmacological targets for reducing inflammation or treating obesity, we can now turn to understanding how various dietary components can be used to interact with the same molecular targets as pharmacological agents.

13.1. Pro-Inflammatory Nutrients. Anti-inflammatory drugs work by inhibiting the formation of downstream pro-inflammatory eicosanoids derived from AA. A more elegant approach is to go upstream and reduce the levels of AA in the cells thereby reducing the substrate required for the production of pro-inflammatory eicosanoids. There is no known drug that can reduce AA. Only the diet can [94, 95]. The most obvious solution is the reduction of the direct dietary intake of AA. Dietary sources richest in AA include organ meats and egg yolks. Other food sources that are slightly lower in AA content include all animal protein sources including fish. It has also been shown that AA has a direct effect on the activation of NF-κB [96]. This may be mediated by increased leukotriene production [46, 47].

However, even if individuals were following even a strict vegetarian diet, they would be able to produce large amounts of AA if that vegetarian diet is rich in both omega-6 fatty acids and high glycemic index carbohydrates that stimulate insulin secretion. As described earlier, the combination of these two dietary factors will increase the production of AA. Therefore in addition to reducing the direct intake of AA, one should also have dietary strategy for the simultaneous reduction of the dietary intake of omega-6 fatty acids, such as linoleic acid, and lowering of the levels of insulin generated by the diet. Reduction of linoleic acid can be achieved by using fat sources low in omega-6 fatty acids, such as olive oil or nuts as well as reducing the consumption of red meat. Reducing insulin requires a reduction of the glycemic load of the diet by increasing the consumption of vegetables and fruits as the primary sources of carbohydrates and the simultaneous reduction of the consumption of high-glycemic carbohydrates, such as grains and starches. Another inflammatory nutrient is saturated fatty acids. Saturated fats can bind to TLR-4 and thus indirectly activate NF-κB [97–102].

Finally, what is often not appreciated is that the total calorie content of a meal can also raise insulin levels and increase inflammation. Therefore maintaining a calorie-restricted diet is also essential for insulin control. It has been demonstrated that overnutrition causes inflammation in the hypothalamus and disrupts the precise signaling balance of satiety and hunger hormones and thus increases appetite [103].

13.2. Anti-Inflammatory Nutrients. The omega-3 fatty acid eicosapentaenoic acid (EPA) will have little direct impact on the reduction of AA because it is a weak inhibitor of the delta 5-desaturase enzyme; however, at high dietary intakes, the EPA can dilute out the concentration of AA in the cell membrane thereby decreasing its potential of being converted into a pro-inflammatory eicosanoids, such as leukotrienes. As stated earlier, high intakes of EPA can also reduce inflammation in the adipose tissue [70–72]. Thus by either directly inhibiting the formation of AA or diluting it out by the presence of high levels of EPA in target cells (especially in the adipose tissue), overall inflammation will be automatically reduced as long there is constant supplementation with fish oils rich in EPA.

13.3. Gene Transcription Factors. Inhibition of the NF-κB is another key anti-inflammatory nutrient target. AA can directly activate NF-κB [96] or serve as substrate for the production of leukotrienes that also appear to activate NF-κB [46, 47]. Thus, the overall reduction of AA will not only reduce the production of pro-inflammatory eicosanoids, but also inhibit the release of gene products (COX-2 enzymes and inflammatory cytokines such as TNFα, IL-1, and IL-6) that are expressed if NF-κB is activated.

Omega-3 fatty acids and polyphenols can also inhibit the activation of NF-κB [104, 105]. However, one requires a therapeutic level of these nutrients to have any significant inhibition of NF-κB. Omega-3 fatty acids can also inhibit the binding of saturated fats to the TLR-4 on the cell surface thus also indirectly inhibiting NF-κB activation [106].

PPARα is the gene transcription factor activated by fibrates thus increasing the expression of fat oxidation enzymes needed to lower triglyceride levels. The same transcription factor can be activated by omega-3 fatty acids, such as EPA and docosahexaenoic acid (DHA). Both omega-3 fatty acids appear to provide a similar activation of this gene transcription factor [107]. By reducing the circulating levels of triglycerides, one also reduces the potential development of lipotoxicity. In essence, one is using fat (if it is rich in EPA and DHA) to burn fat by activating PPARα.

PPARγ is anti-inflammatory gene transcription factor. Its activation creates the stimulus for the production of new healthy fat cells that enhance the capacity of the adipose tissue for resequestering accumulated fat in other tissues thus reversing lipotoxicity. This will reverse much of the metabolic damage caused by the lipotoxicity, but it may possibily make the individual fatter in the process. PPARγ is stimulated by adiponectin whose release from fat cells can be enhanced by the increased consumption of omega-3 fatty acids [76–78]. In addition, EPA and DHA can also directly activate PPARγ [108].

13.4. Hormones. Many of the hormones involved in the control of hunger and satiety are generated by diet. Insulin is a key hormone in the development of obesity. If insulin levels remain elevated, the stored fat in the adipose tissue will remain sequestered due to its inhibition of the hormone sensitive lipase in healthy fat cells. In addition, insulin in the blood is a hunger hormone because of its ability to lower blood glucose levels. Although a diet rich in high-glycemic carbohydrates will increase the post-prandial levels of insulin, hyperinsulinemia caused by silent inflammation in the muscle cells will keep insulin constantly elevated.

This sets up a cycle of increased calorie (primarily carbohydrate) consumption generating increased inflammation in the hypothalamus that dissociates hunger and satiety signals [103].

Ironically, insulin can also function as a satiety hormone if it can reach the hypothalamus [109–111]. But if one has insulin resistance (induced by silent inflammation), then the high levels of insulin in the blood are unable to relay their message to the key cells in the hypothalamus and potential satiety effects of insulin are blunted. Leptin is a hormone released from the fat cells that is also involved in satiety. Like insulin, it must also reach the hypothalamus to exert its satiety actions. Obese individuals are characterized by both insulin and leptin resistance [110–113].

Inhibition of endocannabinoid binding to its receptors in the brain is the mechanism action of rimonabant. Since endocannabinoids are derived from AA, reduction of its levels in the brain should reduce hunger. Unfortunately, the half-life of AA in the brain is long in humans [114]. However, increasing the levels of EPA in the brain can inhibit the binding of endocannabinoids [115]. Since the half-life of EPA in the brain appears to be very short [116], this requires maintaining a therapeutic level of EPA in the blood to create a constant gradient necessary for the constant flow of the EPA into the brain. This gradient can only be maintained by a diet either very high in fatty fish consumption or supplemented by fish oil rich in EPA.

As mentioned earlier, much of the success of gastric bypass surgery is related to the increase in satiety hormones (PYY and GLP-1) released from the ileum. The release of these hormones can be enhanced by slowing down the rate of digestion and absorption of protein and carbohydrate in a meal so that greater amounts of these nutrients can reach the L-cells in the ileum. Lowering the glycemic load of the diet by the inclusion of fiber-rich carbohydrate sources (especially those rich in soluble fiber) can slow the digestion and absorption process of both protein and carbohydrate. At the same time, increasing the protein content of that meal will also increase PYY levels [117]. The slower rates of digestion and absorption mean more of these hormone agonists will appear in the ileum and thus generate higher levels of these satiety hormones.

13.5. Neurotransmitters. Amphetamines increase dopamine levels and decrease hunger [92]. EPA and DHA can also increase dopamine levels in animal models [118]. It has been demonstrated that dietary supplementation of high-dose EPA and DHA can further reduce the symptoms of attention-deficit hyperactivity disorder (ADHD) in children already on their optimal level of drugs used to treat this condition [119, 120].

13.6. AMP Kinase. This particular enzyme is stimulated by metformin [86, 87]. High levels of polyphenols can also stimulate the same enzyme [121–123]. Its activation is a key for not only increasing ATP levels, but also to regulate lipid and carbohydrate metabolism. As with EPA, therapeutic levels of polyphenols are required due to their low bioavailability probably due to their rapid degradation in the small intestine.

13.7. Increased Thermogenesis. The higher the protein content of the diet is, the more thermogenesis is increased [124, 125]. The underlying cause may be activation of protein synthesis. To activate such protein synthesis requires a combination of adequate levels of leucine and a consistent level of insulin in the blood to activate mTOR to stimulate protein synthesis during the post-prandial period [126]. Data suggest that it takes 20–30 grams of protein at a meal to stimulate this protein synthesis [127].

14. Developing an Anti-Inflammatory Diet Based on Anti-Inflammatory Nutrition

Anti-inflammatory nutrition is the understanding how individual nutrients affect the same molecular targets affected by pharmacological drugs. This is only the first step in developing an anti-inflammatory diet. Such a diet should contain all nutrient considerations described above, as well as being a diet that can promote compliance for a lifetime.

The first question would concern the protein, carbohydrate, and fat composition for such an anti-inflammatory diet. Currently the dietary recommendations for suggested fat content in diet range from 20 to 35% of total calories [128]. Lower-fat diets are simply too difficult to maintain for a sustained basis as demonstrated in long-term studies [129–131]. Therefore long-term dietary compliance appears to be more likely at 30% of total calories as dietary fat. However, the fat composition must also be low in both omega-6 and saturated fats because of their ability to increase silent inflammation by their interaction with various components of the innate immune system. The only common dietary fats that are low in both omega-6 and saturated fats are olive oil and nuts. Thus they should constitute the bulk of the dietary fat in any anti-inflammatory diet.

The next question is the amount of protein. Most dieticians would recommend consumption of no more low-fat protein than would fit on the palm of your hand. This translates into about 3 oz. of a low-fat protein source (this would contain about 20 grams of amino acids) for a typical female and approximately 4 oz. of low-fat protein (this would about 30 grams of amino acids) for a typical male. These traditional dietary recommendations are supported by recent research that indicates that this level of high-quality protein will contain enough of the branched-chain amino acid leucine to initiate protein synthesis [132]. Furthermore the absolute intake of protein should be between 20–30 grams at every meal to activate protein synthesis to increase thermogenesis [124, 127, 132]. If one takes into account the protein content of carbohydrate sources and potential snacks, then the average female should be consuming about 80–90 grams of low-fat protein per day and the average male about 100–110 grams of low-fat protein per day. This amount of protein would have to be equally spaced at each meal to provide the necessary levels of protein for

the enhanced release of PYY from the L-cells in the ileum after each meal thereby controlling satiety.

The carbohydrate content should be able to maintain a stable level of insulin between meals. This can be achieved with about 40 grams of low glycemic load carbohydrates at each meal. The vast bulk of the carbohydrates should come from sources with the highest content of polyphenols with the least amount of carbohydrates, meaning the consumption of primarily colorful nonstarchy vegetables, moderate amounts of fruits, limited amounts of whole-grains, and a radical reduction of the dietary intake of refined carbohydrates. Although there is some controversy to whether a low glycemic load diet leads to improved weight loss [133, 134], there is no question that a low glycemic load diet will generate a lower inflammatory burden [135–137].

Finally, there is the question of meal timing. The hormonal control benefits of any meal will last only about five hours. To achieve this hormonal control would require three low-calorie meals and two even lower-calorie snacks spaced throughout the day so that five hours never pass before consuming another meal or snack.

Such a proposed anti-inflammatory diet would consist of about 1,500 calories per day (about 50 grams of monounsaturated fat, 100 grams of low-fat protein, and 150 grams of low glycemic load carbohydrates per day). This would represent a 1 : 2 : 3 ratio of fat to protein to carbohydrate on a weight basis. On a calorie basis, that is about 30% of the calories as fat, 30% as protein, and 40% as carbohydrates. These are the dietary recommendations made by one of the authors in 1995 [94]. Similar dietary recommendations were made by the Joslin Diabetes Research Center at Harvard Medical School for the treatment of obesity, metabolic syndrome, and diabetes in 2005 [138] and confirmed by their own pilot studies [139]. It should be noted that we believe total calorie consumption should be driven by protein requirements necessary to maintain a positive nitrogen balance of essential amino acids, such as leucine. As an example, a typical female might require a total of 90 grams of protein per day to maintain adequate leucine levels to stimulate protein synthesis, whereas a male might require 110 grams of total protein per day. Thus using the macronutrient balance described above, the typical female would require slightly more than 1300 calories per day, whereas the typical male would require about 1600 calories per day to generate enough chemical energy for daily metabolic needs. Obviously, higher levels of physical activity in either the female or male would require higher protein intake to compensate for the breakdown of muscle protein during exercise with a corresponding increase in total calorie consumption [140].

To this anti-inflammatory diet foundation should be added supplemental omega-3 fatty acids at the level of 2-3 grams of EPA and DHA per day either by an increased consumption of fatty fish or supplementation with fish oil supplements rich in EPA. Finally, a diet rich in colorful, non-starchy vegetables also would contribute adequate amounts of polyphenols to help not only to inhibit NF-κB, but also activate AMP kinase.

In many respects, this proposed anti-inflammatory diet has similarities to a Mediterranean diet. Both are rich in vegetables and fruits. Both emphasize the moderate intake of low-fat protein sources, such as chicken and fish. Both recommend the use of monounsaturated fats, like olive oil and nuts. The differences are in the carbohydrate composition. An anti-inflammatory diet radically restricts the use of bread and grains (especially refined grain products) and makes up for it with increased consumption of more colorful (i.e., rich in polyphenols) vegetables and fruits. That one seemingly small difference will have tremendous hormonal and genetic consequences leading to a lowered inflammatory burden.

The goal of an anti-inflammatory diet is not weight loss *per se*, but the reduction of silent inflammation. Of course, this same reduction of silent inflammation should also result in consistent fat loss if our working hypothesis is correct that silent inflammation is a driving force for the accumulation of body fat. This is accomplished by affecting the same molecular targets that have been elucidated by pharmacological agents. The success of this anti-inflammatory diet can be measured clinically by various markers of silent inflammation as mentioned earlier as well as improvement of metabolic conditions (i.e., metabolic syndrome, diabetes, cardiovascular disease, etc.) that are associated with obesity.

15. Why Not Simply Eat Less and Exercise More?

Obesity is far more complex than simply it is caused by calories in being greater than calories out. This is why just telling obese individuals to eat less and exercise will rarely work. Obesity is a metabolic condition in which affects those who are genetically predisposed. Any increase in silent inflammation will cause disruption in metabolic use of fat coming from the adipose tissue to make adequate levels of ATP and also cause a dissociation of hunger and satiety signals in the brain. The combination will leave the obese individual constantly hungry and thus seeking food. Our working hypothesis is that the increased levels of silent inflammation generated by the diet cause a disruption in the signaling mechanisms in both the adipose tissue and the hypothalamus that leads to the accumulation of excess body fat. Unless those inflammatory driving forces are consistently reduced by the diet, the success rate for obese individuals trying to "eat less and exercise more" will remain abysmal.

This is perhaps best demonstrated by the data derived from the National Weight Control Registry on individuals who have lost considerable amounts of weight and have kept it off for more than five years [141, 142]. The average calorie intake for these individuals is approximately than 1,400 calories per day, which is in agreement with our general caloric recommendations. Although the National Weight Control Registry represents a biased reporting population, it is indicative that our suggested calorie intake is in line with long-term weight maintenance as long as our recommended protein amounts are being consumed on a consistent basis throughout the day. It should also be noted that these individuals also exercise for approximately one hour per day which would increase their protein and calorie requirements.

This suggests that our calorie recommendations for sedentary individuals may actually be generous.

Implementation of an anti-inflammatory diet would reduce the silent inflammation that will make it easier to not only maintain a reduced weight with less effort, but also markedly reverse the driving force (i.e., lipotoxicity) for the development of chronic disease. That is the real reason to lose excess body fat.

16. Summary

The ultimate treatment of obesity lies in re-establishing hormonal and genetic balance that generates satiety instead of constant hunger. This can be achieved by reducing silent inflammation induced by the diet. Pharmacological agents can pinpoint what those molecular targets are that induce inflammation. The purpose of anti-inflammatory nutrition is to determine which food ingredients can affect the same molecular targets as drugs and determine what the therapeutic concentrations are for those nutrients required to affect the same molecular targets. Only then can you develop an anti-inflammatory diet that has to be used like a drug at the right time and right level to keep silent inflammation under control.

Obesity is not a consequence of a drug deficiency or a lack of will power, but a lack of knowledge of which nutrients are necessary and which concentrations that are required to control silent inflammation and therefore control appetite. Silent inflammation is generated by the mismatch of our current diet and our genes. Anti-inflammatory nutrition should be considered as a form of gene silencing technology, in particular the silencing of the genes involved in the generation of silent inflammation. Pharmacological agents often work downstream from the true primary molecular target of inflammation (NF-κB), whereas anti-inflammation nutrition works upstream to reduce the dietary factors that activate NF-κB to generate silent inflammation. Not only is upstream targeting a more elegant way to treat obesity with an almost infinite therapeutic index compared to pharmacological agents, but it also provides a new approach to treat chronic diseases ultimately caused by silent inflammation.

Financial Disclosures

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Review Article

Weight Considerations in Pharmacotherapy for Type 2 Diabetes

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Obesity has been increasing in prevalence worldwide and the majority of patients with type 2 diabetes are either overweight or obese. Diabetes management in this population has been difficult since a number of antidiabetes agents are associated with weight gain. The effects of various antidiabetes agents and antiobesity agents on glycemic control and body weight will be reviewed. Briefly, sulfonylureas, thiazolidinediones, and insulin are associated with weight gain, whereas metformin and amylin analogs are weight neutral or associated with modest weight loss. Dipeptidyl-peptidase-4 inhibitors are weight neutral, whereas glucagon-like peptide-1 analogs are associated with weight loss. The effect of orlistat and sibutramine in type 2 diabetes is also evaluated. The treatment of diabetes should not only focus on glycemic control as its sole intention, but it should factor in the effect of these various agents on weight, as well, since obesity aggravates insulin resistance, beta cell failure, and cardiovascular risk.

1. Introduction

Obesity has been increasing in prevalence worldwide and is strongly associated with the development of type 2 diabetes [1]. Both obesity and diabetes are associated with an increase in morbidity and mortality primarily from cardiovascular disease. Currently, about 86% of patients with type 2 diabetes are either overweight or obese [2]. Obesity, particularly intra-abdominal obesity, induces insulin resistance in muscle and liver that leads to glucose intolerance [3]. Consequently, insulin resistance has long been targeted for diabetes control. Additionally, progressive loss of pancreatic beta cell function hallmarks the central defect in diabetes and is related to loss of incretin stimulation, amylin production, and the effects of glucotoxicity and lipotoxicity [4]. Hence, medical treatment of diabetes has collectively targeted these various pathophysiological mechanisms.

Intensive lifestyle modification with diet and exercise to achieve weight loss of at least 5–10% has been shown to improve glycemic control with improvements in insulin sensitivity and beta cell function [5]. Intensive glucose lowering of HbA1c levels to less than 7% is associated with significant reduction in microvascular complications [6]. However, as has been shown in a number of prospective trials including the United Kingdom Prospective Diabetes Study (UKPDS), intensive glucose control is associated with a median weight gain of 3–3.5 kg. A more recent example of this effect is illustrated by the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial which compared standard therapy (HbA1c 7–7.9%) with intensive glucose control (<6%) in over 10,000 subjects [7]. More patients in the intensive group had significant weight gain of >10 kg (28% versus 14%). For every 1% decrease in HbA1c, there was a corresponding weight gain of about 2 kg [8]. Thus, the effect of antidiabetes agents on weight and obesity status is an important consideration when treating patients with diabetes since obesity, itself, aggravates insulin resistance and cardiovascular risk.

A number of medications including sulfonylureas, thiazolidinediones, and insulin are associated with weight gain particularly when used in combination, whereas metformin and amylin analogs are weight neutral or associated with modest weight loss. DPP4-inhibitors are weight neutral whereas GLP-1 analogs are associated with weight loss (Figure 1). The effect of various antidiabetes medication classes on glycemic control and weight will be reviewed here. Based on a practice-based approach, a therapeutic strategy that balances weight and glucose control for obese type 2 diabetic patients will be presented in Figure 2 that is distinct from the conventional glucose lowering approach [9].

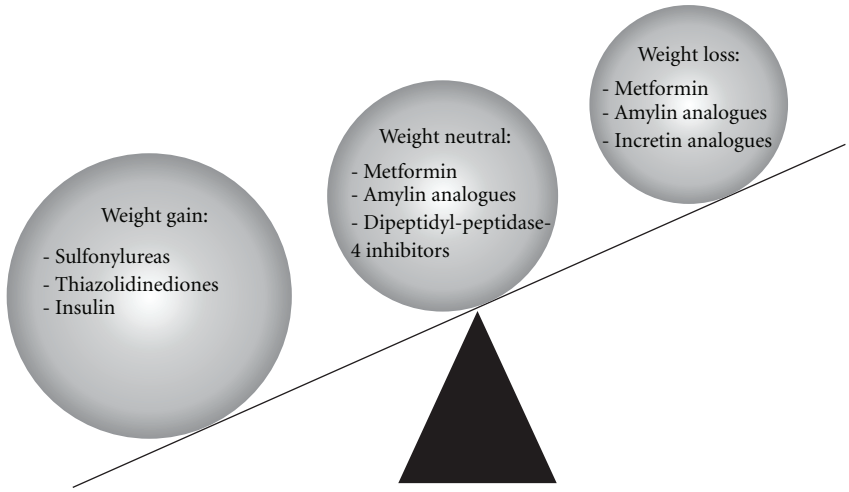

Figure 1: Effects of various antidiabetes agents on body weight.

2. Antidiabetes Agents Associated with Weight Gain

2.1. Sulfonylureas. The sulfonylureas (SUs) have been extensively used in the treatment of type 2 diabetes and are associated with weight gain. SUs stimulate pancreatic betacells directly to secrete insulin independent of glucose concentrations. SUs, when used as monotherapy, are expected to decrease HbA1c by 1-2% [10, 11]. In UKPDS, 3,867 newly diagnosed patients with type 2 diabetes were randomized to intensive treatment with sulfonylurea (chlorpropamide 100–500 mg or glibenclamide 2.5–20 mg), insulin, or conventional dietary management [6]. The median HbA1c of 7.0% in the intensive group was significantly lower compared with the conventional treatment (7.9%) over the 10-year study period. However, patients on the intensive treatment group had greater weight gain (mean 2.9 kg; $P < .001$) than the conventional group. Patients on glibenclamide gained 1.7 kg more than the conventional group whereas those on insulin gained 4.0 kg more.

To determine the effects of SU when combined with metformin, Garber et al. conducted a multicenter, double-blind study in which 486 type 2 diabetes patients (mean HbA1c ~8.7%) were randomized to glyburide/metformin therapy (1.25/250 mg), glyburide (2.5 mg), or metformin (500 mg) monotherapy with titration to maximum doses for 16 weeks [12]. There was greater reduction in HbA1c (−2.27%) in the glyburide/metformin group compared with both glyburide (−1.90%) and metformin (−1.53%) monotherapy ($P = .0003$). Patients on glyburide/metformin treatment had a weight increase of 1.6 kg compared with 2.0 kg in the glyburide group (P = NS). In contrast, patients on metformin had a weight loss of 1.1 kg. Thus, the combination of SU with metformin results in superior glycemic control with less weight gain than SU monotherapy.

2.2. Thiazolidinediones. Thiazolidinediones (TZDs) reduce hyperglycemia primarily by improving insulin sensitivity in adipose, skeletal muscle, and liver tissues. These compounds may also have some favorable effects to preserve beta cell function and prevent diabetes development in patients at high risk [13, 14]. TZDs are used as monotherapy or in combination with metformin or SUs in type 2 diabetes. In the Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) Study, 5,238 patients (mean HbA1c ~7.8%) were randomized to pioglitazone 15 mg to 45 mg ($n = 2,605$) or placebo ($n = 2,633$) in addition to their antidiabetes agents. Following the 34.5-month study period, the pioglitazone group achieved a reduction in HbA1c of 0.8% compared with 0.3% in the placebo group despite the addition of more antidiabetes agents in the placebo group [15]. There was an associated weight gain of 3.6 kg in the pioglitazone group as compared to a 0.4 kg weight loss in the placebo group. Despite weight gain, pioglitazone improved some secondary cardiovascular outcomes with regard to composite of all-cause mortality, nonfatal myocardial infarction, and stroke with multiple effects of lowering cholesterol and blood pressure.

The Diabetes Outcome Progression Trial (ADOPT) was done to evaluate rosiglitazone monotherapy on glycemic control compared with metformin or glyburide monotherapy. Patients (mean HbA1c ~7.4%) were randomized to receive maximum daily doses of rosiglitazone 8 mg ($n = 1,456$), glyburide 15 mg ($n = 1,441$), or metformin 2 g ($n = 1,454$). At 4 years, 40% of patients in the rosiglitazone group had HbA1c <7%, as compared with 36% in the metformin group and 26% in the glyburide group [16]. Weight gain was a major consistent finding with the use of TZDs. Over a period of 5 years, treatment with rosiglitazone resulted in an average weight gain of 4.8 kg versus 1.6 kg in the glyburide group whereas patients on metformin had a decrease in weight (−2.9 kg). The weight gain associated with TZDs was postulated to be due to an increase in subcutaneous fat as a result of fat redistribution from visceral adipose tissues, which is related to major improvements in insulin sensitivity in hepatic and peripheral tissues [17].

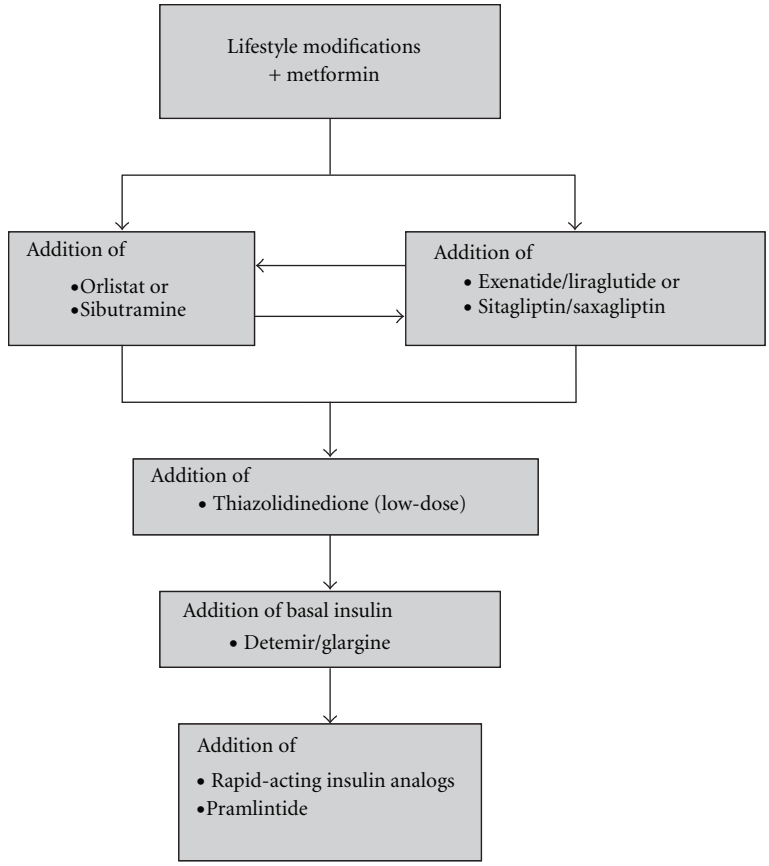

Figure 2: Practice-based approach for balancing weight loss and glycemic control (HbA1c <7%) in uncomplicated type 2 diabetes.

The use of TZDs and metformin as an insulin-sensitizing strategy as compared to an insulin-providing strategy with SUs and insulin employed in diabetic patients with coronary artery disease enrolled in the BARI 2D trial had a greater likelihood of achieving glycemic control with HbA1c <7% (insulin-sensitizing 55% versus insulin-providing 40%). Insulin-sensitizing therapy also was associated with less weight gain and hypoglycemia compared with the insulin-providing therapy [18].

The combination of TZDs with incretin agonists discussed below balances both glycemic and weight control. The addition of exenatide 10 μg twice daily to rosiglitazone 4 mg twice daily resulted in weight loss of 1.2 kg compared with weight gain of 1.5 kg with rosiglitazone monotherapy. There was also a greater reduction in HbA1c in the combination therapy group (-1.3% versus -1%; $P < .05$) [19].

2.3. Insulin. By way of progressive loss of beta cell function, the majority of patients with diabetes will require insulin therapy to achieve glycemic control. Early initiation of multiple daily insulin injections allowed rapid achievement of glucose control and improvement in beta cell function [20]. This effect was sustained for up to two years after discontinuation of insulin [21]. Early intensive glucose control had been repeatedly shown to improve microvascular outcomes, however, at the expense of significant weight gain and hypoglycemia [6]. Weight gain from insulin use is related to its effect to increase body fat and lean mass through reductions in glycosuria, anabolic effects on adipose tissue, as well as to appetite-enhancing effects [22]. However, the type of basal insulin used may have different effects on weight. Rosenstock et al. compared the efficacy of insulin analogs detemir and glargine when added to oral glucose-lowering agents in insulin-naïve type 2 diabetes subjects with baseline HbA1c of 8.6% in a 52-week, open-label treat-to-target trial [23]. Treatment with insulin detemir ($n = 291$) and glargine ($n = 291$) resulted in a comparable decrease in HbA1c from 8.6 to 7.2 and 7.1%, respectively. However, there were modest reductions in weight gain seen with determir versus glargine (+3.0 kg versus + 3.9 kg, $P = .01$). Holman et al. conducted an open-label multicenter trial to evaluate the efficacy of basal insulin, prandial insulin, and biphasic insulin in type 2 diabetes patients inadequately controlled (mean HbA1$\sim$8.5%) on oral hypoglycemic agents [24]. Patients randomized to prandial insulin ($n = 239$) had a greater weight gain of 6.4 ± 0.5 kg compared with patients on biphasic insulin ($n = 235$) with weight gain of 5.7 ± 0.5 kg and basal insulin ($n = 234$) of 3.6 ± 0.5 kg. Clearly, the weight gain associated with insulin is a major drawback for treating diabetes. Further studies examining the effects of antiobesity drugs and GLP-1 agonists to combat insulin-induced weight gain are needed.

3. Antidiabetes Agents that Are Weight Neutral or Weight Reducing

3.1. Metformin. Metformin is an insulin sensitizing agent that serves as the first line agent in treatment of type 2 diabetes and acts by decreasing hepatic glucose production and enhances peripheral tissue sensitivity to insulin. It has been shown to significantly delay the progression of impaired glucose tolerance to diabetes in the Diabetes Prevention Program (DPP) Study. This study was conducted to evaluate metformin in the prevention or delaying of the progression of type 2 diabetes in high-risk patients [5]. Patients who had impaired fasting glucose or impaired glucose tolerance test, were overweight and had a sedentary lifestyle were randomized to receive metformin 850 mg twice daily (n =1,073), lifestyle-modification (goal of 7% weight loss and 150 minutes of physical activity per week) program (n = 1,079), or placebo (n =1,082) with a mean followup of 2.8 years. There was a 58% reduction in incidence of diabetes in the lifestyle-intervention group and 31% reduction in the metformin group. Patients in the metformin group had a greater weight loss of 2.1 kg compared to placebo but less compared with the lifestyle-intervention group of 5.6 kg.

Monotherapy with metformin reduced the HbA1c up to 1.5% [25]. DeFronzo et al. conducted a two-parallel-group, double-blind study to determine the efficacy of metformin in moderately obese patients with diabetes uncontrolled with diet or diet plus glyburide. In the first protocol (n = 289), patients (mean HbA1c ~8.3) were randomized to metformin (n = 143) or placebo (n = 146) after an eight-week hypocaloric dietary phase. The addition of metformin resulted in the reduction of HbA1c by 1.4% versus 0.4% in the placebo group at 29 weeks. The metformin group experienced weight loss of 0.6 kg whereas the placebo group had a weight reduction of 1.1 kg (P = NS). In the second protocol (n = 632), the patients (mean HbA1c ~8.7%) were started or continued on glyburide for five weeks. They were then randomized to glyburide (n = 209), metformin (n = 210), and metformin-glyburide (n = 213). Patients given metformin-glyburide achieved a greater reduction in HbA1c of 1.7% compared with 0.4% with metformin alone. There was a weight loss of 3.8 kg in the metformin group compared with a weight gain of 0.4 kg in the metformin-glyburide group.

In a double-blind, open-label study, metformin-glibenclamide combination was compared with metformin-rosiglitazone in 318 type 2 diabetes patients uncontrolled on metformin monotherapy (mean HbA1c ~8.5%) [26]. Patients randomized to metformin-glibenclamide (n = 160) had a greater reduction in Hba1c of 1.5% compared with metformin-rosiglitazone (n = 158) with a reduction in Hba1c of 1.1% at 24 weeks. There was a greater weight gain with the metformin-glibenclamide group compared with the metformin-rosiglitazone group (+3.0 kg versus +1.4 kg). There is a general consensus that metformin is the treatment of choice in obese type 2 diabetes patients as it has been shown to be either weight neutral or even result in modest weight loss. However, combination with SUs or TZDs may result in weight gain whereas the combination with GLP-1 analogs may enhance weight loss.

3.2. Amylin Analogs. Pramlintide is an injectable synthetic analog of amylin which is a naturally occurring neuroendocrine hormone co-secreted with insulin by pancreatic beta cells in response to food intake. Pramlintide slows gastric emptying [27], attenuates postprandial glucagon secretion [28], enhances satiety, and reduces food intake [29]. In a 52-week, double-blind, multicenter trial, 656 patients (mean HbA1c ~9.1%) were randomized to subcutaneous injections of either placebo or pramlintide (60 μg TID, 90 μg BID, or 120 μg BID) in addition to their existing insulin regimen (alone or in combination with sulfonylureas and/or metformin) [30]. Treatment with pramlintide 120 μg BID produced a significant reduction in HbA1c of 0.68% at week 26 which was sustained at week 52. This effect was accompanied by a mean weight loss of 1.4 kg compared with a weight gain of 0.7 kg in the placebo group (P < .05). The addition of mealtime pramlintide 120 μg (n = 57) was compared with rapid-acting insulin analogs (n = 56) in addition to basal insulin in patients with inadequately controlled type 2 DM (n = 113; mean HbA1c ~ 8.2%) in a 24-week, randomized, open-label parallel-group, multicenter study [31]. Mealtime pramlintide and rapid-acting insulin analog (RAIA) resulted in a reduction of Hba1c of 1.1% versus 1.3%(P = .46), respectively. Pramlintide was weight neutral whereas RAIA use was associated with a weight gain of 4.7 ± 0.7 kg.

The efficacy of pramlintide was evaluated in obese, nondiabetic subjects in a double-blind, placebo-controlled dose-ranging study [32]. Four hundred and eleven obese subjects (BMI ~37.5) were randomized to receive pramlintide (120, 240, and 360 μg BID and TID) or placebo. Pramlintide at doses of 120 μg TID and 360 μg BID achieved weight loss of 3.2 ± 1.2 and 3.3 ± 1.1 kg, respectively, at 4 months. After completion of the 4-month study, 260 subjects continued to participate in the single-blind extension for 8 months. There was a progressive reduction in weight of 6.1 ± 2.1 and 7.2 ± 2.3 kg, respectively, at 12 months. In addition, 40 and 43% of patients treated with pramlintide 120 μg BID and 360 μg TID compared with 12% of patients in the placebo group achieved a reduction of >10% of baseline weight. These data are encouraging in that weight control is possible with the use of amylin analogs in patients inadequately controlled on insulin therapy.

3.3. Glucagon-Like Peptide-1 (GLP-1) Analogs. GLP-1 analogs (exenatide and ligraglutide) are the latest medications used to treat diabetes and have favorable effects on both glycemic and weight control. In diabetes and obesity, the GLP-1 response to an oral glucose load is blunted contributing toward impaired insulin secretion and prandial hyperglycemia [33]. GLP-1 enhances insulin secretion in a glucose-dependent manner, accounting for about 50–70% of the insulin secreted in response to oral glucose [34], and suppresses glucagon secretion [35]. Weight loss from GLP-1 analogs results from the effects of hormone to promote satiety and slow gastric emptying [36, 37]. Further, animal studies have shown beneficial effect of GLP-1 hormone on

beta cell proliferation and inhibiting apoptosis [38]. These effects are of particular significance given that beta-cell failure occurs prior to the onset of diabetes.

Exenatide has a 53% homology to human GLP-1. It is available subcutaneously and due to a short half-life of 2–4 hours, it is administered twice daily and at least 60 minutes before meals. In a 30-week triple-blind, placebo-controlled trial, 336 patients inadequately controlled on maximum doses of metformin (mean HbA1c ~8.2%) were randomized to receive 5 μg exenatide or placebo twice daily for 4 weeks followed by 5 or 10 μg exenatide or placebo twice daily for 26 weeks [39]. Exenatide 5 μg and 10 μg resulted in a significant reduction in HbA1c (−0.40% and −0.78%, resp.) compared with placebo (+0.085%). Moreover, there was a dose-dependent reduction in body weight. Patients on exenatide 10 μg had a weight loss of 2.8 kg compared with 1.6 kg on exenatide 5 μg. In an uncontrolled open-label extension of the prior 30-week study, 150 patients opted to continue exenatide treatment for another 52 weeks for a total of 82 weeks [40]. Exenatide was associated with a reduction in HbA1c of 1.0 ± 0.1% and with weight loss of 3.0 ± 0.6 kg at 30 weeks, effects were sustained at the end of 82 weeks with a final HbA1c reduction of 1.3 ± 0.1% and weight loss of 5.3 ± 0.8 kg.

The long-term efficacy of exenatide was assessed in an open-ended, open-label clinical trial in which patients from three placebo-controlled trials and their open-label extensions were enrolled for 3 years. A total of 217 patients completed the study [41]. The reduction in HbA1c from baseline to week 12 (−1.1 ± 0.1%) was sustained up to 3 years (−1.0 ± 0.1%). There was progressive reduction in body weight from baseline (−5.3 ± 0.4 kg at 3 years from −1.6 ± 0.2 kg at week 12). In addition to weight reduction, there was also a reduction in total body fat mass and truncal fat mass compared to the use of glargine [42]. The use of exenatide (10 μg BID with a 4-week 5 μg dose initiation) in addition to decreased calorie intake in nondiabetic obese subjects ($n = 152$) resulted in weight loss of 5.1 ± 0.5 kg from baseline versus 1.6 ± 0.5 kg with placebo at the end of a 24-week randomized study [43].

Liraglutide is more recently approved by FDA (January 2010) to be used as a once-daily subcutaneous injection for type 2 diabetes. In contrast to exenatide, liraglutide has a 97% homology to native human GLP-1 and has a longer half-life of 13 hours due to the presence of a fatty moiety allowing it to bind to albumin rendering it resistant to degradation by the DPP-4 enzyme [44]. Garber et al. conducted a 52-week double-blind, double-dummy, active-control, parallel-group study wherein 746 patients with early type 2 diabetes (mean HbA1c 8.2%) were randomized to once-daily subcutaneous liraglutide (1.2 mg or 1.8 mg) or oral glimepiride 8 mg [45]. Liraglutides 1.2 mg and 1.8 mg were associated with a reduction in HbA1c of 0.84% and 1.14%, respectively, compared with 0.51% with glimepiride. There was a dose-dependent reduction in body weight with 1.2 and 1.8 mg liraglutides (−1.85 kg and −2.26 kg, resp.).

The addition of liraglutide with metformin and TZDs was studied in a 26-week, double-blind, placebo-controlled trial. Subjects ($n = 533$) were randomized to once-daily liraglutide (1.2 mg or 1.8 mg) or placebo in addition to metformin (1 g BID) and rosiglitazone (4 mg BID) [46]. The addition of liraglutide resulted in a mean reduction of HbA1c of 1.5% for both the 1.2 and 1.8 mg liraglutide doses with a dose-dependent weight loss of 1-2 kg. In a 26-week, multinational, parallel-group trial, liraglutide was compared to glargine in addition to metformin and sulfonylurea [47]. Five-hundred-eighty-one patients were randomized (2:1:2) to liraglutide 1.8 mg ($n = 232$), placebo ($n = 115$), and open-label insulin glargine ($n = 234$) in addition to metformin (1 g BID) and glimepiride (4 mg once daily). There was a greater reduction in HbA1c with liraglutide compared with insulin glargine (−1.33% versus −1.09%). Liraglutide 1.8 mg resulted in a mean weight loss of 1.8 kg from baseline compared with 0.42 kg in the placebo group. Patients on insulin glargine gained 1.6 kg. In addition, waist circumference decreased significantly in the liraglutide group (−1.50 cm) whereas patients on insulin glargine had an increase in waist circumference (+0.89 cm).

Buse et al. compared the efficacy of liraglutide 1.8 mg once daily with exenatide 10 μg twice daily in a 26-week open-label, parallel-group multinational study [48]. Patients ($n = 464$) randomized to liraglutide ($n = 233$) had a greater reduction in HbA1c than to exenatide (−1.12% versus −0.79%, resp.) and more patients achieved HbA1c value of <7% with the liraglutide group (54% versus 43%). Both groups achieved weight loss (−3.24 kg versus −2.87 kg; P = NS). The efficacy of liraglutide (1.2 mg, 1.8 mg, 2.4 mg, or 3.0 mg) was assessed in obese nondiabetic individuals in a 20-week double-blind, placebo-controlled trial with an open-label orlistat comparator [49]. Individuals in the liraglutide group significantly lost more weight compared to placebo at all doses ($P < .003$) and orlistat group ($P = .003$ for liraglutide 2.4 mg and $P < .001$ for liraglutide 3 mg). Mean weight loss with liraglutide at different doses ranged from an average of 4.8–7.2 kg compared with 2.8 kg with placebo and 4.1 kg with orlistat. About 76% of individuals ($n = 70$) on liraglutide 3.0 mg lost >5% of their weight compared with 30%($n = 29$) with placebo or 44%($n = 42$) with orlistat. Liraglutide decreased the prevalence of prediabetes (impaired fasting glucose or impaired glucose tolerance) by 84–96% with 1.8 to 3 mg per day.

3.4. Dipeptidyl-Peptidase- (DPP-) 4 Inhibitors. Sitagliptin, saxagliptin, and vildagliptin are DPP-4 inhibitors that inhibit the degradation of endogenous GLP-1. These are available as oral agents. Sitagliptin and saxagliptin are FDA approved for monotherapy and use with metformin for type 2 diabetes. Vildagliptin is approved for use in Europe. Aschner et al. evaluated the efficacy of sitagliptin as monotherapy in type 2 diabetes patients in a double-blind, placebo-controlled study [50]. A total of 741 patients with baseline HbA1c 8.0% were randomized to sitagliptin 100 mg or 200 mg once daily or placebo for 24 weeks. Sitagliptin at doses of 100 or 200 mg had a significant reduction in HbA1c of 0.69 and 0.94%, respectively, with no significant change in body weight.

In another double-blind, placebo-controlled, parallel-group study in which 1091 patients with type 2 diabetes (mean HbA1c 8.8%) were randomized to sitaglipin

100 mg/metformin 1000 mg (S100/M1000 group), sitagliptin 100 mg/metformin 2000 mg (S100/M2000 group), metformin 1000 mg (M1000 group), metformin 2000 mg (M2000 group), sitagliptin 100 mg (S100 group), or placebo [51]. All treatment groups achieved a statistically significant reduction in HbA1c from baseline compared with placebo and respective monotherapies at 24 weeks ($P < .001$). Patients in the S100/M2000 group had a reduction in Hba1c of 2.07%, S100/M1000 group with a reduction of 1.57%, M2000 group with a reduction of 1.30%, M1000 group with a reduction of 0.99%, and S100 group with reduction of 0.83%. There were significant reductions in body weight from baseline (−0.6 to 1.3 kg) in all groups, except in the sitagliptin group with no change in weight. Thus, as opposed to using SUs with metformin that may promote weight gain and run the risk of hypoglycemia, the use of DPP-4 inhibitors with metformin would be expected to result in less weight gain without the risk of hypoglycemia.

Similar to the effects of sitagliptin, saxagliptin at doses of 2.4, 5, and 10 mg, in addition to metformin, also similarly produced a significant reduction in HbA1c from baseline (−0.59 to 0.69%) with no significant change in weight [52].

4. Use of Antiobesity Agents in Type 2 DM

At present, there are only two antiobesity agents approved for long-term use by the FDA. These agents may be used with lifestyle modification and metformin. Orlistat and sibutramine have been studied in type 2 diabetes populations which will be briefly reviewed here.

4.1. Orlistat (Xenical). Orlistat is a lipase inhibitor that inhibits the absorption of dietary fat in the gastrointestinal tract by approximately 30% at dosage of 120 mg three times daily in combination with a mildly hypocaloric diet [53]. Xenical in the Prevention of Diabetes in Obese Subjects (XENDOS) Study, a 4-year double-blind, prospective study, was conducted to determine the long-term effect of orlistat in reducing progression to type 2 diabetes [54]. Obese patients (n =3,305; BMI ~37 kg/m^2) with normal (79%) or impaired (21%) glucose tolerance were randomized to lifestyle changes plus either orlistat 120 mg or placebo, three times daily. Patients treated with orlistat achieved significantly greater weight loss (5.8 kg) compared to placebo (3.0 kg). More patients in the orlistat group achieved a weight loss of ≥10% compared to the placebo group (26% versus 15.6%). There was also a greater reduction in incidence of type 2 diabetes with the orlistat group versus the control group of 6.2% versus 9.0% corresponding to a risk reduction of 37.3%.

Several studies were also conducted to determine the effect of orlistat in patients with type 2 DM. Jacob et al. conducted a multicenter double-blind, placebo-controlled study in which overweight or obese patients with type 2 diabetes (mean HbA1c ~8.5%; BMI ~37 kg/m^2) on either metformin, sulfonylurea, and/or insulin were randomized to treatment with orlistat 120 mg three times daily (n = 1279) or placebo (n = 1271) [55]. Patients treated with orlistat had a statistically significant greater decrease in body weight (−3.8 kg) than placebo-treated patients (−1.4 kg) and a larger decrease in HbA1c compared with placebo (−0.74% versus −0.31%). In addition, patients with minimal weight loss (<1% of baseline body weight) were also found to have a significant improvement in glycemic control with orlistat (HbA1c −0.29%) compared with placebo (±0.14%) suggesting that improvement of glycemic control associated with orlistat may be independent of weight loss. The greater reduction in weight loss and improvement in glycemic control with orlistat has also been shown consistently in combination with metformin and sulfonylureas [56, 57] or insulin [58] in individual studies. In a 52-week, randomized, double-blind placebo-controlled study in which type 2 diabetes patients (n = 220) treated with metformin alone or in combination with sulfonylurea were randomized to orlistat versus placebo [56]. Orlistat-treated patients achieved greater reduction in weight (5%) versus placebo (1.8%) as well as greater reduction in HbA1c (−1.1% versus −0.2%). These were also accompanied by greater improvements in beta cell function and insulin resistance. Thus, treatment with orlistat may counteract the weight gain effects associated with SUs and insulin and is currently available over the counter.

4.2. Sibutramine. Sibutramine is a serotonin-norepinephrine reuptake inhibitor. It acts by decreasing food intake by reducing appetite and increasing satiety [59]. In a multicenter, double-blind, placebo-controlled study, 348 obese patients (BMI~35 kg/m^2) were randomized to sibutramine 15 mg daily or placebo [60]. The sibutramine-treated group had greater weight loss compared to placebo (8.1 kg versus 5.1 kg). More patients on the sibutramine group lost more than 5% (62% versus 41%) and 10% (40% versus 19%) of their baseline weight compared to placebo. Aronne et al. conducted a study to evaluate the effects of coadministration of pramlintide with sibutramine or phentermine in a 25-week multicenter trial [61]. Overweight or obese, nondiabetic subjects (n = 244) were randomized to pramlintide (120 μg TID), pramlintide (120 μg TID) + oral sibutramine (10 mg once daily), or pramlintide (120 μg TID) + phentermine (37.5 mg once daily). Subjects who received combination treatment achieved greater weight loss (−11.3 kg) compared with pramlintide alone (−3.6 kg) or placebo (−2.1 kg).

McNulty et al. conducted a 12-month, prospective, double-blind study in which 195 subjects with type 2 diabetes (mean HbA1c ~ 9.5%; BMI ~ 36 kg/m^2) treated with metformin were randomized to sibutramine 15 or 20 mg daily or placebo [62]. There was significant weight loss in the sibutramine group with both 15 mg (−5.5 ± 0.6 kg) and 20 mg (−8.0 ± 0.9 kg) doses compared to placebo (−0.2 ± 0.5 kg) with no significant change in HbA1c. However, in subjects who lost more than 10% of their body weight, there was a significant decrease in HbA1c averaging 1.2%. Gokcel et al. evaluated the efficacy of sibutramine in combination with oral hypoglycemic agents in obese patients with poorly controlled type 2 diabetes (mean HbA1c ~9.9%; BMI ~ 38 kg/m^2) [63]. Sixty patients were randomized to sibutramine 10 mg twice daily or placebo. Sibutramine induced a greater mean reduction in weight than placebo (−9.61 kg versus −0.91 kg). HbA1c reduced significantly at 6 months

by 2.73% in the sibutramine group compared with 0.53% in the placebo group. A recent randomized control trial of sibutramine compared with placebo on cardiovascular effects in obese patients has shown an increase in risk for myocardial infarction, however this increase wasn't noted in patients with diabetes [64]. Careful consideration for risk versus benefit is needed for the use of sibutramine for weight loss in diabetes.

4.3. Summary. In recent years, an increasing number of antidiabetes agents, which have varying effects on glycemic control and weight, have become available. The treatment of diabetes should not only focus on glycemic control as its sole intention, but it should factor in the effect of these various agents on weight as well, since obesity aggravates insulin resistance, beta cell failure, and cardiovascular risk. SUs, TZDs, and insulin, when used alone or in combination, are usually associated with modest weight gain, whereas metformin and amylin analogues are weight neutral or result in weight loss. DPP-4 inhibitors are weight neutral whereas GLP-1 analogues are associated with weight loss. Therefore, in contrast to the conventional glycemic control strategy that results in significant weight gain, we propose that aggressive weight loss with lifestyle modification and metformin remain the foremost strategy followed by the use of incretin analogs. If glycemic control remains inadequate, the addition of low-dose TZDs would be a reasonable approach followed by the addition of basal insulin as necessary. Alternatively, the use of antiobesity agents such as orlistat, phentermine, or sibutramine has been shown to prevent or delay the onset of type 2 diabetes in obese patients and minimize the weight gain associated with SUs and insulin. These strategies should help balance glycemic and weight control in patients with obesity and diabetes.

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Research Article

Roles of Adiponectin and Oxidative Stress in the Regulation of Membrane Microviscosity of Red Blood Cells in Hypertensive Men—An Electron Spin Resonance Study

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This study was undertaken to investigate possible relationships among plasma adiponectin, 8-iso-prostaglandin F2α (8-iso-PG F2α: an index of oxidative stress), and membrane fluidity (a reciprocal value of microviscosity) in hypertensive and normotensive men using an electron spin resonance-method. The order parameter (S) for the spin-label agent (5-nitroxide stearate) in red blood cell (RBC) membranes was higher in hypertensive men than in normotensive men, indicating that membrane fluidity was decreased in hypertension. Plasma adiponectin and NO metabolites levels were lower in hypertensive men than in normotensive men. In contrast, plasma 8-iso-PG F2α levels were increased in hypertensive men compared with normotensive men. Plasma adiponectin concentration was correlated with plasma NO-metabolites, and inversely correlated with plasma 8-iso-PG F2α. The order parameter (S) of RBCs was inversely correlated with plasma adiponectin and plasma NO metabolite levels, and positively correlated with plasma 8-iso-PG F2α, suggesting that the reduced membrane fluidity of RBCs might be associated with hypoadiponectinemia, endothelial dysfunction, and increased oxidative stress. In a multivariate regression analysis, adiponectin and 8-iso-PG F2α were significant determinants of membrane fluidity of RBCs after adjustment for general risk factors. These results suggest that adiponectin and oxidative stress might have a close correlation with rheologic behavior and microcirculation in hypertension.

1. Introduction

It has been shown that dysregulation of adipocytokines may be accompanied by obesity, diabetes mellitus, dyslipidemia, and hypertension, and finally result in atherosclerotic vascular diseases [1–4]. Recently, it is strongly suggested that adiponectin, the most abundant secretory protein of adipose tissue in human plasma, might actively participate in the regulation of cardiovascular functions in humans, because hypoadiponectinemia might be observed in subjects with hypertension and other cardiovascular diseases [1–4]. It has been demonstrated that plasma adiponectin levels increased during weight reduction or blockade of the renin-angiotensin system [5], indicating that adiponectin might be beneficial for preventing the development of atherosclerotic changes.

On the other hand, it has also been shown that oxidative stress might be involved in the pathophysiology of obesity, hypertension, and atherosclerosis, and might be associated with increased risk of cardiovascular diseases, vascular dysfunction, and the metabolic syndrome [6–8]. Evidence indicates that plasma 8-iso-prostaglandin F2α (8-iso-PG F2α) may be a reliable index of oxidative stress in humans [9–12]. It was demonstrated that plasma concentration of 8-iso-PG F2α was significantly increased in subjects with essential hypertension compared with normotensive subjects [9, 10]. It was shown that plasma 8-iso-PG F2α levels were elevated in patients with coronary artery disease, particularly in those with hypertension [11, 12]. Moreover, it was shown that oral administration of vitamin E significantly decreased 8-iso-PG F2α concentrations in overweight/obese individuals,

suggesting that a decrease in plasma 8-iso-PG F2α has the potential to reduce the risk of cardiovascular disease in obesity [13].

Many studies have focused on the cardioprotective effects attributable to nitric oxide (NO) and have shown that hypertension and other circulatory disorders may be associated with insufficient NO production and availability [14, 15]. Chen et al. [16] demonstrated that adiponectin may stimulate production of NO in vascular endothelial cells. It has been shown that plasma adiponectin was correlated with endothelium-dependent vasodilation of the brachial artery in humans [2, 17]. In contrast, it was shown that endothelium-dependent vasodilation was impaired in subjects with elevated oxidative stress levels [18, 19]. These findings suggest that adiponectin and 8-iso-PG F2α might have a role in the production and bioavailability of NO.

It has been proposed that abnormalities in physicochemical properties of the cell membranes may underlie the defects that are strongly linked to hypertension, stroke, and other cardiovascular diseases [20–22]. An electron spin resonance (ESR) and spin-labeling method has been developed to evaluate the membrane fluidity and perturbations of the membrane function by external agents [21, 22]. The membrane fluidity is a reciprocal value of membrane microviscosity and is an important factor in modulating the cell rheological behavior [21, 22]. We have shown that the membrane fluidity of red blood cells (RBCs) was significantly lower in both spontaneously hypertensive rats (SHR) and patients with essential hypertension than in the normotensive controls [23–26], and proposed that abnormal membrane fluidity of RBCs might contribute to the pathogenesis of hypertension. In a study presented recently, we showed that adiponectin alone [27] or 8-iso-PG F2α alone [28] might be determinants of membrane fluidity of RBCs. In addition, it has been demonstrated that NO may be involved in the regulation of cell membrane fluidity [29]. Our previous in vitro study showed that an NO donor significantly improved membrane fluidity of RBCs in subjects with essential hypertension [30], indicating that NO could have a beneficial effect on the rheologic behavior of RBCs and the microcirculation in hypertension. The present study was performed to assess the relationships among adiponectin, oxidative stress, and NO, and their roles in the regulation of membrane fluidity of RBCs in hypertensive men using the ESR and spin-labeling method.

2. Subjects and Methods

2.1. Subjects. A total of 26 men with untreated essential hypertension were studied and compared with 17 age-matched normotensive men. The characteristics and laboratory findings in both groups were shown in Table 1. All subjects had no history of haematologic or hepatic disorders. All men were nonsmokers. They had similar life styles and dietary habits, and were instructed to avoid any changes in dietary habits at least 12 weeks before the study. The study was approved by a local research committee of Kansai University of Health Sciences. Written informed consent was obtained from all participants after they were informed about the nature and objective of the study.

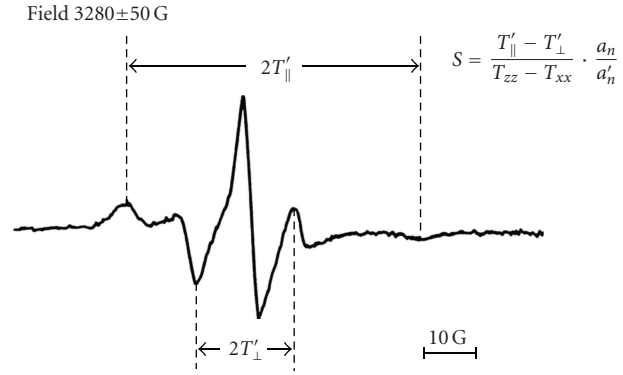

Figure 1: Representative electron spin resonance spectrum of red blood cells (RBCs) for the fatty acid spin-label agent (5-nitroxide stearate). S: order parameter, $2T'_{\parallel}$: outer hyperfine splitting, $2T'_{\perp}$: inner hyperfine splitting, T_{zz} and T_{xx}: hyperfine constants, a_n/a'_n: isotropic coupling constant, G: gauss. The greater the value of the order parameter (S) was, the lower the membrane fluidity of RBCs was.

2.2. Measures. Blood sampling was performed by venipuncture after a 30 minutes of bed rest while fasting. The procedures of RBC preparation and ESR measurements were shown previously [22–27]. We evaluated the values of outer and inner hyperfine splitting ($2T'_{\parallel}$ and $2T'_{\perp}$ in G, resp.) in the ESR spectra for the spin label agent (5-nitroxide stearate, Aldrich Co. Ltd., Milwaukee, Wisconsin, USA) and calculated the order parameter (S) [23–28] (Figure 1). The greater the value of the order parameter (S) was, the lower was the membrane fluidity of RBCs [22–27].

Plasma 8-iso-PG F2α levels were determined by using an enzyme immunoassay (Cayman Chemicals Co., Ann Arbor, Michigan, USA) [10, 11]. The plasma levels of adiponectin and NO-metabolites (nitrite and nitrate) were measured according to the method described previously [1, 27, 28, 31].

2.3. Statistical Analysis. Values are expressed as mean ± SEM. The differences between hypertensive and normotensive men were analyzed using an unpaired Student's t-test. Linear regression analysis was performed to assess the relationships among membrane fluidity (order parameter; S) of RBCs, plasma adiponectin, plasma 8-iso-PG F2α, and plasma NO-metabolite levels. Multivariate regression analysis with membrane fluidity (order parameter; S) of RBCs as a dependent variable and plasma adiponectin, plasma 8-iso-PG F2α, age, body mass index (BMI), fasting plasma glucose, systolic blood pressure, and plasma total cholesterol as independent variables was also performed. A P value less than .05 was accepted as the level of significance.

3. Results

3.1. Membrane Fluidity of RBCs in Hypertensive and Normotensive Men. The order parameter (S) for 5-nitroxide

Table 1: Clinical Characteristics and Laboratory Findings of Hypertensive (HT) and Normotensive (NT) Men.

	NT	HT
Number of subjects	17	26
Age (y.o.)	65 ± 3	63 ± 2
Body mass index (kg/m^2)	24.5 ± 0.6	24.1 ± 0.6
Systolic blood pressure (mmHg)	124 ± 2	147 ± 1*
Diastolic blood pressure (mmHg)	69 ± 2	87 ± 1*
Heart rate (beats/min)	75 ± 1	73 ± 1
Erythrocyte counts (10^4 cells/μL	458 ± 11	475 ± 9
Hemoglobin (g/dL)	14.1 ± 0.3	14.1 ± 0.2
Hematocrit (%)	43.1 ± 1.0	43.0 ± 0.6
Leucocyte counts (10^3 cells/μL)	5.5 ± 0.3	5.4 ± 0.2
Platelets (10^4 cells/μL)	20 ± 1	22 ± 1
Total cholesterol (mg/dL)	203 ± 7	210 ± 7
High density lipoprotein cholesterol (mg/dL)	50 ± 3	53 ± 4
Low density lipoprotein cholesterol (mg/dL)	127 ± 6	126 ± 6
Triglycerides (mg/dL)	137 ± 18	143 ± 15
Serum sodium (mmol/L)	140.7 ± 0.4	140.1 ± 0.3
Serum potassium (mmol/L)	4.1 ± 0.1	4.0 ± 0.1
Serum creatinine (mg/dL)	0.8 ± 0.1	0.9 ± 0.1
Fasting plasma glucose (mg/dL)	120 ± 10	119 ± 9

Values are means ± SEM. *$P < .05$ between HT and NT.

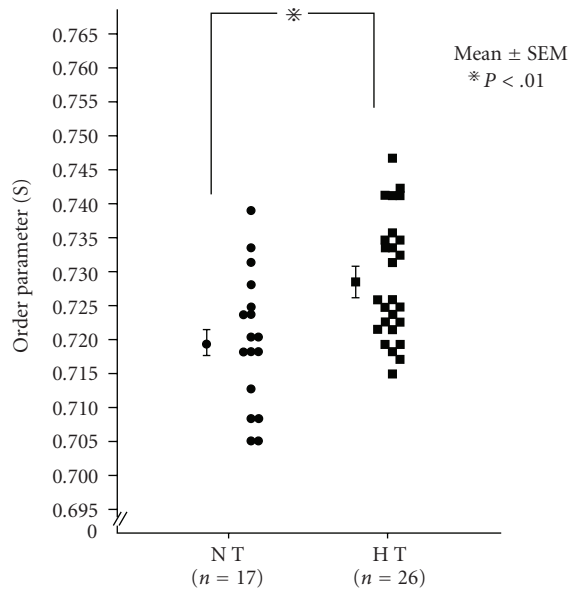

Figure 2: Membrane fluidity of red blood cells (RBCs) in hypertensive and normotensive men. The greater the value of the order parameter (S) was, the lower the membrane fluidity of RBCs was.

stearate in the ESR spectra of RBC membranes was significantly higher in hypertensive men (HT) than in normotensive men (NT) (HT 0.728 ± 0.002, mean ± SEM, $n = 26$, NT 0.719 ± 0.002, $n = 17$, $P < .01$) (Figure 2). The finding indicated that membrane fluidity of RBCs was decreased in hypertensive men compared with normotensive men.

3.2. Plasma Adiponectin, Plasma 8-iso-PG F2α, and Plasma NO-Metabolite Levels in Hypertensive and Normotensive Men. Plasma adiponectin concentration was lower in hypertensive men than in normotensive men (HT 7.0 ± 0.3 μg/mL, $n = 26$, NT 8.3 ± 0.4 μg/mL, $n = 17$, $P < .05$). The plasma NO-metabolites were also lower in hypertensive men than in normotensive men (HT: 36.3 ± 2.6 μmol/L, $n = 26$, NT: 54.6 ± 5.0 μmol/L, $n = 17$, $P < .01$). In contrast, the plasma 8-iso-PG F2α levels were significantly higher in hypertensive men than in normotensive men (HT: 3.33 ± 0.27 nmol/L, $n = 26$, NT: 2.32 ± 0.18 nmol/L, $n = 17$, $P < .05$). In the overall analysis of hypertensive and normotensive men, plasma adiponectin concentration was positively correlated with plasma NO-metabolites ($r = 0.334$, $n = 43$, $P < .05$) (Figure 3). It was also clearly shown that the plasma 8-iso-PG F2α levels were inversely correlated with plasma adiponectin ($r = -0.313$, $n = 43$, $P < .05$) (Figure 4), and plasma NO-metabolites ($r = -0.396$, $n = 43$, $P < .01$) (Figure 5).

3.3. Relationship among Membrane Fluidity of RBCs, Plasma Adiponectin, Plasma 8-iso-PG F2α, and NO-Metabolite Levels in Hypertensive and Normotensive Men. The order parameter (S) of RBCs was inversely correlated with plasma adiponectin ($r = -0.405$, $n = 43$, $P < .01$) (Figure 6) and plasma NO metabolite levels ($r = -0.342$, $n = 43$, $P < .05$) (Figure 7), and positively correlated with plasma 8-isoPG F2α ($r = 0.318$, $n = 43$, $P < .05$) (Figure 8). These results might suggest that the reduced membrane fluidity of RBCs might be associated with hypoadiponectinemia, endothelial dysfunction, and increased oxidative stress. In a multivariate regression analysis, both of adiponectin and 8-iso-PG F2α

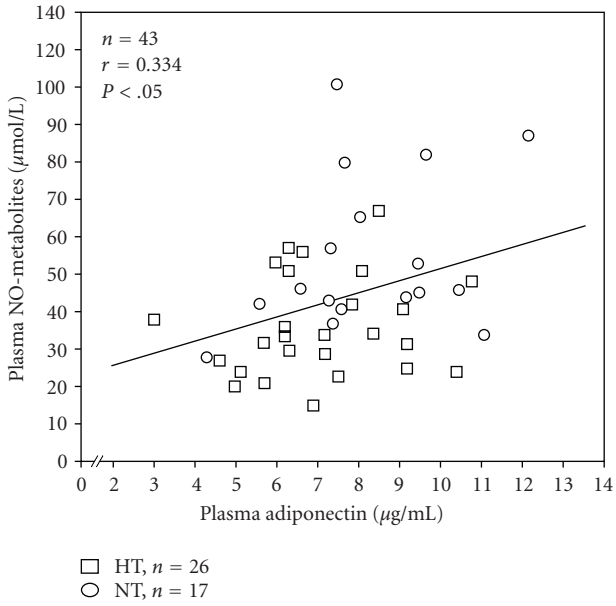

Figure 3: Correlation between plasma adiponectin and plasma nitric oxide (NO) metabolite levels in hypertensive and normotensive men.

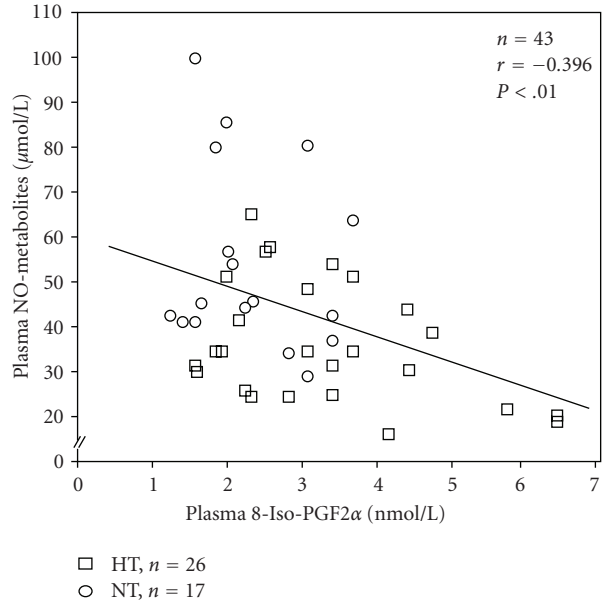

Figure 5: Inverse correlation between plasma 8-iso-PG F2α and plasma nitric oxide (NO) metabolite levels in hypertensive and normotensive men.

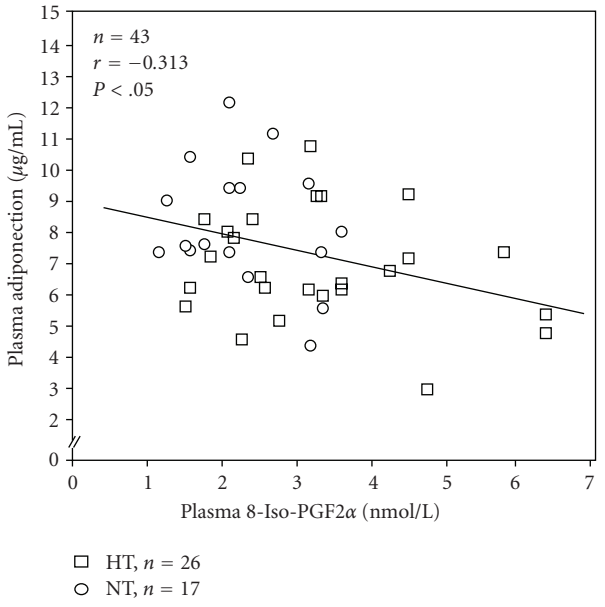

Figure 4: Inverse correlation between plasma 8-iso-PG F2α and plasma adiponectin levels in hypertensive and normotensive men.

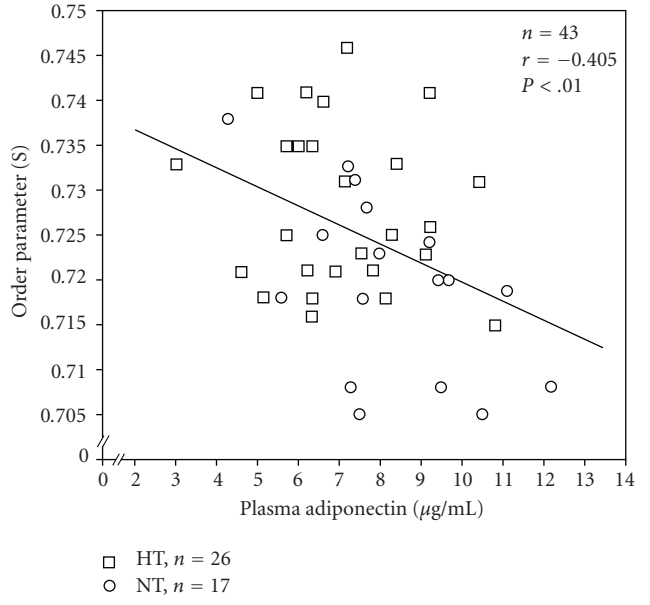

Figure 6: Inverse correlation between plasma adiponectin levels and the order parameter (S) of red blood cells (RBCs) in hypertensive and normotensive men.

were significant determinants of membrane fluidity of RBCs after adjustment for general risk factors (Table 2).

4. Discussion

Recent studies have shown that both of adiponectin and oxidative stress might actively participate in the pathophysiology of obesity, hypertension, atherosclerosis, and other cardiovascular and metabolic disease conditions [1–8]. The present study was performed to evaluate the possible relationship among adiponectin, oxidative stress, and membrane fluidity of RBCs in hypertensive and normotensive men using the ESR method. The results showed that the order parameter (S) of RBC membranes in the ESR spectra was significantly higher in hypertensive men than in normotensive men, indicating that the membrane fluidity of

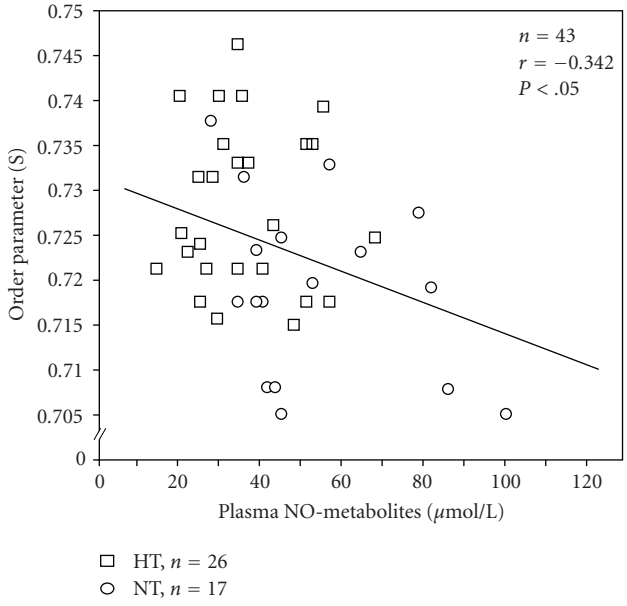

Figure 7: Inverse correlation between plasma nitric oxide (NO) metabolite levels and the order parameter (S) of red blood cells (RBCs) in hypertensive and normotensive men.

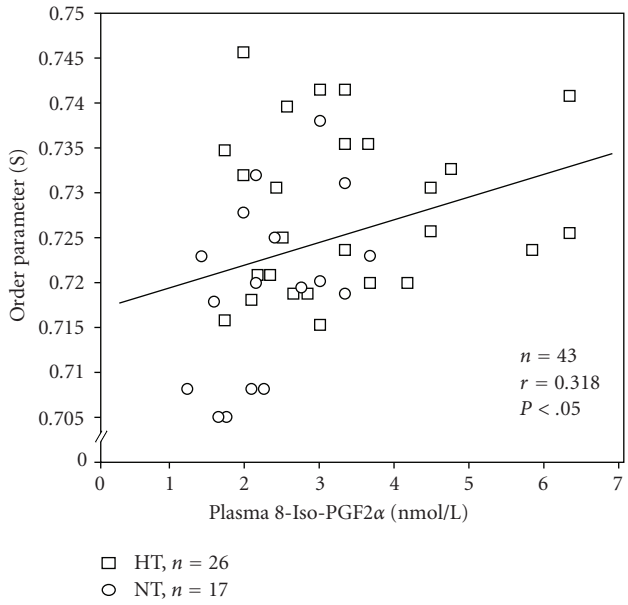

Figure 8: Correlation between plasma 8-iso-PG F2α levels and the order parameter (S) of red blood cells (RBCs) in hypertensive and normotensive men.

RBCs was decreased in hypertension. The finding might be consistent with our previous findings showing that the cell membranes were stiffer and less fluid in hypertension [23–28].

In the present study, plasma adiponectin levels were significantly lower in hypertensive men than in normotensive men. In contrast, plasma 8-iso-PG F2α levels (an index of oxidative stress) were significantly higher in hypertensive men than in normotensive men. Furthermore, it was shown that the order parameter (S) of RBCs was inversely correlated with plasma adiponectin levels, and positively correlated with plasma 8-iso-PG F2α levels, indicating that the reduced membrane fluidity of RBCs was associated with hypoadiponectinemia and increased oxidative stress. Multivariate regression analysis also demonstrated that both adiponectin and plasma 8-iso-PG F2α were independent determinants of membrane fluidity of RBCs after adjustment for general risk factors. Because the deformability of RBCs might be highly dependent on the membrane fluidity [21, 22], the reduction in membrane fluidity of RBCs could cause a disturbance in the blood rheologic behavior and the microcirculation in hypertension.

Le Quan Sang et al. [32] demonstrated that shear rate, shear stress, and blood viscosity were correlated with membrane fluidity of RBCs. They proposed that in vivo shear forces might participate in the control of RBC membrane fluidity, and that RBCs might adapt their membrane properties to blood flow conditions [32]. Saldanha et al. [33] examined the RBC membrane fluidity of acute myocardial infarction, and showed that RBC membranes might become more rigid after myocardial infarction, which could contribute to the decreased RBC deformability and the increased blood viscosity in this group of patients. Cazzola et al. [34] also reported that membrane fluidity of RBCs was decreased in the obese subjects, and that RBC membranes in obese subjects had higher susceptibility to peroxidation. They proposed that a decrease in RBC membrane fluidity could contribute to a reduction of the rate of blood flow and the oxygen diffusion through the RBC membrane and its exchange with tissues. It might be possible that alterations in RBC membrane fluidity with elevated oxidative stress would be strongly linked to the progression of obesity and cardiovascular diseases.

Recently, it was demonstrated that adiponectin may stimulate production of NO in vascular endothelial cells in vitro [16]. In addition, it has been shown that plasma adiponectin was correlated with endothelium-dependent vasodilation of the brachial artery, suggesting that plasma adiponectin may be a useful marker of endothelial function in hypertensive subjects [2, 17]. The present study demonstrated that the plasma levels of the NO metabolites were significantly lower in hypertensive men than in normotensive men. Furthermore, we showed that the plasma adiponectin levels were correlated with plasma NO metabolites in the overall analysis of normotensive and hypertensive men. It is, therefore, strongly suggested that hypoadiponectinemia could be associated with the reduced NO production and endothelial dysfunction. In an in vitro study presented earlier, we showed that NO significantly improved membrane fluidity of RBCs in hypertensive subjects [30]. The finding might propose that NO could have a crucial role in the regulation of membrane fluidity of RBCs, and further support the hypothesis that adiponectin might be associated with alterations in membrane fluidity of RBCs, at least in part, via the NO-dependent mechanism. However, the influence of adiponectin on the membrane lipid-protein interactions [35, 36] cannot be fully excluded.

Table 2: Multivariate regression analysis for predicting order parameter (S) of RBCs.

	SRC	t-value	P value
Age	−0.025	−0.142	.8878
Body mass index	−0.101	−0.616	.5417
Fasting plasma glucose	0.283	2.016	.0515
Systolic blood pressure	0.201	1.390	.1733
Total cholesterol	−0.356	−1.939	.0607
Plasma 8-iso-PG F2α	0.332	2.133	.0400
Plasma adiponectin	−0.322	−2.195	.0349

$R^2 = 0.389$, $n = 43$, $F = 3.186$, $P = .0102$.
SRC: standard regression coefficient.

The precise mechanisms by which oxidative stress could affect the membrane functions remain still unclear. Recently, it was shown that endothelium-dependent vasodilation was impaired in subjects with elevated oxidative stress levels [18, 19]. The present study demonstrated that the plasma 8-iso-PG F2α levels were inversely correlated with plasma NO-metabolite concentration in the overall analysis of hypertensive and normotensive men. One hypothesis is that elevated oxidative stress could be associated with the reduced NO-production and endothelial dysfunction. In the present study, we demonstrated that the lower membrane fluidity of RBCs was associated with decreased plasma NO-metabolite levels. It is suggested that the effects of oxidative stress on membrane fluidity of RBCs might be mediated, at least in part, by reducing the NO-bioavailability, although direct actions of oxidative stress on membrane structural and functional properties cannot be excluded [37, 38].

Jubelin and Gierman [39] showed that RBCs of rats and humans are positive for NO synthase, which indicated that RBCs possess all the cellular machinery to synthesize their own NO. Chen and Mehta [40] provided direct evidence that human RBCs possess endothelium-type NO synthase in the cytosol. It would be possible that the membrane action of NO could be one of the mechanisms responsible for its beneficial effects in improving the rheological behavior of RBC membranes and the microcirculation. Further studies should be performed to assess more precisely the relationships among adiponectin, oxidative stress, NO, and membrane functions, and their contribution to the pathophysiology of hypertension.

Recently, it was shown that pharmacologic elevation of serum adiponectin with natural compounds derived from a medicinal herb, such as astragaloside II and isoastragaloside I, significantly ameliorated hyperglycemia, glucose intolerance, and insulin resistance in obese mice [41]. In addition, it was demonstrated that direct administration of adiponectin into the coronary artery reduced the myocardial infarction size and improved left ventricular function in pigs after ischemia-reperfusion injury [42]. These findings support the idea that adiponectin could be useful for the treatment of obesity and obesity-related cardiovascular disorders.

5. Conclusion

The results of the present study demonstrated that plasma adiponectin was significantly lower in hypertensive men than in normotensive men. In addition, plasma adiponectin concentration was positively correlated with plasma NO metabolite levels, and inversely correlated with plasma 8-iso-PG F2α levels. Furthermore, it was shown that the reduced membrane fluidity of RBCs was correlated with the decreased plasma adiponectin and NO metabolite levels and the increased plasma 8-iso-PG F2α levels, suggesting that abnormalities in RBC membranes in hypertension might be associated with hypoadiponectinemia, endothelial dysfunction, and elevated oxidative stress. Although this is a cross-sectional and correlative study in Japanese men, the results of the present study could provide a hypothesis that both adiponectin and oxidative stress might have a close correlation with the rheologic behavior of RBCs and the microcirculation, and contribute to the pathophysiology of hypertension in men. Furthermore, it is possible that adiponectin could be a useful pharmacologic tool to improve membrane microviscosity in hypertension via the NO-dependent mechanisms.

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Case Report

Refsum's Disease—Use of the Intestinal Lipase Inhibitor, Orlistat, as a Novel Therapeutic Approach to a Complex Disorder

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Refsum's Disease is an inherited metabolic disorder in which a metabolite of branched chain fatty acids accumulates due to lack of appropriate oxidative enzymes. Patients have elevated plasma phytanic acid levels and high concentrations of phytanic acid in a variety of tissues leading to progressive tissue damage. Besides retinal degeneration or retinal dystrophy associated with adult onset retinitis pigmentosa, additional symptoms include chronic polyneuropathy, cerebellar ataxia, sensorineural hearing loss, anosmia, ichthyosis, as well as skeletal, cardiac, hepatic, and renal abnormalities. Current management includes avoidance of dietary sources of branched chain fatty acids and regular plasmapheresis to prevent accumulation of these compounds to ameliorate progressive neurological deficits. Two brothers with Refsum's disease who experienced progressive symptoms despite optimal diet and plasmapheresis were commenced on a novel therapy. We report the effect of the intestinal lipase inhibitor, Orlistat, which led to significant reduction (P-value < 0.001 on 2-sample unpaired t-test) of mean preplasmapheresis phytanic acid levels with retardation of the progression of most of their dermatological and neurological symptoms.

1. Introduction

Refsum's Disease, also known as heredopathia atactica polyneuritiformis (HAP), was described by Norwegian neurologist Sigvald Refsum in 1946. It is a rare complex disorder that affects many organs. It has an autosomal recessive pattern of inheritance due to mutations on chromosome 10p13. Carriers are unaffected, however they may asymptomatically exhibit slightly elevated phytanic acid levels, whereas Refsum's disease patients have markedly elevated levels (normal <0.70 mg/dL) [1].

Phytanic acid is a branched-chain fatty acid (BCFA), formed by bacterial degradation of chlorophyll in the intestinal tract of ruminants, invertebrates and, pelagic fish [2]. Individuals with Refsum's disease are unable to metabolize phytanic acid by the β-oxidation pathway due to deficiency of the peroxisome enzyme phytanoyl-CoA hydroxylase (PAHX) [2–5] (Figure 1). It is essential for the 3-methyl group in the β-position of this BCFA to be removed by an α-oxidation step, activated by PAHX (within the endoplasmic reticulum) in order to proceed with the β-oxidation pathway. Peroxisomal β-oxidation is the most efficient mechanism for the metabolism of phytanic acid. As a result, high levels of phytanic acid accumulate in blood and other tissues, especially adipose tissue, neural tissue, and astrocytes, where they cause oxidative stress in mitochondria and oxidative damage during chronic exposure [3, 6, 7]. In a subset of patients, a mutation of a second gene encoding for PEX7- peroxin 7 receptor protein, involved in peroxisomal import of proteins, has been identified as a cause for the phenotype of Refsum's disease [2, 5].

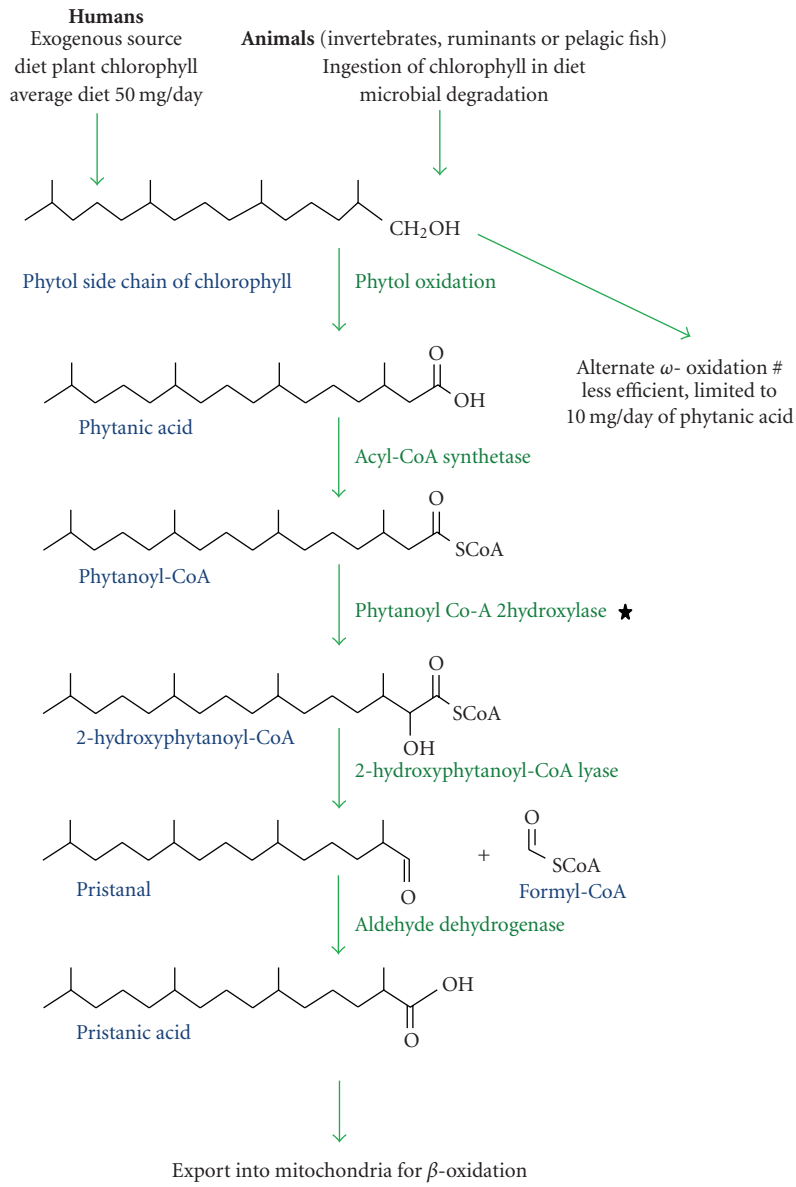

Figure 1: Metabolic pathway of phytanic acid. Phytanic acid is derived from microbial degredation of the phytol side chain of chlorophylls ingested by ruminants, invertebrates, or pelagic fish. In humans the source is phytol from diet chlorophyll, or from meat, pelagic fish, or diary. When digested it is in-cooperated into chylomicrons/VLDL and then transported to liver and tissues for further metabolism. Most fatty acids are metabolised by β-oxidation pathways in peroxisomes and mitochondria. $\star$ denotes the enzyme deficient in patients with Refsum disease. # denotes the alternate less efficient ω-oxidation pathway.

Early diagnosis of HAP or Refsum's disease is important because treatment is available to minimize progression. Classical Refsum's disease is usually diagnosed during childhood or early adulthood when visual problems due to retinitis pigmentosa become apparent [1, 8]. Accumulation of phytanic acid beneath the retina results in progressive visual impairment. The presenting symptom is usually night blindness followed by gradual loss of peripheral vision. Cataracts, which are common in patients with retinitis pigmentosa, may develop. Refsum's disease leads to other sensory complications, including impaired sense of smell, usually occurring in early childhood but some times undiagnosed until other symptoms become apparent. Gradual or sudden hearing loss can occur in adulthood, usually after the 3rd decade. Cardiac abnormalities include cardiomyopathy or even fatal arrhythmias. Other neurological manifestations include peripheral neuropathy, paraesthesia, and cerebella ataxia. Ichthyosis, malaise, anorexia, and skeletal bone abnormalities such as bony prominences around elbows, knees and ankles and short digits of tubular bones of hands or feet (especially the metatarsal of the fourth toe) are also common. Renal and hepatic manifestations include tubular

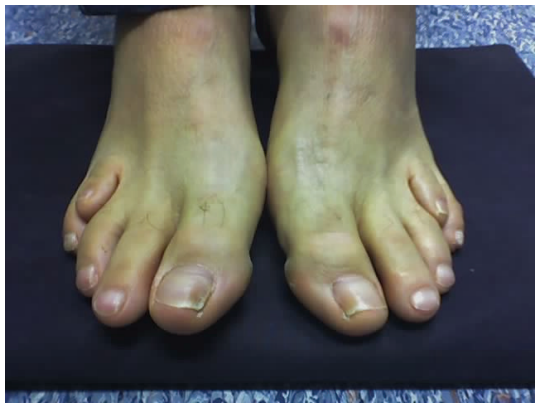

Figure 2: Shortening of AF's fourth toe, characteristic of Refsum's Disease.

dysfunction, aminoaciduria, and fatty degeneration [1, 5, 8–11].

Humans have a secondary, less efficient pathway for phytanic acid metabolism via ω-oxidation, which is not affected in these patients [2, 5] (Figure 1). However the capacity of ω-oxidation is limited and it is only sufficient to process the reduced supply of phytanic acid associated with dietary restriction. It is reported in animal studies that fibrate drugs may induce this ω-oxidation pathway of phytanic acid metabolism [2].

1.1. Current Management. Patients with Refsum's disease require multidisciplinary monitoring to detect cardiac, ophthalmic, and neurological manifestations. Humans do not synthesize phytanic acid, obtaining it almost exclusively from their diet. Phytanic acid is found in meat, pelagic fish, and dairy products [2]. Humans also convert phytol, a side chain of chlorophyll found in green leafy vegetables, to phytanic acid. It is impossible to achieve a diet that is completely free of phytanic acid. Management of Refsum's disease requires a diet restriction of intake of phytanic acid to <10–20 mg/day (i.e., about 10% of that in a normal western diet) [1]. These low phytanic acid (<10 mg/dL) diets are very stringent [1].

Lowering of plasma phytanic acid levels by the long-term adherence to diets low in phytanic acid and phytol may be enhanced by serial plasma exchange to prevent development or progression of neuropathy, ataxia, cardiac arrhythmias, and ichthyosis [1, 10]. It is less certain whether progression of retinitis pigmentosa, anosmia or deafness can be prevented. It is important that patients maintain body weight, since rapid weight loss releases phytanic acid stored in body tissues and increases symptoms. Similarly fevers, pregnancy, and catecholamine released during plasmapheresis have been associated with acute or subacute presentations that mimic Guillain-Barre Syndrome or chronic inflammatory demyelinating polyneuropathy.

1.2. Rationale for Treatment with Orlistat. Orlistat (Xenical) is an inhibitor of intestinal lipase that blocks the digestion of triglycerides. We hypothesised that it would therefore reduce absorption of dietary branched chain fatty acids, in particular phytanic acid. Orlistat is usually prescribed for weight loss and has a favourable safety profile which has contributed to the decision to make it available across the counter. Side effects associated with Orlistat therapy include diarrhoea, faecal incontinence following excessive fat ingestion, and a slight decrease in absorption of fat soluble vitamins. It must be noted that Orlistat-induced weight loss might release adipose stores of phytanic acid, thereby increasing plasma levels. We guarded against this possibility by advising our patients to increase their calorie intake so as to maintain weight.

2. Methods and Patients

The family comprised five children, four brothers and one sister, born of consanguineous parents. There is no clear history of a similar disorder in other generations of the family. Brothers AF (50 years) and VF (48 years) were diagnosed following the detection of the disorder in their older brother ALF (56 years) who was living overseas. The diagnosis of Refsum's disease was made when ALF presented to an ophthalmologist with progressive visual symptoms due to retinitis pigmentosa. The family was screened, and the younger brothers AF and VF were found to have elevated plasma phytanic acid levels (AF 18.5–36 mg/dL and VF 33–41 mg/dL). In retrospect, brothers AF and VF reported long standing symptoms of poor sense of smell, tinnitus, loss of peripheral vision, and clumsiness. Examination revealed anosmia, retinitis pigmentosa, constricted visual fields, nystagmus, impaired coordination, and ataxia on heel-toe walking. AF also had an episode of nonsustained cardiac arrhythmia, long-standing irritable bowel syndrome, and a characteristic deformity in his fourth toes (Figure 2) which was reported to be a feature in brother ALF as well. The additional features in younger brother VF included hearing impairment, ichthyosis, long slender toes, and multiple bony prominences which are associated with Refsum's disease.

Following confirmation of the diagnosis by serum phytanic acid measurements, both brothers commenced a low phytanic diet and plasmapheresis. The plasma phytanic acid levels at base-line and with dietary treatment plus plasmapheresis are shown in Figure 3(a). Despite this intensive treatment, the two brothers continued to have progressive symptoms and incomplete control of plasma phytanic acid levels (greater than 10 times the upper limit of normal). Substantially lower treatment goals were recommended to minimize complications or progression of disease.

At this stage they were referred to the lipid and metabolic disorder clinic at Royal Prince Alfred Hospital in Sydney for further optimisation of treatment.

2.1. Method. AF and VF commenced treatment with Orlistat at the standard dose of 120 mg three times a day before meals. However their compliance was incomplete and they managed only two doses per day over the first few months. They continued a suitable low-phytanic acid diet with adequate calorie intake to avoid weight loss and regular

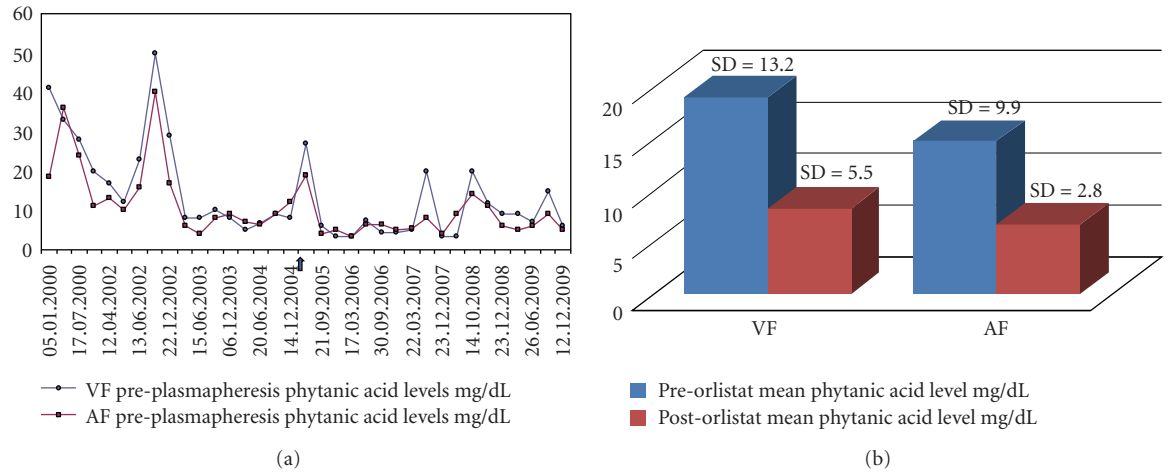

Figure 3: (a) Pre-plasmapheresis plasma phytanate (phytanic acid) levels. AF's and VF's preplasmapheresis phytanic acid levels before and after addition of Orlistat (Xenical) therapy to diet and regular plasmapheresis (shown by arrow in June 2005) showing good control of phytanic acid levels except during periods of non compliance with Orlistat therapy (in 2008) and weight loss (in 2007 and 2009). (b) AF's and VF's mean plasma phytanic acid levels (mg/dL) before and after addition of Orlistat therapy to regular plasmapheresis and stable low phytanic acid diet. AF's and VF's mean phytanic acid level before addition of Orlistat was 14.8 mg/dL (SD 9.9) and 19.0 mg/dL (SD 13.2), respectively. AF's and VF's mean phytanic acid level after addition of Orlistat therapy was 6.7 mg/dL(SD 2.8) and 8.2 mg/dL (SD5.5), respectively, with a P-value <0.05 on two-sample t-Test.

(every 3 weeks) plasma exchanges. The mean pre plasmapheresis phytanic acid levels were calculated for the periods before (April 2000–June 2005) and during (June 2005–January 2010) Orlistat therapy, (Figure 3(b)). Nutritional biochemical markers including fat soluble vitamin levels were monitored at baseline and at regular intervals but supplements were not required. Phytanic acid was measured by gas chromatography using a 25 m × 0.32 mm i.d. SGE BP-20 capillary column; nonadecanoic acid (19 : 0) methylester was used as internal standard and calibrated against phytanic acid methyl ester (Ultra Scientific, USA).

3. Results

In AF, mean plasma phytanic acid level (Figure 3(b)) on diet and plasmapheresis every 3 weeks was 14.8 mg/dL (SD 10 mg/dL), falling to 6.7 mg/dL (SD 2.8 mg/dL) after the addition of unblinded orlistat therapy ($P < 0.05$ on two-sample t-Test). He reported clinical improvement in symptoms of polyneuropathy, ataxia, ichthyosis, irritable bowel syndrome, and cardiac arrhythmia. In VF, mean plasma phytanic acid level (Figure 3(b)) on diet and plasmapheresis was 19.0 mg/dL (SD 13.0 mg/dL), falling to 8.2 mg/dL (SD 5.5 mg/dL) during unblinded orlistat therapy ($P < 0.05$, two-sample t-Test). This was associated with improvement in symptoms of ataxia, hearing loss, and pruritus. However VF continued to suffer progressive impairment of vision, which has improved following bilateral cataract surgery. During this period AF and VF maintained stable weight most of the time with brief periods of weight loss associated with a slight increase in measured phytanic acid levels resolving with weight stabilisation, (Figure 3(a)).

4. Discussion

Early diagnosis of HAP or Refsum's disease is important because early treatment will minimize accumulation of phytanic acid and progression of functional impairment [1, 2]. Specific treatment for Refsum's disease is limited. We considered the use of Orlistat (Xenical), an intestinal lipase inhibitor, hypothesising that it has the potential to reduce the bioavailability of dietary phytanic acid. This occurs because the inhibition of intestinal lipase by Orlistat results in intestinal fat accumulation. Lipid soluble materials such as phytanic acid are likely to partition into the triglyceride phase and remain there until excreted. Indeed, significant reductions in mean pre-plasmapheresis plasma phytanic acid levels were demonstrated, (Figure 3(b)) in these two patients, without significant adverse effects or sustained weight loss. Liberalisation of the restrictive diet or reduction in the frequency of plasmapheresis may be feasible in the setting of continued Orlistat therapy. Orlistat reduces dietary triglyceride absorption by approximately 30%. In future, it may be possible to intensify reduction in intestinal lipolysis by the additional inhibition of lingual lipase. This offers the prospect of greater reductions in phytanic acid absorption, but this must be balanced against the possibility that associated weight loss might release tissue stores. It has provided the most effective means of reducing phytanic acid levels and disease progression.

Orlistat might be useful in the treatment of other metabolic disorders in which lipid soluble materials from the intestine contribute to pathology. More specific treatment for sitosterolaemia is available via the NPC1-L1 inhibitor, ezetimibe. We have used Orlistat to treat chylomicronaemia associated with massive hypertriglyceridaemia, which poses

a risk of acute pancreatitis. These patients remained free of pancreatitis during Orlistat therapy, but triglyceride levels and the clinical course of this condition are notoriously variable. A large-scale randomised clinical trial of the use of Orlistat would be required to assess its potential for the prevention of pancreatitis in chylomicronaemia. This report of the therapeutic effect of Orlistat in Refsum's disease requires confirmation in other patients. The use of Orlistat to reduce plasma phytanic acid levels may permit a reduction in the intensity of diet therapy and plasmapheresis, which would result in significant benefit to the patient and reduction in the cost burden to health systems. It may also favourably modify the progression of the clinical manifestations of Refsum's disease.

Conflict of Interest

The authors report no conflict of interest.

Acknowledgments

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Research Article

Switching to Aripiprazole as a Strategy for Weight Reduction: A Meta-Analysis in Patients Suffering from Schizophrenia

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Weight gain is one of the major drawbacks associated with the pharmacological treatment of schizophrenia. Existing strategies for the prevention and treatment of obesity amongst these patients are disappointing. Switching the current antipsychotic to another that may favorably affect weight is not yet fully established in the psychiatric literature. This meta-analysis focused on switching to aripiprazole as it has a pharmacological and clinical profile that may result in an improved weight control. Nine publications from seven countries worldwide were analyzed. These encompassed 784 schizophrenia and schizoaffective patients, 473 (60%) men and 311 (40%) women, mean age 39.4 ± 7.0 years. The major significant finding was a mean weight reduction by −2.55 ± 1.5 kgs following the switch to aripiprazole ($P < .001$). Switching to an antipsychotic with a lower propensity to induce weight gain needs be explored as a strategy. Our analysis suggests aripiprazole as a candidate for such a treatment strategy.

1. Introduction

Excessive bodyweight gain is not a new clinical observation amongst patients treated with antipsychotics and was already reported during the 1950's as an adverse effect of typical antipsychotic drug treatment. Hence, one of the major difficulties facing patients suffering from schizophrenia and health care professionals is obesity. In comparison to the general population, these patients have higher rates of morbidity and mortality, which are thought to be due, in part, to increased rates of obesity. This problem has been exacerbated more recently with the introduction and increased use of second generation antipsychotics (SGA), several of which are associated with the risk of weight gain and metabolic disturbance [1]. The magnitude of bodyweight gain reported in the largest naturalistic treatment studies (the CATIE study) of patients treated by SGAs with bodyweight gain of >7% compared with baseline was in the range of 12% to 30% [2, 3].

In many obese patients suffering from schizophrenia, treatment based on only lifestyle interventions is limited by the core symptomatology of the disease, medication side effects, and patient's nonadherence and thus may not result in desirable weight loss. Nevertheless, antipsychotic-induced weight gain is an important modifiable parameter in the high rates of obesity in this population. The need for an effective complementary drug treatment would be advantageous specifically for this large group of vulnerable patients.

The Schizophrenia Patient Outcomes Research Team (PORT) psychopharmacological treatment recommendations provide a wide-ranging summary of current evidence-based pharmacological treatment practices. The latest report of the PORT group was recently published, and the pharmacological prevention and treatment of antipsychotic-associated weight gain in schizophrenia were specifically addressed [4]. The PORT approach for obesity associated with antipsychotic medications is important in treatment and prevention. Three pharmacological strategies have been evaluated by this group: (1) switching current antipsychotic to an antipsychotic with a lower propensity for weight gain, (2) employing a medication to prevent weight gain, and (3)

addition of a medication to patients who have gained weight during therapy in order to promote weight loss. Regrettably, there is currently insufficient evidence to recommend any of these strategies.

The equivocal recommendations of the PORT group have been challenged by researchers and clinicians who have focused on "switching" antipsychotic medications as a viable strategy to induce weight loss. Development of obesity in patients suffering from schizophrenia who were stabilized on SGA has been the framework by these researchers. Risks and benefits of staying on current treatment versus switching to another agent and switching strategies were evaluated, concluding that switching to another antipsychotic with more favorable side effects is called for if other weight-loss strategies fail [3]. However, there is little evidence to support which is the best switching strategy [5]. There are several SGAs that may be feasible candidates for switching strategy. These may include ziprasidone, amisulpride, paliperidone, and aripiprazole [6]. Although the debate about the potential relationship between aripiprazole therapy and the risk of weight gain is not yet resolved, it has been also stated that this specific aspect of the safety profile of aripiprazole assumed on the basis of its unusual pharmacological characteristics may result in a decreased adverse impact on weight that often complicates management for a large number of patients suffering from schizophrenia treated chronically with antipsychotics [7, 8]. Clinical characteristics of aripiprazole in comparison to other SGAs have been recently evaluated by the Cochrane Collaboration. The authors observed that aripiprazole was associated with fewer side effects such as weight gain, cholesterol increase, sedation, and prolactin-associated side effects, concluding that aripiprazole may be more tolerable in terms of metabolic effects and sedation [9].

The primary objective of this meta-analysis was to examine the efficacy of switching to aripiprazole as a strategy for achieving weight loss in schizophrenia patients currently using other antipsychotics.

2. Materials and Methods

2.1. Study Selection and Data Collection. At the beginning of this project, we drafted a study protocol. For our analysis, we included all trials in which patients suffering from schizophrenia or schizoaffective disorder (DSM-IV criteria) treated with any antipsychotic were switched to aripiprazole monotherapy in acute-phase or maintenance treatment. We excluded placebo groups where present.

The authors independently reviewed references and abstracts retrieved by the search, assessed the completeness of data abstraction, and confirmed quality rating. We used a structured data abstraction form to ensure consistency of appraisal for each study. Investigators were contacted and asked to provide data to supplement the incomplete reporting of the original articles [10].

2.2. Outcome Measures. We defined mean change from baseline weight (and standard deviation) as the primary efficacy outcome measure in the present analysis.

Studies were included only if mean change in weight was reported as well as the following variables: (a) design: industry support, sample size, previous antipsychotic treatment, aripiprazole dose, and treatment duration. (b) demographic: age, gender, and diagnosis.

2.3. PubMed. A PubMed literature search (last search April 2010) was performed to identify all original research articles using aripiprazole to determine weight change in adult patients.

The search terms were ("aripiprazole" or "abilify"), ("schizophrenia" or "schizoaffective disorder") and ("switch", "weight", "metabolic", "BMI", or "obesity") in the abstract, title, or index terms.

2.4. Additional Electronic Searches. Studies were searched for using the following search strategy: Diagnosis = Schizophrenia* or Schizoaffective* and Intervention = aripiprazole. References were searched using the same search strategy with the addition of Free-Text = weight. The researchers conducted searches on the MEDLINE and PsychInfo and checked various meta-analyses and review articles on the 8th of April 2010.

Searching other resources includes the following.

(1) Hand searches. We also searched the ongoing trial register http://clinicaltrials.gov/ in the USA.

(2) Personal communication. Experts in this field were asked if they knew of any study that met the inclusion criteria of this review.

(3) Reference checking. Reference lists of the included studies, previous systematic reviews, and major textbooks of schizophrenia written in English were checked for published reports and citations of unpublished research

2.5. Data Extraction and Management. Both authors extracted data from the included studies. Again, any disagreement was discussed, and decisions were documented. Retrieved data was rated dichotomously by each author as either "sufficient" or "inadequate". Only studies for which both authors' ratings were "sufficient" were included in the present analysis.

If necessary, we contacted the authors of the studies for clarification. We extracted the following data:

(i) participant characteristics,

(ii) intervention details,

(iii) outcome measures of interest from the included studies.

2.6. Data Synthesis and Statistical Analysis. Our primary method for the assessment of weight reduction was through pooled estimates using fixed-effects meta-analyses methodology. This allowed the integration of information to provide an estimate with a 95% confidence interval (CI). The results also allow us to make probabilistic statements about the

effect size; that is, we are able to answer the following question: "Given the observed data, what is the probability that switching treatment to aripiprazole will induce weight loss?"

Analyses were performed on those antipsychotic medications with published studies that were completed in the period from 2003 to 2010. Also, pooled results were estimated by subgroups of diagnosis, sample size, and gender.

Data analysis was carried out with the use of the Statistical Analysis System software, SAS Institute, version 9.1.3 from 2007.

3. Results and Discussion

3.1. Results. The literature search yielded 47 articles, of which 11 manuscripts fulfilled the inclusion criteria for the present meta-analysis. These studies spanned the period from 2003 to 2010. Taken altogether, the cumulative sample size included 786 patients [11–21]. There were also 2 case reports [13, 14] which were excluded from the final analysis. Characteristics of the final 784 analyzed participants are presented in Table 1.

The sample analyzed was composed mainly of adult males suffering from schizophrenia. Mean age for the sample was 39.4 ± 7.0 years (range: 30 to 54); there were 473 (60%) men and 311 (40%) women, of whom 644 (82%) were diagnosed as suffering from schizophrenia, and 140 (18%) were suffering from schizoaffective disorder. Half of the studies were conducted in the USA (5/9), two in Korea, one each in the Netherlands, Japan, and Eastern Europe. It should be noted that one of the studies was an international collaboration between USA and East European researchers. Thus, 422 (54%) of patients were of Caucasian ethnicity, and 148 (19%) were Korean.

Antipsychotic medications resulting in weight gain for which the later switch to aripiprazole was undertaken were distributed as follows: olanzapine 352 (46%), risperidone 226 (30%), typical—first generation 168 (22%), sulpiride 17 (3%), clozapine 11 (1%), and quetiapine 10 (1%). Mean weight for the sample prior to switching to aripirazole was 83.8 ± 15.4 kgs (range: 63 to 104); mean aripirazole dose following the switch was 20.2 ± 5.0 mgs/daily (range: 15 to 30), and mean duration of aripirazole treatment was 26.2 ± 16.9 weeks (range: 8 to 56). In all studies herein analyzed, a minimal reduction in weight of 1.2 kgs was reported. In the four largest studies included [11, 12, 18, 19], the reduction in weight was statistically significant, $P < .001$. It is of interest to note that the weight reduction observed in the two case reports (10.5 and 16.8 kgs) was extremely higher than that reported in the large-scale studies [13, 14].

Body mass index (BMI) was reported only in 2 studies, and thus we were unable to analyse the significance in it's change. In one study [11], reduction in BMI following aripiprazole treatment was −1.3, and in the second study [16] it was −0.5.

Following the switch to aripirazole, a reduction in weight of 7% or more was observed in 52/484 patients (10.7%) while an increase of 7% or more in weight was observed in 22/484 (4.5%); this was highly statistically significant, $P < .0004$.

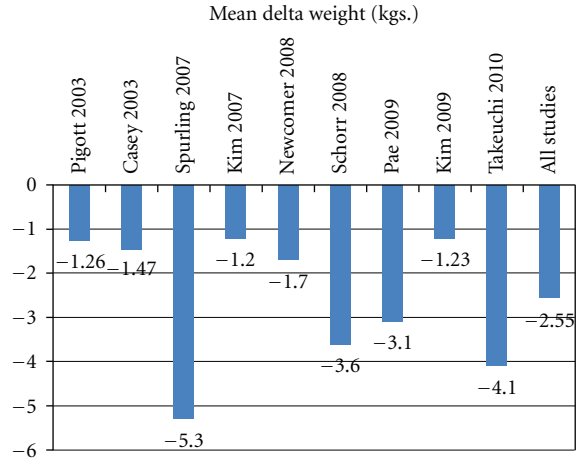

Figure 1: Mean reduction in weight (kgs) across analyzed studies.

Correlations computed for weight reduction and gender, age, ethnicity, or aripiprazole dose did not reach statistical significance. Duration of aripiprazole treatment was negatively correlated with the weight change (longer time = greater weight loss) not reaching statistical significance ($R^2 = -0.48$, $P = .085$).

The major significant findings of the present analysis were the following:

(i) mean weight reduction by −2.55 ± 1.5 kgs 95% CI: −3.7 to −1.4 (range: −1.2 to −5.3) following the switch to aripiprazole was statistically significant, $P < .001$. See Figure 1.

(ii) mean weight reduction was statistically greater for patients diagnosed as suffering from schizophrenia (−2.67 kgs) when compared to mean reduction in patients suffering from schizoaffective disorder (−2.18 kgs), $P < .022$.

(iii) the most significant mean weight reduction (−2.74 kgs) was noted in patients who were exposed to olanzapine prior to switching treatment to aripiprazole, $P < .001$.

3.2. Discussion. The introduction of the SGAs has brought to the forefront the awareness that antipsychotic treatment is associated with an increase in weight, adversely affecting cardiac and metabolic risks. However, not all antipsychotics are thought to contribute to the same degree, and the possibility of switching antipsychotic medications to reduce the induced weight gain has been intriguing [22].

Several reports on studies in which already overweight, antipsychotic-treated patients were allocated to switch to aripiprazole have been published. Aripiprazole treatment was then associated with significant improvements in weight. However, to the best of our knowledge, no meta-analysis encompassing all switching studies has been carried out to date. The strength of weight reduction effect was deemed

Table 1: Description of analyzed studies.

Reference in text []=in text	Industry-supported study	Sample size	Age	Diagnosis		BAT		Baseline weight (kilograms)	Aripiprazole treatment duration	Weight change (P value)
				Schizophrenia	Schizoaffective	T	SGA			
Pigott 2003 [11]	+	155	42.2	155 (100%)	0	116	39	75	26	−1.26 ($P < .05$)
Casey 2003 [12]	+	311	38.7 ± 10.3	210 (65.3%)	101	24	287	90.2	8	−1.47 ($P = .02$)
Spurling 2007 [15]	−	24	44.6 ± 9.1	9 (37.5%)	15	0	24	—	24	−5.3 ($P < .001$)
Kim 2007 [16]	+	10	34 ± 13	10 (100%)	0	0	10	104.1	16	−1.2 ($P = .05$)
Newcomer 2008 [18]	+	88	39.7 ± 10.1	68 (77.3%)	20	0	88	92	16	−1.7 ($P = .02$)
Schorr 2008 [17]	−	16	34 ± 10.7	12 (75%)	4	1	15	95	56	−3.6 ($P < .001$)
Pae 2009 [19]	+	87	36.5 ± 10.3	87 (100%)	0	13	74	—	12	−3.1 ($P < .001$)
Kim 2009 [20]	+	61	30.8 ± 7.9	61 (100%)	0	6	55	67	26	−1.23 ($P < .05$)
Takeuchi 2010 [21]	−	32	54.6 ± 16.9	32 (100%)	0	6	26	63.1	52	−4.1 ($P < .001$)

BAT = Baseline antipsychotic treatments (prior to switching to aripiprazole; T = typical antipsychotics; SGA = second generation antipsychotics).

clinically meaningful in the present analysis and is consistent with previous research in weight-unselected patients [23]. Our results support the suggestion that switching might be considered for any antipsychotic-treated patients who had gained weight. More so, for those possessing pre-existing cardiac and metabolic risk factors, an early switch to a more metabolically neutral antipsychotic such as aripiprazole might serve best.

In general, switching studies are carried out to evaluate whether switching results in further symptom improvement [24]. The BETA and EU-BETA studies [25], as well as other switching studies [12, 16, 24], have focused mainly on the degree of clinical improvement, tolerability, and safety profiles. However, data from these studies was herein analyzed to determine whether weight reduction may be achieved, as clinicians who switch patients from one antipsychotic agent to another are often concerned about the potential for reducing this serious side effect. Nevertheless, some patients gain weight when treatment with an antipsychotic is switched to aripiprazole. A clinically meaningful change in weight is usually defined as a change greater than 7% of body weight before an intervention [26]. In the Kim et al. naturalistic switch study [27], the authors reported that the rate of patients switched to aripiprazole who had gained weight was lower than that of patients continuing standard-of-care antipsychotics. In the present analysis, the rate of patients who had lost more than 7% of their weight following the switch to aripiprazole was more than twice that of patients who had gained more than 7% of their weight prior to the switch.

3.3. Limitations. Some of the analyzed studies lacked data as to BMI and to the rates of the 7% change in weight. There were two single cases that were statistically "outliers" in the reported weight reduction and were thus excluded from the final analysis. Age ranges limit generalization of our findings to adolescents or the elderly. Finally, all of the studies with the largest sample size were industrially supported.

4. Conclusions

Patients taking antipsychotic medications for psychiatric disorders have also many risk factors for medical comorbidities. There is a pressing need for effective interventions to address problems related to the additional iatrogenic burden from weight gain caused by antipsychotic medications. For patients suffering from schizophrenia, discontinuation of antipsychotic medication is not advisable, and thus switching to an antipsychotic with a lower propensity to induce weight gain needs be explored as a strategy. Our analysis suggests, in accord with previous findings [28], that aripiprazole could be useful for patients treated with other antipsychotics who show significant weight gain, a well-established side effect of many antipsychotics.

Disclosure

Authors (Y. Barak and D. Aizenberg) have received an unrestricted educational Grant by Biotis Ltd (Israel).

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Research Article

Effect of Treatment of Sprague Dawley Rats with AVE7688, Enalapril, or Candoxatril on Diet-Induced Obesity

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The objective of this study was to determine the effect of AVE7688, a drug that inhibits both angiotensin converting enzyme (ACE) and neutral endopeptidase (NEP) activity, on neural and vascular defects caused by diet induced obesity (DIO). Rats at 12 weeks of age were fed a standard or high fat diet with or without AVE7688 for 24 weeks. DIO rats had impaired glucose tolerance and developed sensory neuropathy. Vascular relaxation to acetylcholine and calcitonin gene-related peptide was decreased in epineurial arterioles of DIO rats. Rats fed a high fat diet containing AVE7688 did not become obese and vascular and sensory nerve dysfunction and impaired glucose tolerance were improved. DIO is associated with increased expression of NEP in epineurial arterioles. NEP degrades vasoactive peptides which may explain the decrease in neurovascular function in DIO.

1. Introduction

Previously, we demonstrated that high-fat fed rats, a model for diet-induced obesity, develop microvascular and neural deficits independent of hyperglycemia and this was accompanied by an increase in the expression of neutral endopeptidase in epineurial arterioles, vessels that provide circulation to the sciatic nerve [1]. In the present study, we sought to determine whether treatment of high-fat fed rats with AVE7688, a vasopeptidase inhibitor, for 24 weeks beginning at 12 weeks of age could improve microvascular dysfunction and prevent the slowing of sensory nerve conduction velocity. Vasopeptidase inhibitors are a new class of drugs that simultaneously inhibits neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE) activity [2]. Recent studies have shown increased expression of angiotensin II-forming enzymes in adipose tissue, and increased activity of the renin-angiotensin system has been implicated in the development of insulin resistance and type 2 diabetes [3]. Neutral endopeptidase is found in many tissues including vascular and nerve tissue and its activity is increased by fatty acids and glucose in human microvascular cells [4–8]. Neutral endopeptidase degrades many vasoactive peptides including natriuretic peptides, adrenomedullin, bradykinin, and calcitonin gene-related peptide [9, 10]. Therefore, inhibition of ACE and NEP activity would be expected to improve vascular function. In this regard, we have demonstrated that treating type 1 and type 2 diabetic rats as well as a genetic rat model of obesity with AVE7688 improves vascular and neural dysfunction [11–13]. However, no information is available about the effect of vasopeptidase inhibitors in an animal model of diet-induced obesity.

2. Materials and Methods

Unless stated otherwise, all chemicals used in these studies were obtained from Sigma Chemical Co. (St. Louis, MO).

2.1. Animals. Male Sprague-Dawley (Harlan Sprague Dawley, Indianapolis, IN) rats 10-11 weeks of age were housed in a certified animal care facility and food (Harlan Teklad, #7001, Madison, WI) and water were provided ad libitum. All institutional (ACURF #0691101), VAMC, and NIH guidelines for use of animals were followed. At 12 weeks of age the rats were weighed and randomly divided into six groups. One group was maintained on a normal diet.

A second group was maintained on a normal diet containing 500 mg/kg AVE7688 (Ilepatril, Sanofi Aventis). Group 3 was placed on a high-fat diet. Group 4 was placed on a high-fat diet containing 500 mg/kg AVE7688, which inhibits both ACE and NEP activity. In order to determine the role of ACE and NEP inhibition independently rats (Groups 5 and 6) were fed a high-fat diet containing Enalapril (500 mg/kg, ACE inhibitor) or Candoxatril (300 mg/kg, NEP inhibitor), respectively. The high-fat diet contained 24 gm% fat, 24 gm% protein, and 41 gm% carbohydrate (D12451; Research Diets, New Brunswick, NJ). The primary source of the increased fat content in the diet was soybean oil and lard. The average fat content of the control diet was 4.25 gm% (Harlan Teklad, #7001, Madison, WI).

2.2. Glucose Tolerance. Glucose tolerance was determined by injecting rats with a saline solution containing 2 g/kg glucose, i.p., after an overnight fast. Immediately prior to the glucose injection and for 240 minutes afterwards blood samples were taken to measure circulating glucose levels using glucose oxidase reagent strips (Lifescan Inc., Milpitas, CA). Fasting basal levels of insulin and leptin was also determined using Luminex technology.

2.3. Thermal Nociceptive Response. The day before terminal studies, thermal nociceptive response in the hindpaw was measured using the Hargreaves method as previously described [14].

2.4. Motor and Sensory Nerve Conduction Velocity and Biological and Oxidative Stress Markers. On the day of terminal studies, rats were weighed and anesthetized with Nembutal i.p. (50 mg/kg, i.p., Abbott Laboratories, North Chicago, IL). Serum samples were collected for determination of free fatty acid, triglyceride, free cholesterol, adiponectin, 8-hydroxy deoxyguanosine (8-OH DG), and angiotensin converting enzyme activity, using commercial kits from Roche Diagnostics, Mannheim, Germany; Sigma Chemical Co., St. Louis, MO; Bio Vision, Mountain View, CA; ALPCO diagnostics, Windham, NH, Cell Biolabs, Inc., San Diego, CA; ALPCO diagnostics, Windham, NH, respectively. Serum thiobarbituric acid reactive substances (TBARS) levels were also determined as an additional marker of oxidative stress as previously described [15].

Motor and sensory nerve conduction velocity was determined as previously described and afterwards the left gastrocnemius muscle, epididymal fat pad, interscalpel brown fat pad, and tissue containing the epineurial arterioles was collected [16–18]. The gastrocnemius muscle, epididymal fat pad and interscalpel brown fat pad were weighed.

2.5. Intraepidermal Nerve Fiber Density in the Hindpaw. Immunoreactive intraepidermal nerve fiber profiles were visualized using confocal microscopy. Samples of skin of the right hindpaw were fixed, dehydrated, and embedded in paraffin. Sections (7 μm) were collected and immunostained with anti-PGP9.5 antibody (rabbit antihuman, AbD serotic, Morpho Sys US Inc., Raleigh, NC) over night followed by treatment with secondary antibody Alexa Fluor 546 goat antirabbit (Invitrogen, Eugene, OR). Profiles were counted by two individual investigators that were blinded to the sample identity. All immunoreactive profiles within the epidermis were counted and normalized to epidermal length [1, 19].

2.6. Vascular Reactivity. Videomicroscopy was used to investigate *in vitro* vasodilatory responsiveness of arterioles vascularizing the region of the sciatic nerve as previously described [1, 16, 17]. Cumulative concentration-response relationships were evaluated for acetylcholine (10^{-8}–10^{-4} M) and calcitonin gene-related peptide (10^{-11}–10^{-8} M) (CGRP) using vessels from each group of rats. At the end of the acetylcholine concentration response curve a maximal dose of sodium nitroprusside (10^{-4} M) was added in order to determine the endothelium-independent relaxation potential. At the end of each dose response curve for acetylcholine or CGRP papaverine (10^{-5} M) was added to determine maximal vasodilation.

2.7. Data Analysis. Results are presented as mean ± SEM. Comparisons between the groups were conducted using one-way ANOVA and Bonferroni post-test (Prism software; GraphPad, San Diego, CA). Concentration response curves for acetylcholine and CGRP were compared using a two-way repeated measures analysis of variance with autoregressive covariance structure using proc mixed program of SAS [16, 17]. A P-value of less than .05 was considered significant.

3. Results

3.1. Effect of AVE7688 Treatment on Weight and Metabolic Parameters Induced by a High-Fat Diet. Sprague Dawley rats at 12 weeks of age were placed on a normal diet with or without 500 mg/kg AVE7688 or a high-fat diet (45% kcal fat) with or without AVE7688, Enalapril, or Candoxatril for 24 weeks. During the course of the study the rats on the control diet ate 54 g/kg rat/day and the rats on the high-fat diet or high-fat diet containing AVE7688, Enalapril, or Candoxatril ate 33, 35, 33 or 32 g/day/kg rat, respectively. At the end of the study period rats on the high-fat diet weighed significantly more then rats on the control diet (Table 1). However, high-fat fed rats treated with AVE7688 or Enalapril weighed significantly less than the high-fat fed rats. In contrast, high-fat fed rats treated with Candoxatril weighed significantly more than the control or high-fat fed rats. All groups of rats weighed approximately the same at the beginning of the study and nonfasting blood glucose levels at the end of the study were similar for rats in all six groups. Adding AVE7688 to the control diet also reduced weight gain over 24 weeks but this difference was not significant compared to control. The weight of the left gastrocnemius muscle was not different for the 6 groups of rats. In contrast, weight of the epididymal fat pad was significantly increased in high-fat fed rats and high-fat fed rats treated with Enalapril or Candoxatril compared to control. Weight of the epididymal fat pad was significantly less in rats fed a high-fat diet containing AVE7688 or

Table 1: Effect of high-fat diet ± AVE7688, Enalapril or Candoxatril on blood glucose and change in body weight, fat pad and muscle mass.

Determination	Control (23)	Control + AVE7688 (8)	High Fat (24)	High Fat + AVE7688 (28)	High Fat + Enalapril (6)	High Fat + Candoxatril (6)
Start Body Weight (g)	338 ± 3	327 ± 5	340 ± 2	336 ± 3	353 ± 7	354 ± 4
End Body Weight (g)	485 ± 7	454 ± 5	560 ±9[a]	459 ±7[b]	495 ± 15[b]	643 ±20[a,b]
Blood glucose (mg/dL)	93 ± 6	93 ± 7	96 ± 6	88 ± 5	111 ± 6	116 ± 11
Epididymal fat pad (g)	5.6 ± 0.4	4.2 ± 0.2	12.0 ± 0.6[a]	6.5 ± 0.4[b]	8.3 ± 1.2[a,b]	17.3 ± 1.6[a,b]
Interscapular Brown fat pad (g)	0.38 ± 0.02	0.34 ± 0.03	0.68 ± 0.04[a]	0.44 ± 0.01[b]	ND	ND
Gastrocnemius muscle (g)	3.3 ± 0.1	3.2 ± 0.1	3.4 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.6 ± 0.1

Data are presented as the mean ± SEM. [a]$P < .05$ compared to control; [b]$P < .05$ compared to high-fat. ND equals not determined. Parentheses indicate the number of experimental animals in each group.

Enalapril, compared to high-fat fed rats.. The weight of the interscapular brown fat pad was significantly increased in high-fat fed rats compared to control and this was prevented when the rats on the high-fat diet were treated with AVE7688.

Serum was collected from rats fed the control, high-fat, and high-fat with AVE7688, Enalapril, or Candoxatril diets and used to measure a number of physiological parameters. Data in Table 2 demonstrate that fasting insulin and leptin levels are significantly increased in rats fed a high-fat diet and that this was prevented when the high-fat diet contained AVE7688. Feeding rats a high-fat diet containing Enalapril also significantly reduced serum insulin levels and leptin levels (but not significantly) compared to high-fat fed rats. Serum insulin and leptin levels were significantly increased in rats fed a high-fat diet containing Candoxatril compared to control. Data in Figure 1 show that glucose tolerance was impaired in high-fat fed rats compared to control rats and this was improved by adding AVE7688 to the high-fat diet. In contrast, adding Enalapril or Candoxatril to the high-fat diet did not improve glucose tolerance. Combined these data indicate that high-fat fed rats show signs of insulin resistant and this can be prevented by AVE7688.

Serum cholesterol levels are significantly increased in high-fat fed rats and this was prevented by AVE7688, Enalapril or Candoxatril (Table 2). Serum free fatty acids and triglycerides levels are elevated in high-fat fed rats and this was not prevented by adding AVE7688, Enalapril, or Candoxatril to the high-fat diet (Table 2). Serum markers for oxidative stress, 8-OH- deoxyguanosine and thiobarbituric acid reactive substances (TBARS) and adiponectin are significantly increased in rats fed a high-fat diet and this was prevented by adding AVE7688 to the high-fat diet (Table 2). Adding Enalapril or Candoxatril to the high-fat diet reduced 8-OH- deoxyguanosine levels but not TBARS levels. In contrast, adding Candoxatril to the high-fat diet reduced both 8-OH- deoxyguanosine levels but not TBARS levels.

3.2. Effect of a High-Fat Diet and AVE7688 Treatment on Neural and Vascular Function. Data in Figure 2 demonstrate that sensory nerve conduction velocity was decreased in rats fed a high-fat diet and this was prevented by AVE7688 and

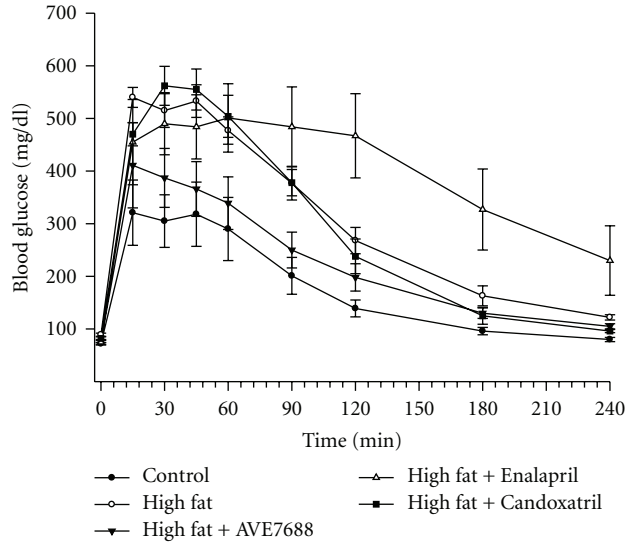

Figure 1: Effect of a high-fat diet and treatment with AVE7688 on glucose tolerance. Rats were fed a standard or high-fat diet with or without AVE7688, Enalapril, or Candoxatril for 24 weeks. Afterwards glucose tolerance was determined as described in Section. Data are presented as the mean ± SEM in mg/dL. The area under the curve (AUC) was significantly different $P < .01$ for high-fat fed rats versus control. There was no significant difference for AUC between rats fed a high-fat diet containing AVE7688 and control or high-fat fed rats. Feeding rats a high-fat diet containing Enalapril or Candoxatril provided no improvement in impaired glucose tolerance caused by the high-fat diet. The number of rats in each group was the same as shown in Table 1.

Candoxatril treatment. Enalapril treatment of high-fat fed rats also improved sensory nerve conduction velocity but the difference between the high-fat fed group and Enalapril treated group was not significant. Motor nerve conduction velocity was not significantly affected by high-fat diet or treatments Data in Figure 3 demonstrate that rats fed a high-fat diet for 24 weeks become thermal hypoalgesic and that intraepidermal nerve fiber profiles in the hindpaw are decreased (Figure 3) and these significant changes are prevented by adding AVE7688 to the high-fat diet. Adding

Table 2: Effect of high fat diet ± AVE7688, Enalapril or Candoxatril on change in serum insulin, leptin, cholesterol, triglycerides, free fatty acids, adiponectin, 8-hydroxy deoxyguanosine, TBARS, and ACE activity.

Determination	Control (23)	High Fat (24)	High Fat + AVE7688 (28)	High Fat + Enalapril (6)	High Fat + Candoxatril (6)
Insulin (ng/mL)	1.28 ±.09	3.60 ± 0.43[a]	1.78 ± 0.17[b]	0.67 ± 0.05[b]	3.74 ± 1.19[a]
Leptin (pM)	446 ± 81	2052 ± 389[a]	592 ± 75[b]	1442 ± 430	2852 ± 260[a]
Cholesterol (mg/dL)	303 ± 31	680 ± 29[a]	347 ± 15[b]	201 ± 22[b]	255 ± 49
Triglycerides (mg/dL)	41.8 ± 4.1	71.5 ± 11.7	72.0 ± 9.1	67.3 ± 5.3	72.2 ± 8.9
Free fatty acids (mmol/L)	0.10 ± 0.01	0.20 ± 0.03[a]	0.21 ± 0.02[a]	0.28 ± 0.04[a]	0.20 ± 0.04
Adiponectin (μg/mL)	7.9 ± 0.6	11.0 ± 0.7[a]	9.5 ± 0.5	7.7 ± 0.7	7.8 ± 0.7
8-OH DG (ng/mL)	1.88 ± 0.10	2.58 ± 0.17[a]	1.91 ± 0.17[b]	1.85 ± 0.09	1.88 ± 0.14
TBARS (μg/mL)	0.13 ± 0.01	0.50 ± 0.07[a]	0.26 ± 0.05[b]	0.71 ± 0.03[a]	0.55 ± 0.02[a]
ACE activity (mU/mL/min)	94 ± 8	75 ± 6	23 ±2[a,b]	4 ±1[a,b]	111 ± 14

Data are presented as the mean ± SEM. [a]$P < .05$ compared to control; [b]$P < .05$ compared to high-fat. ND equals not determined. Parentheses indicate the number of experimental animals in each group.

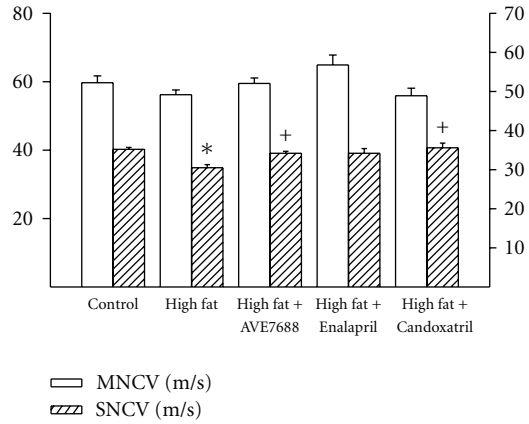

Figure 2: Effect of a high-fat diet and treatment with AVE7688 on motor and sensory nerve conduction velocity. Rats were fed a standard or high-fat diet with or without AVE7688, Enalapril or Candoxatril for 24 weeks. Data are presented as the mean ± SEM for motor and sensory nerve conduction velocity in m/sec. The number of rats in each group was the same as shown in Table 1. $^*P < .05$, compared to rats fed the standard diet (control), $^+P < .05$, compared to rats fed the high-fat diet.

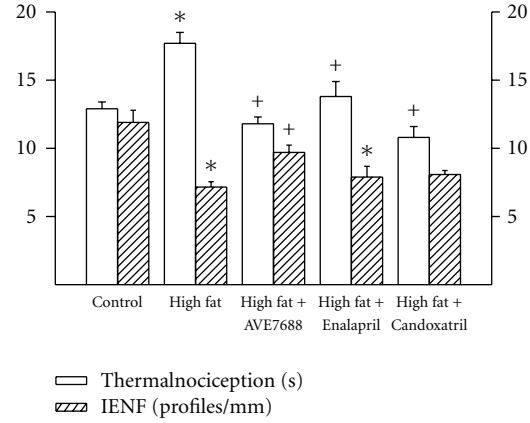

Figure 3: Effect of a high-fat diet and treatment with AVE7688 on thermal nociception and intraepidermal nerve fiber profiles in the hindpaw. Rats were fed a standard or high-fat diet with or without AVE7688, Enalapril or Candoxatril for 24 weeks. Data are presented as the mean ± SEM for thermal nociception in sec and intraepidermal nerve fiber profiles per mm. The number of rats in each group was the same as shown in Table 1. $^*P < .05$, compared to rats fed the standard diet (control), $^+P < .05$, compared to rats fed the high -fat diet.

Candoxatril to the high-fat diet also prevented the impairment in thermal nociception and the significant decrease in intraepidermal nerve fiber profiles. Supplementing the high-fat diet with Enalapril also improved thermal nociception compared to high-fat fed rats but to a lesser extent than AVE7688 or Candoxatril. In contrast, adding Enalapril to the high-fat diet did not improve the reduction in intraepidermal nerve fiber profiles compared to high-fat fed rats. Feeding control rats a standard diet containing AVE7688 did not affect motor and sensory nerve conduction velocity, thermal nociception or innervations, of the hindpaw (data not shown).

Vascular relaxation in response to acetylcholine and calcitonin gene-related peptide by epineurial arterioles from high-fat fed rats are significantly decreased compared to rats fed a control diet or a high-fat diet containing AVE7688 (Figures 4 and 5, resp.). Treating rats with a high-fat diet containing Enalapril, or Candoxatril also improved vascular relaxation in response to acetylcholine and calcitonin gene-related peptide with Candoxatril having a better efficacy than Enalapril. Feeding control rats a standard diet containing AVE7688 did not affect vascular reactivity activity in epineurial arterioles (data not shown).

4. Discussion

Previously, we have demonstrated that treatment of types 1 and 2 diabetic rats and nondiabetic obese Zucker rats with AVE7688, a vasopeptidase inhibitor, is effective in improving microvascular and neural complications [11–13]. We have also reported that treatment of diabetic rats with AVE7688 may be more efficacious then monotherapy

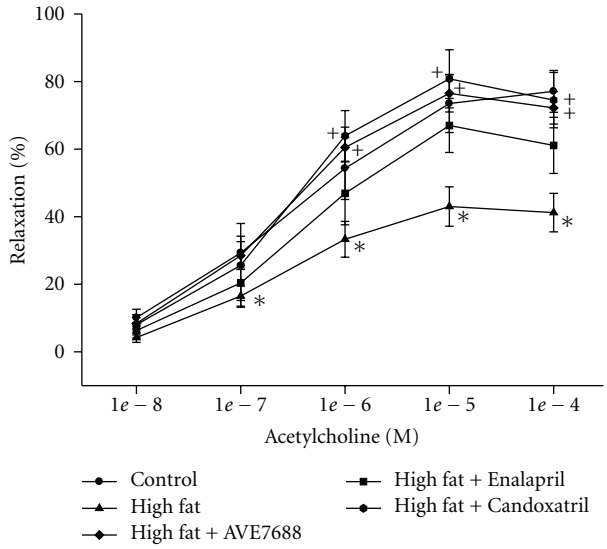

Figure 4: Effect of a high-fat diet and treatment with AVE7688 on acetylcholine-mediated vascular relaxation of epineurial arterioles. Rats were fed a standard or high-fat diet with or without AVE7688, Enalapril, or Candoxatril for 24 weeks. Pressurized arterioles (40 mm Hg and ranging from 60–100 μm luminal diameter) were constricted with U46619 (30%–50%) and incremental doses of acetylcholine were added to the bathing solution while recording steady state vessel diameter. Data are presented as the mean of % relaxation ± SEM. For these studies, two vessels were collected from each rat, studied, and the data combined. The number of rats in each group was the same as shown in Table 1. $^{*}P < .05$, compared to rats fed the standard diet (control), $^{+}P < .05$, compared to rats fed the high-fat diet.

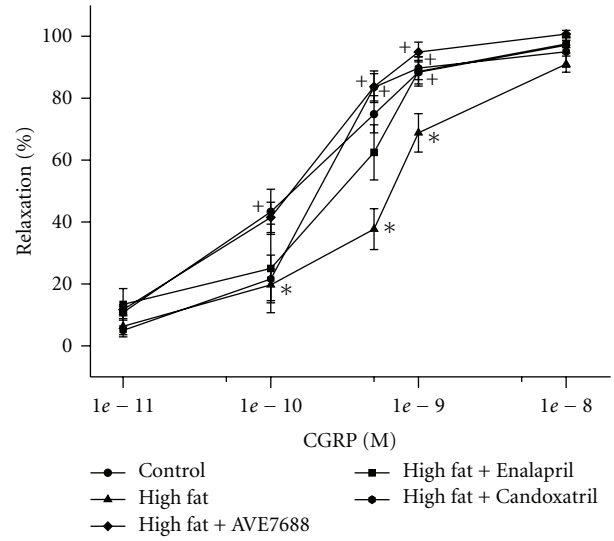

Figure 5: Effect of a high-fat diet and treatment with AVE7688 on calcitonin gene-related peptide-(CGRP)-mediated vascular relaxation of epineurial arterioles. Rats were fed a standard or high-fat diet with or without AVE7688, Enalapril, or Candoxatril for 24 weeks. Arterioles were derived from rats as described in Figure 4. Incremental doses of CGRP were added to the bathing solution while recording steady state vessel diameter. Data are presented as the mean of % relaxation ± SEM. The number of rats in each group was the same as shown in Table 1. $^{*}P < .05$, compared to rats fed the standard diet (control), $^{+}P < .05$, compared to rats fed the high-fat diet.

using an angiotensin converting enzyme inhibitor [20]. Given these results, we were interested in determining the effect AVE7688 treatment would have on microvascular and neural complications in a rat model of diet-induced obesity [1]. The primary hypothesis to be examined was that treatment of rats fed a high-fat diet with AVE7688 will prevent vascular and neural dysfunction and that dual inhibition of angiotensin converting enzyme and neutral endopeptidase would be more effective than monotherapy. To test this hypothesis high-fat fed rats were treated with or without AVE7688, Enalapril, or Candoxatril. After 24 weeks a number of endpoints were examined to determine the effect of treatments on obesity related changes in weight, insulin resistance, oxidative stress, and vascular and neural function.

The characteristics of the diet induced obesity rat model are insulin resistance, impaired glucose intolerance, dyslipidemia and increased fat deposit but not hyperglycemia [21–26]. Studies have also demonstrated an increase in oxidative stress [27, 28]. In a recent study, we found that rats fed a high-fat diet develop sensory neuropathy as indicated by slowing of sensory nerve conduction velocity, thermal hypoalgesia, and decrease in intraepidermal nerve fiber profiles. In contrast, endoneurial blood flow of the sciatic nerve and motor nerve conduction were not changed [1]. We also found that relaxation of epineurial arterioles to acetylcholine and CGRP was decreased in high-fat fed rats, but unlike diabetic rats, oxidative stress in epineurial arterioles was not increased [11, 12, 15, 16]. Moreover, we found that expression and activity of neutral endopeptidase in epineurial arterioles was increased in high-fat fed rats. This led us to speculate that treating high-fat fed rats with AVE7688 could be efficacious in improving vascular and neural defects associated with diet induced obesity. Since AVE7688 is an inhibitor of both angiotensin converting enzyme and neutral endopeptidase we also investigated the role of Enalapril, an inhibitor of angiotensin converting enzyme, and Candoxatril, an inhibitor of neutral endopeptidase, on weight gain and vascular and neural function in rats fed a high-fat diet.

The most important findings of this study was that rats fed a high-fat diet containing AVE7688 do not become obese and treatment prevented development of the vascular and neural dysfunction associated with diet induced obesity. Treatment of high-fat fed rats with AVE7688 over a period of 24 weeks using a prevention protocol prevented weight gain and mass of white and brown fat pads from high-fat fed rats treated with AVE7688 was significantly less compared to high-fat fed rats. Treating high-fat fed rats with Enalapril also reduced weight gain and the weight of the epididymal fat pad was significantly less compared to high-fat fed rats but was also significantly more than control rats. In contrast, treating high-fat fed rats with Candoxatril caused a greater weight gain and accumulation of epididymal fat pad mass compared to high-fat fed rats. Analysis of glucose tolerance revealed

that treatment of high-fat fed rats with AVE7688 prevented impairment of glucose utilization in rats fed a high-fat diet. Glucose tolerance of high-fat fed rats treated with Enalapril or Candoxatril remained significantly impaired.

Previous studies have shown that diet induced obesity in rodent models can be prevented by angiotensin converting enzyme inhibitors and angiotensin II receptor blockers [29–32]. In addition, diet induced weight gain and fat mass is reduced, energy expenditure increased, and glucose tolerance improved in mice lacking angiotensin converting enzyme or the angiotensin II type 1a receptor [33, 34]. Therefore, we were surprised to find that glucose tolerance was not improved in high-fat fed rats treated with Enalapril. Studies showing improved insulin resistance with angiotensin converting enzyme inhibitor treatment of chronic diet-induced obesity have been done primarily with mice [30]. In rats de Kloet et al. [35] demonstrated that Captopril treatment of high-fat fed rats improved glucose tolerance. However, the duration of the high-fat diet in this study was 35 days. In comparison, the duration of high-fat diet in our study was 168 days. Other studies demonstrating improved glucose tolerance in rats treated with angiotensin converting enzyme inhibitor were performed using rat models of type 2 diabetes [36–38]. Therefore, the long term period of the high-fat diet in our study may explain why we failed to see an improvement in glucose tolerance in Enalapril treated high-fat fed rats compared to the studies by de Kloet et al. [35].

Treating high-fat fed rats with Candoxatril provided no benefit toward reducing weight gain and fat mass or improving glucose tolerance. At the end of the study period, weight gain and epididymal fat pad mass were significantly higher in Candoxatril treated high-fat fed rats compared to high-fat fed rats. Therefore, inhibition of angiotensin converting enzyme or neutral endopeptidase alone is not sufficient to prevent obesity or insulin resistance in chronic high-fat fed rats.

The mechanisms proposed for the improvement in obesity and glucose tolerance with treatment of rodent models with angiotensin converting enzyme inhibitors are increased energy expenditure, liver and adipose tissue metabolic modulation, lower concentration of leptin, improved insulin signaling, and increased glucose and fatty acid utilization by muscle [29–34, 37–42]. In a study comparing the effects of Ramipril, an angiotensin converting enzyme inhibitor, to AVE7688 in JCR:LA-cp rats, an obese, insulin-resistant, hyperinsulinemic, normoglycemic model, it was found that both compounds reduced the surge of plasma insulin in a meal tolerance test by about 50% but AVE7688 was more beneficial in improving vascular reactivity [43]. In another study using obese Zucker rats, it was found that dual inhibition of angiotensin converting enzyme and neutral endopeptidase improved insulin mediated glucose disposal more effectively than monotherapy and this effect was linked to increased activation of the kinin-nitric oxide pathway [44]. In a similar independent study, it was found that Omapatrilat, a vasopeptidase inhibitor, induced insulin sensitization and increased myocardial glucose uptake in obese Zucker rats and that the effect of Omapatrilat was greater than Ramipril in part due to stimulation of the B_2 receptor [45]. Later this group reported that treatment of obese Zucker rats with a vasopeptidase inhibitor increased muscle glucose uptake independent of insulin signaling [46]. In two of these studies, protection of bradykinin from degradation by neutral endopeptidase was found to improve insulin action [44, 45]. Interestingly, it has been shown that natriuretic peptides promote muscle mitochondrial biogenesis and fat oxidation as to prevent obesity and glucose intolerance [47]. The natriuretic peptides are also degraded by neutral endopeptidase [20]. Because neutral endopeptidase is expressed in skeletal muscle in relatively large amounts and being located on the cell surface, neutral endopeptidase is able to hydrolyze peptides in the vicinity of their receptors thereby neutralizing their bioactivity [20, 48]. Since bradykinin and natriuretic peptides may have a role in regulating glucose and fatty acid metabolism by muscle protecting, their bioactive function by preventing degradation may be a therapeutic approach for treatment of obesity and insulin resistance [20, 48].

The second major finding of this study was that treatment of diet-induced obese rats with AVE7688 prevented vascular and neural complications associated with obesity. Previously, we reported that treatment of types 1 and 2 diabetic rat models with AVE7688 improved vascular and neural complications and AVE7688 was more effective than angiotensin converting enzyme inhibition [11–13, 20]. In these studies, treating high-fat fed rats with AVE7688 was generally more efficacious then Enalapril treatment in improving sensory neuropathy and vascular relaxation to acetylcholine and calcitonin gene-related peptide. We did not achieve significant differences between the AVE7688 and Enalapril treatment groups but we consistently found that treatment with AVE7688 and Candoxatril had better outcome for vascular and neural function than Enalapril.

It is well known that patients with impaired glucose tolerance are at increased risk for myocardial infarction, stroke, and large-vessel disease [49]. However, impaired glucose tolerance is also independently associated with traditional microvascular complications of diabetes including retinopathy, nephropathy, and polyneuropathy. Several mechanisms including inhibition of nitric oxide vasodilation, endothelial injury from hyperlipidemia and cytokines, and increased formation of reactive oxygen species have been cited as pathogenic causes for microvascular dysfunction associated with obesity and insulin resistance [49, 50]. However, in our studies with high-fat fed rats and examination of the effect on vascular reactivity of epineurial arterioles, we have not been able to document an increase in reactive oxygen species in these vessels [1]. Thus, we cannot conclude that quenching of nitric oxide by superoxide is responsible for reducing acetylcholine-mediated vascular relaxation in epineurial arterioles. There is an increase in oxidative stress in high-fat fed rats as evidence by the increase of markers of oxidative stress in the serum that can be corrected by treatment with AVE7688 and to a lesser extent with Enalapril or Candoxatril. It is thought that a primary source of reactive oxygen species in the vasculature derived from obese models

is NAD(P)H oxidase [51, 52]. We have demonstrated that epineurial arterioles from diabetic rats generate reactive oxygen species and the primary source is the mitochondria [53]. We propose that in addition to increased oxidative stress other mechanisms may be responsible for microvascular dysfunction in obesity. One of these mechanisms is the increased expression/activity of neutral endopeptidase [20]. We have shown that diet-induced obesity causes an increase in the expression of neutral endopeptidase in epineurial arterioles [1]. In addition, it has been shown that hyperlipidemia and hyperglycemia increases neutral endopeptidase activity in human microvascular endothelial cells [6]. In these studies, we propose that AVE7688, Candoxatril, and to a lesser extent Enalapril may be protecting vascular reactivity and neural function by inhibiting neutral endopeptidase and the degradation of C-type natriuretic peptide and calcitonin gene-related peptide [11–13, 20].

5. Conclusion

Feeding rats a high-fat diet causes weight gain, impaired glucose tolerance, increased markers of oxidative stress, and vascular and sensory nerve dysfunction. Each of these obesity-induced pathogenic conditions was prevented by treating the high-fat fed rats with AVE7688 and treatment with AVE7688 was generally more effective than treatments with Enalapril or Candoxatril. Thus, we conclude that dual inhibition of angiotensin converting enzyme and neutral endopeptidase may be more effective than monotherapy in reducing insulin resistance and the complications associated with diet induced obesity.

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Research Article

Assessment of Acute and Chronic Pharmacological Effects on Energy Expenditure and Macronutrient Oxidation in Humans: Responses to Ephedrine

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Evidence of active brown adipose tissue in human adults suggests that this may become a pharmacological target to induce negative energy balance. We have explored whole-body indirect calorimetry to detect the metabolic effects of thermogenic drugs through administration of ephedrine hydrochloride and have assessed ephedrine's merits as a comparator compound in the evaluation of novel thermogenic agents. Volunteers randomly given ephedrine hydrochloride 15 mg QID ($n = 8$) or placebo ($n = 6$) were studied at baseline and after 1-2 and 14-15 days of treatment. We demonstrate that overnight or 23-hour, 2% energy expenditure (EE) and 5% fat (FO) or CHO oxidation effects are detectable both acutely and over 14 days. Compared to placebo, ephedrine increased EE and FO rates overnight (EE 63 kJ day 2, EE 105 kJ, FO 190 kJ, day 14), but not over 23 h. We conclude that modest energy expenditure and fat oxidation responses to pharmacological interventions can be confidently detected by calorimetry in small groups. Ephedrine should provide reliable data against which to compare novel thermogenic compounds.

1. Introduction

Obesity usually develops gradually as a result of a modest but sustained energy surplus. A continuing positive energy imbalance of just 1% predicts a body mass increase of around 1 kg per year. Clinical management of obesity presents a particular challenge because lifestyle interventions are poorly effective [1–3] and there are few drug targets that have yielded outstanding clinical efficacy. Theoretically, such targets could be in systems regulating energy intake or energy expenditure, although it is clear that these are closely integrated within the central nervous system (CNS).

Most pharmacological approaches to obesity management in humans have focussed on reducing energy intake. Even when pharmacological interventions have been postulated to increase energy expenditure, it seems that much of the effect on body weight was mediated through decreased energy intake [4]. However, it is interesting that the ephedrine-caffeine combination [4] and, more recently, tesofensine [5], a triple central monoamine uptake inhibitor, which have been shown to produce weight loss, do have measurable effects on energy expenditure, though again most of the weight loss observed appears to be attributable to effects on energy intake [6]. This demonstrates that mechanistic specificity is hard to achieve. This is exemplified by sibutramine, a drug that predominantly enhances satiety, but which, in some studies, increases energy expenditure acutely and reduces the fall in resting energy expenditure seen during weight loss [7–9], although in others it was without measurable effect [9, 10]. This contrasts with the substantial energy expenditure effects of sibutramine in animal studies [11, 12].

Thus, three clear messages emerge from the literature. First, the degree of energy expenditure-related weight loss that can be induced pharmacologically in rodents is generally much greater than that seen in humans. Secondly, modest thermogenic effects in man could make a valuable direct or indirect contribution to body weight management by reducing the propensity for weight loss to plateau due to metabolic compensation [13]. Thirdly, poor mechanistic specificity of previous weight loss compounds suggests that energy expenditure should arguably be included in the mechanistic studies of developmental compounds.

From this introduction, it will follow that studying the responses to novel pharmacological interventions requires measurement of changes in body composition, changes in energy expenditure, and changes in food intake. Well-documented reference compounds are of value in providing positive control.

Demonstrating the efficacy of compounds in early-phase drug development requires particularly sensitive and precise measurement techniques. We have recently reported the potential for quantitative magnetic resonance (QMR) methodology to enhance the precision of body composition change measurements [14] and have documented its precision in a way which will contribute to the design and powering of our studies of pharmacological agents. Here we present a complementary analysis of the precision with which calorimetric methods may be used to detect changes in energy expenditure and macronutrient oxidation rates. To provide a pharmacological context for this analysis, we have adopted ephedrine, an established compound with documented thermogenic properties, to generate the manipulation of energy expenditure in this study.

Recent observations of active brown adipose tissue (BAT) in man may offer new targets through which energy expenditure contributions to weight loss and its maintenance may be enhanced (refs) and may involve attempts to promote the development of BAT from precursor cells [15].

We anticipate that our findings will inform the design of protocols to evaluate novel therapies in the near future.

2. Methods

The study was conducted at Addenbrooke's Centre for Clinical Investigation (ACCI), Addenbrooke's Hospital, Cambridge, UK. Within the ACCI, investigations were undertaken in the GlaxoSmithKline Clinical Unit in Cambridge (CUC) and the Wellcome Trust Clinical Research Facility (WTCRF).

2.1. Participants. Participants were recruited from the GSK CUC panel of healthy volunteers. All were healthy males, with body mass index (BMI, weight/height^2) ranging from 25 to 30 kg/m^2. Participants were screened by review of medical history, physical examination, and laboratory tests. Exclusion criteria included history of metabolic disorders, substantial change, or modification of body weight, regular strenuous exercise, or use of recreational drugs. Fourteen subjects completed this study.

The study was performed in accordance with Good Clinical Practice guidelines and the 1996 version of the Declaration of Helsinki. Protocols were approved by an internal GSK review panel, Addenbrooke's Hospital R&D office, the WTCRF Scientific Advisory Board, and the Cambridge Local Research Ethics Committee (LREC04/Q0108/6). Each subject gave written informed consent prior to participation.

2.2. Treatment. The study followed a double-blind, placebo controlled design. The active treatment was ephedrine hydrochloride 15 mg administered four times per day (E). Eight participants received ephedrine and six received placebo in a randomized order.

2.3. Study Design. Participants attended on two occasions separated by 2 weeks. The days of the study visits are identified as follows: Day −1, Day 1, Day 2, Day 14, and Day 15. Two participants were studied at each visit. Alcohol, caffeine, and strenuous exercise were prohibited 48 hours prior to the first visit and throughout the study.

Participants arrived fasting at 08:00 on Day −1. Breakfast was given at 09:45 and lunch at 13:30. Following lunch, body composition was measured by dual-energy X-ray absorptiometry (DXA; GE Lunar Prodigy, GE Healthcare, Madison, WI, USA) and by BOD POD (Life Measurement Inc., Concord, CA). Dinner was served at 19:00. After dinner, participants were fitted with an ActiHeart combined activity and heart rate monitor (CamNtech Ltd., Cambridge, UK). The ActiHeart monitors were worn until the end of the visit. At 20:00, each participant entered a room calorimeter, where he remained until 09:00 on Day 2.

Participants followed a strictly defined activity protocol within the calorimeter. During the first evening (Day −1), participants remained sedentary. They prepared for bed at 23:00, and "lights out" was at 23:30. At 07:00 (Day 1), they were woken for blood pressure measurements, to pass urine and to provide fasting blood samples. They returned to bed until 08:00 when basal metabolic rate (BMR) was measured over 1h. At 9:00, they rose and dressed, and breakfast was served at 9:30. To obtain postprandial measures of energy expenditure (not reported here), participants remained recumbent on the bed between 10:00 and 13:00, but they were free to read or watch television. Lunch was served at 13:30. After lunch, participants undertook sedentary activities until a period of cycling exercise at 17:00. During each exercise period, a work rate of 50 watts was maintained for 30 minutes, equivalent to 3 kJ/min external work, costing to the subject typically 13 kJ/min. Dinner was served at 19:00. Following this, the first treatment dose was given at 20:00. A further period of exercise began at 21:00. Participants prepared for bed at 23:00 as on the previous night. The protocol described above was then repeated until participants left the calorimeter at 22:00 following the exercise on the evening of Day 2. During Day 2, ephedrine or placebo was administered at 07:00, 13:00, 16:00, and 20:00. At each treatment time point, blood pressure was recorded and a blood sample was drawn. Treatment administration continued until the end of the second visit.

During the second visit, Day 14 events were as those for Day −1 with the exception that body composition measurements (DXA and BOD POD) were not repeated. Day 15 events were as those for Day 2.

2.4. Calorimetry. The room calorimeters, in the WTCRF, are comfortable bed-sitting rooms. They provide measures of oxygen consumption and carbon dioxide production. Urine passed by the subject is collected and analysed for urea and creatinine, from which nitrogen excretion is calculated. From these measures, macronutrient oxidation rates (fat, CHO, protein) are computed and energy expenditure is derived. Calculations, calibrations, and performance have been described in previous publications [16–18].

The rooms are approximately 4 m × 2.5 m in area and 2.15 m high and have a window to the outside world. Two air-lock hatches allow entry of food and exit of urine samples. A porthole, sealed with a latex sleeve, enables a participant to present an arm for venepuncture. The rooms are carpeted and are furnished with a bed, table, armchair, and desk chair. They have a wash-basin and a caravan-type flushable chemical toilet. A television with DVD and games console and a computer with internet access are provided for recreation. Exercise is performed on a Seca Cardiotest CT100 cycle ergometer (Seca GMBH, Hamburg, Germany).

2.5. Diet. Prior to attendance for the study, each participant's daily energy expenditure within the calorimeter was predicted using an estimate of BMR from the regression equations of Schofield [19] scaled to account for sedentary activity, and with an addition for the cost of programmed exercise. Diets were prescribed to match these individual predictions. Diets were presented as three meals of equal energy content and composition, with 35% of energy as fat, 13% as protein, and 52% as carbohydrate. Decaffeinated drinks were provided ad libitum, but the associated energy was fixed by the provision of a standardised milk allowance and low-energy sweeteners.

2.6. Statistical Analysis. Ephedrine was compared with placebo by comparing the difference in active treatment measurements between days or visits with the corresponding difference in placebo treatment measurements. Analysis was performed using the general linear model implemented in the DataDesk 6.0 statistical analysis application (Data Description Inc., Ithaca, NY, USA). The significance level, α, was set at 0.05.

3. Results

The physical characteristics of the participants are presented in Table 1. Mean values were quite well matched between the ephedrine and placebo participants, with no significant difference between groups in any measured characteristic.

Variability in repeated measures of energy expenditure and macronutrient oxidation was explored in the placebo group. Two repeat intervals were explored: successive days and two days separated by 14 days. Analysis was performed between 00:00 and 07:00 representing sleep, and over a 23-hour interval between 22:00 and 21:00 the following day. Due to a technical problem during the data acquisition on day 14, data for one placebo subject was excluded from the 23-hour variability analyses and from the treatment effect analysis. Figures 1 and 2 illustrate the findings of the variability analysis. From this analysis, we estimate that the methodology has the power to detect the changes in energy expenditure and macronutrient oxidation presented in Tables 2(a) and 2(b).

Figure 3 illustrates the concentrations of ephedrine hydrochloride in the circulation of the actively treated participants. Though the plasma concentration during the night following the initial dose was lower than on the

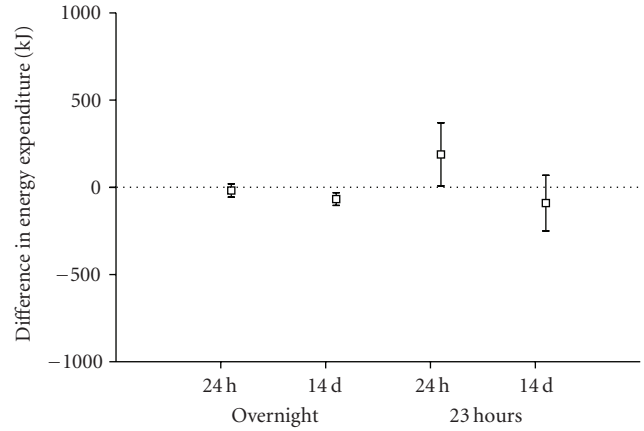

Figure 1: Mean differences and standard deviations of differences between measures of energy expenditure, made overnight and over 23 hours, and repeated after a 24-hour or 14-day interval. Untreated (placebo) subjects, $n = 6$.

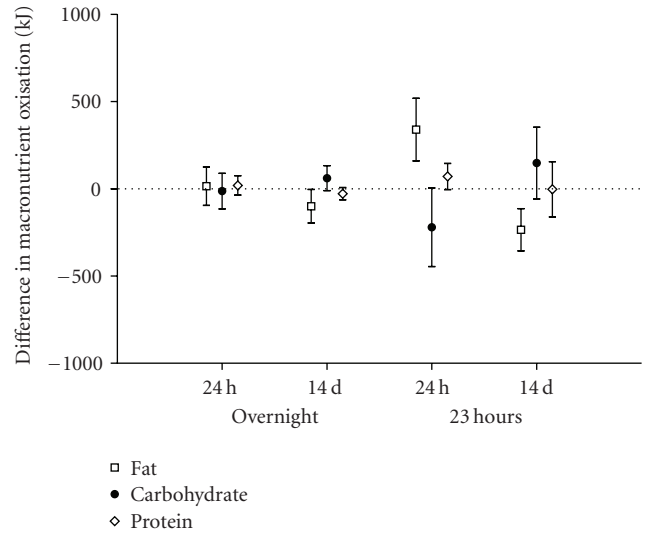

Figure 2: Mean differences and standard deviations of differences between measures of fat, carbohydrate, and protein oxidation, made overnight and over 23 hours, and repeated after a 24-hour or 14-day interval. Untreated (placebo) subjects, $n = 6$.

Table 1: Physical characteristics of ephedrine study subjects.

Study	Treated: $n = 8$		Placebo $n = 6$	
	Mean	SD	Mean	SD
Age (y)	29.42	3.69	30.60	2.03
Height (m)	1.81	0.07	1.75	0.03
Weight (kg)	86.76	5.01	81.00	3.62
BMI (kg/m^2)	26.57	1.48	26.49	1.24
Fat-free mass (kg)	63.70	7.32	62.61	3.30
Fat mass (kg)	23.05	4.04	18.39	5.71
Fat (%)	26.73	5.26	22.52	6.15

Table 2

(a) The predicted change in energy expenditure detectable in response to an intervention in a group of 6 subjects.

	Consecutive days change in EE kJ	14-day-apart change in EE kJ
Overnight (7 h)	40	38
24 h	190	202

(b) The predicted change in macronutrient oxidation detectable in a group of 6 subjects.

	Consecutive days change in kJ			14-day-apart change in kJ		
	Protein	CHO	Fat	Protein	CHO	Fat
Overnight (7 h)	58	107	116	37	76	101
24 h	79	237	189	163	212	125

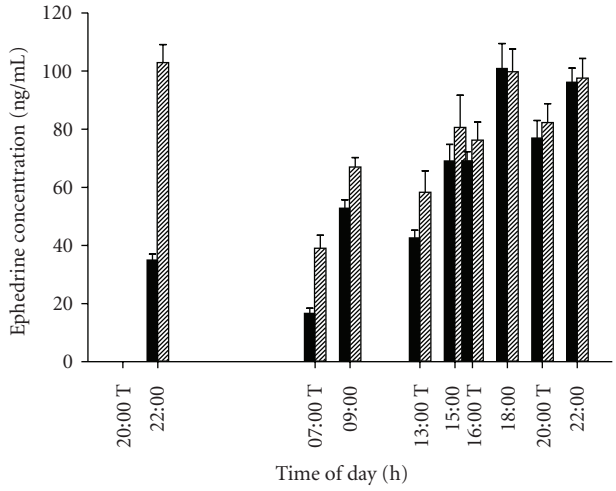

Figure 3: Circulating concentrations of ephedrine hydrochloride in treated subjects ($n = 8$). Solid bars: days 1-2; hatched bars days: 14-15. T indicates administration times.

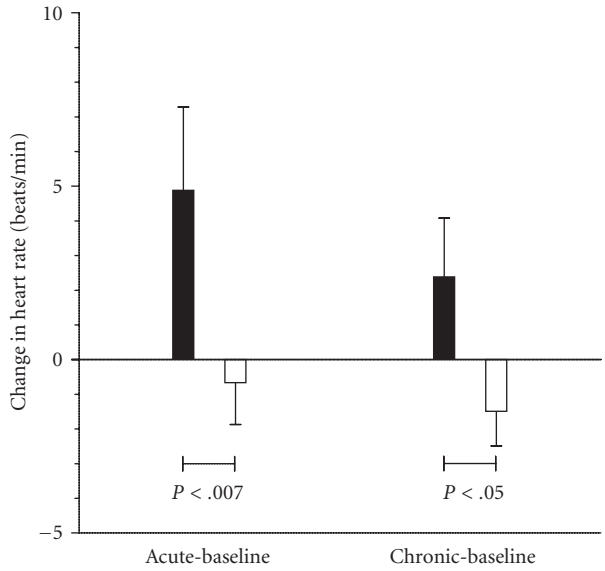

Figure 4: Influence on 22.5-hour average heart rate of acute or chronic (14 day) treatment with ephedrine hydrochloride (solid bars) or placebo (open bars).

corresponding night of the second visit, the daytime concentrations were similar on both visits.

Figure 4 shows the influence of ephedrine treatment on heart rate, measured over 22.5 hours, between 23:00 and 21:30 the next day. Treatment significantly increased heart rate both acutely (5.5 beats/min) and chronically (3.8 beats/min) when compared to the placebo treated group.

Table 3 presents the average macronutrient oxidation and energy expenditure for all subjects, overnight and over 23 hours, on the untreated day (Day −1). This provides a context for interpreting the magnitude of the treatment effects, which are presented in Table 4.

The differences in overnight energy expenditure and macronutrient oxidation between ephedrine- and placebo-treated participants are presented in Table 4(a). Ephedrine elevated energy expenditure significantly relative to placebo between both the acute- and chronic-treated nights and

Table 3: Macronutrient oxidation and energy expenditure—means for all subjects in the untreated condition.

	Protein (kJ)		CHO (kJ)		Fat (kJ)		Energy (kJ)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Overnight (7 h)	358	154	684	220	1033	253	2075	145
23h	1356	240	4463	680	4137	938	9957	942

Table 4

(a) Differences between ephedrine- and placebo-related changes in overnight macronutrient oxidation and energy expenditure, between treated (days 1 and 14) and untreated (day 0), and between chronic and acute treatments (days 14 and 1)

Interval	Protein		Carbohydrate		Fat		Energy	
days	difference (kJ)	*P*	difference (kJ)	*P*	difference (kJ)	*P*	difference (kJ)	*P*
1-0	−46.7	.75	−41.4	.86	150.8	.14	62.7	.05
14–0	−95.9	.32	10.8	.98	190.3	.05	105.2	.001
14–1	−46.2	.73	52.2	.78	39.5	.86	42.5	.23

(b) Differences between ephedrine and placebo treatment effects on changes in macronutrient oxidation and energy expenditure over 23 h, from 22:00 to 21:00, between treated (days 1 and 14) and untreated (day 0), and between chronic and acute treatments (days 14 and 1)

Interval	Protein		Carbohydrate		Fat		Energy	
days	difference (kJ)	*P*	difference (kJ)	*P*	difference (kJ)	*P*	difference (kJ)	*P*
1-0	131.9	.52	14.5	.99	36.7	.99	183.2	.48
14–0	285.8	.06	−61.0	.93	−94.9	.92	129.9	.69
14–1	153.8	.41	−75.6	.90	−161.6	.85	−53.3	.94

the untreated night, but there was no significant difference between acute and chronic nights. The magnitude of the treatment effect was 3.0% of the group average untreated overnight energy expenditure (shown in Table 3) for the acute response and 5.1% for the chronic response. The energy expenditure increments were associated with increments in fat oxidation which reached significance when chronic treatment was compared to the untreated state. There were no significant effects of treatment on carbohydrate or protein oxidation.

Differences in 23-hour energy expenditure and macronutrient oxidation between ephedrine- and placebo-treated participants are shown in Table 4(b). No treatment effects reached significance, though the changes in energy expenditure reflected those which were significant in the overnight observations and represented 1.8% and 1.3% for acute and chronic treatment effects, respectively.

4. Discussion

Weight loss agents may act on both sides of the energy balance equation, through central anorexigenic effects or modulation of energy expenditure. The recent resurgence of interest in BAT as a potential mediator of thermogenesis in adult humans [15, 20–23] has refocussed interest on thermogenic targets for potential antiobesity drugs. Increased thermogenesis might be achieved by targeting BAT with small molecules or possibly by increasing the number of brown adipocytes and their related oxidative capacity [24] via either transdifferentiation of white adipocytes [25] and/or shifting the developmental fate of adipocyte progenitors [26].

The evaluation of potential thermogenic compounds requires measures of changes, energy expenditure, macronutrient oxidation, and body composition. Modest rates of body mass reduction yield beneficial clinical outcomes [27], and so high measurement precision is essential to identify compounds with favourable profiles during early stages of development. We have recently reported on the favourable precision of QMR for elucidating changes in body composition [14]. Now, we have explored the changes in energy expenditure and macronutrient oxidation induced by ephedrine administered both acutely and over a 2-week "chronic" interval, an interval over which we might expect to be able to detect the beneficial body composition changes using QMR. We have explored ephedrine as a potential comparator compound and have documented the sensitivity with which whole-body indirect calorimetry can detect modest changes in energy expenditure and macronutrient oxidation following its administration.

Ephedrine has been shown to have a complex mechanism of action which includes central sympathetic activation, direct actions on adrenoreceptors [28], and peripheral noradrenalin release [29]. Both alone [30] and in combination with caffeine [4], it has been shown to increase thermogenesis in man, with much of this activity contributed by skeletal muscle in addition to BAT [31]. We selected ephedrine as our test compound in the light of this literature and, in doing so have extended the literature on what may well become a valuable "positive control" against which new compounds are evaluated.

Our study extends the surprisingly limited literature documenting the effects of ephedrine on energy expenditure

over the day [30]. Shannon et al. administered ephedrine 50 mg three times per day over 24 hours. This was a rather higher dose than the 15 mg × 4 per day which we administered and which we chose to minimise side effects during the longer period (14 days versus 1 day) of administration necessary to study ephedrine's chronic effects. We found a 3% increase in overnight energy expenditure after acute administration, rising to 5.1% after 15 days. This compares with 8.4% acute response reported by Shannon et al. at their higher dose. When we analysed the 23-hour data (Table 4(b)), we found our effects reduced below the level of significance, in both the acute or chronic conditions, to 1.8 and 1.3%. However, Shannon et al. also reported attenuated 24-hour effects, to a just-significant 3.6%. Given the robust response in the overnight measurements, this suggests a substantial compensation in daytime energy expenditure for the effects of the ephedrine treatment, at least at the thermoneutral temperatures of our studies.

Our study extends the report of Shannon et al. by demonstrating that the increment in overnight energy expenditure was funded by increased fat oxidation, significantly so over the chronic study period. This supports the inference from early, short-duration resting gas exchange measurements made on directly-expired air [32]. The recruitment of fat oxidation seen overnight was not present in the 23 hours analysis.

The effect of ephedrine on heart rate was significant and was maintained to the end of the 14-day intervention (Figure 4), suggesting little tachyphylaxis of its cardiac chronotropic effects during chronic ephedrine administration. The magnitude of the rise in heart rate is similar to that seen with sibutramine treatment [33]. The lack of tachyphylaxis is confirmed by the overnight energy expenditure response to ephedrine. Compared to the placebo-treated group, the energy expenditure of the ephedrine group was significantly elevated during the overnight period (Table 4(a)). During the first night, after a single ephedrine dose (Figure 3), the elevation was 3%. After 14 days, this had increased to 5% ($P < .001$), by which time circulating ephedrine levels (at steady state) were quantifiable 2 hours post dose (Figure 3).

Data from the control group in our study extends the literature documenting the intraindividual variability of 24-hour or overnight energy expenditure and macronutrient oxidation rates, both from day-to-day and across longer-time intervals [34–36]. Though such information is essential to the design and powering of studies seeking to document the effects of interventions which target energy expenditure or macronutrient balance, we found insufficient published work to enable us to power studies with confidence. Murgatroyd et al. [37] reported day-to-day variability in energy expenditure over 14-day continuous confinement in a calorimeter. Schoffelen and Westerterp [35] focussed on variability in overnight observations. Rumpler et al. [36] looked at short- and longer-term repeatability, as we have done here, but the longer-term analysis may have been influenced by energy imbalance. None of these reports extend to analysis of variability in macronutrient oxidation rates.

Taking data from the placebo-treated group, we have assessed the repeatability of these measurements and have derived the sensitivity with which putative changes in energy expenditure and macronutrient oxidation might be resolved in an overnight resting state or over a whole day. In the context of the expenditure and oxidation levels in Table 3, we estimate that treatment-induced changes in energy expenditure greater than 2% should be detectable in a group of six subjects (Table 2(a)), while changes in fat or carbohydrate oxidation should be detectable at around the 5% level (Table 2(b)). This suggests that calorimetry offers sufficient sensitivity to detect quite subtle changes in energy expenditure and fuel selection in response to pharmacological interventions, changes which nonetheless could have valuable therapeutic benefits in the medium-to-long term.

5. Conclusions

In conclusion, highly precise methods are paramount for detecting subtle differences in energy expenditure when evaluating the thermogenic properties of drugs that are being evaluated for the treatment of obesity. Our data confirms that modest drug effects, where present, can be detected by whole-body indirect calorimetry, and this technique should be considered when planning small experimental medicine studies to evaluate manipulations of energy balance. The high degree of precision in detecting changes in both energy expenditure and macronutrient utilisation should permit early confirmation of preclinical data and proof of mechanism and help to predict long-term efficacy. We have confirmed that ephedrine is a reliable thermogenic compound against which comparison of novel thermogenic agents may be made after both acute and chronic administration.

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Review Article

Experience (Mostly Negative) with the Use of Sympathomimetic Agents for Weight Loss

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Sympathomimetic agents have a poor history of long-term success in the treatment of obesity. From earlier experiences with amphetamine and its analogs, to more recent drugs with direct effects on adrenergic receptors or indirect effects from release of catecholamines or inhibition of reuptake, cardiovascular toxicity (strokes and cardiac arrhythmias) has been the major concern. These concerns also extended to food supplements containing ephedra alkaloids and may require consideration for current supplements containing the sympathomimetic drug, synephrine.

Sibutramine is the most recent drug with sympathomimetic activity that has been recognized by regulatory agencies as having cardiovascular adverse effects that may outweigh its potential value as a weightloss drug. Sibutramine is marketed in Europe under several trade names, including Reductil, Reduxade, Ectiva, Sibutral, Zelium, and others. Meridia is the only brand name in the United States. The European Medicines Agency took definitive action on January 21, 2010 in advising against the continued prescribing of the drug, and Abbott Laboratories suspended sales of the drug in Europe [1]. This action was prompted by a preliminary evaluation of results from the Sibutramine Cardiovascular OUTcome (SCOUT) trial reported by the United States FDA [2]. The FDA has not imposed a ban on the drug but has obtained a change in the boxed warning label to contraindicate its use in patients with a history of cardiovascular disease and in all individuals over 65 years of age (updated warning, April 15, 2010). These regulatory actions were prompted by an early review of the SCOUT trial [3] that revealed an incidence of cardiovascular events of 11.4% in patients receiving sibutramine compared to 10% in those receiving placebo. This was an unexpected finding; the hypothesis in the design of the study was that an anticipated weight loss from the use of sibutramine would be associated with a reduction in the incidence of cardiovascular events when compared to that observed in patients receiving the placebo treatment [3].

Sibutramine was not developed as a sympathomimetic drug. In early studies, it was first evaluated for its potential as an antidepressant medication, and its behavioral effects were apparently explained by its inhibition of both 5-hydroxytryptamine (5-HT) and norepinephrine reuptake in the central nervous system (CNS) [4, 5]. The inhibition of monoamine uptake is produced almost completely by two metabolites of sibutramine [5]. The hypophagic effect of sibutramine was recognized later, and this became the focus of its development as an antiobesity drug [6–8]. Its sympathomimetic effects (an increase in heart rate and blood pressure), which are of concern in relation to the observed increase in adverse cardiovascular events, would appear to be primarily related to a peripheral inhibition of norepinephrine reuptake and interrelationships with sympathetic outflow from the CNS [9, 10]. The increases in heart rate and blood pressure would be expected to follow from inhibition of norepinephrine reuptake by sibutramine (mostly its metabolites) at sympathetic nerve endings in the heart and blood vessels. This effect would be magnified during physical activity or other conditions of stress that would increase sympathetic nerve activity and norepinephrine overflow in the periphery. These increases

in heart rate and blood pressure are seen, despite the fact that sibutramine is known to blunt the intensity of central sympathetic outflow by virtue of α_2-adrenergic suppression of sympathetic activity in the CNS. The locus of this action is considered to include α_2-adrenergic receptors in the lower portion of the brain stem (medulla oblongata) [11]. This is also the presumed site of action of the antihypertensive drug, clonidine. The extent to which sympathetic outflow is decreased by this central inhibitory effect of overflow norepinephrine may be limited, because sibutramine has been observed to rapidly (within days) desensitize central α_2-adrenergic receptors in animal studies [12].

Amphetamines, phentermine, and phenyl-propanolamine (PPA) are sympathomimetic amines that were once widely used for the treatment of obesity because of their anorexic effects [13, 14]. They are all contraindicated for this use at this time because of their adverse cardiovascular and/or behavioral profiles. They are primarily indirect acting amines, that is, they release norepinephrine from sympathetic nerve endings. These amines all have an α-methyl substitution, which results in inhibition of monoamine oxidase and an intensification and prolongation of their pharmacological effects. In addition, the *dextro* isomer of amphetamine, dexamphetamine, is a potent inhibitor of norepinephrine reuptake, which may be the basis for its greater potency over the *levo*isomer [13]. The unregulated sale of supplements containing ephedra alkaloids is probably the most recent example of the discontinued use of a group of sympathomimetic entities for weight loss. These supplements were banned by the FDA in 2004 because of their unreasonable risks of cardiovascular toxicity. The complete ban on ephedra sales was challenged in 2005; that challenge was overturned on appeal by the FDA, with confirmation of the ban in 2006. The United States Supreme Court declined to review a further industry appeal in 2007. (I have commented previously on the cardiovascular risks of ephedra alkaloids [15].)

As one of several consultants to the FDA before the imposition of the ephedra ban [16], I conducted an evaluation of the pharmacology and inherent safety concerns of ephedra alkaloids. The principal focus in that analysis concerned ephedrine. Although the natural herb, *Ephedra sinica* (Ma Huang), contains three sympathomimetic agents in addition to ephedrine (pseudoephedrine, methylephedrine, and phenylpropanolamine), ephedrine is the most potent and is typically present in the highest concentration among the four alkaloids [17]. The sympathomimetic impact of ephedrine results from a combination of multiple actions. Ephedrine is classified as both a direct and indirect acting amine. Its indirect effects result primarily from the fact that it causes a displacement release of norepinephrine from sympathetic nerve endings. In addition to releasing norepinephrine, ephedrine blocks the reuptake of norepinephrine into sympathetic nerve terminals. Ephedrine is also an inhibitor of the degradation of norepinephrine by monoamine oxidase. Thus, by three mechanisms, ephedrine magnifies the intensity and duration of action of norepinephrine that is released [13, 14, 18]. Obviously, this entire process is further amplified during periods of physical exercise, where sympathetic nerve activity is increased. In addition to indirect effects of ephedrine, it also has direct vasoconstrictor (α_1-adrenergic), cardiac acceleration and increased contractility (β_1-adrenergic), and bronchodilator (β_2-adrenergic) effects. This triad of effects is similar to that of epinephrine. The direct effects of ephedrine summate with its indirect effects to produce its total cardiovascular impact.

A major negative impact of ephedrine upon the heart is its ability to induce cardiac arrhythmias. The primary mechanism involved in this risk is the fact that ephedrine and all sympathomimetic drugs that activate β_1-adrenergic receptors (either directly or through release, inhibition of reuptake, or inhibition of metabolism of norepinephrine) shorten the refractory period of the conducting system of the heart and cardiac muscle [11]. This is particularly problematic in an individual who is exercising, because the increased sympathetic outflow with exercise augments the release of norepinephrine, which itself has β_1-adrenergic activity. This adds to the shortening of refractoriness of cardiac cells. A shortening of the refractory period of myocardial cells is an essential element in the induction of a re-entrant arrhythmia, that is, an impulse may now encounter receptive cells, cells that would ordinarily be refractory and would not allow for a premature activation. That premature activation can permit an abnormal route of electrical excitation, that is, an arrhythmia [19]. In addition, myocardial ischemia caused by exercise and/or exacerbated by the presence of coronary artery obstruction also results in a shortening of the refractory period of myocardial cells [19, 20]. Since the presence of coronary artery disease may be unrecognized in many individuals during routine activities, the summation of drug and stress effects on the electrophysiology of the heart requires consideration. Recent data presented by the American Heart Association shows that the incidence of cardiovascular disease is 39.6 percent in the age group 40 to 59 years (identical for men and women) and 73.6 percent and 73.1 percent in the age group 60 to 79 years for men and women, respectively [21].

In addition to the risks of stroke associated with blood pressure elevations produced by sympathomimetic drugs, increases in blood pressure may also contribute an arrhythmogenic potential. With increases in blood pressure, compensatory baroreceptor-mediated predominance of the parasympathetic nervous system does result in a slowing of heart rate in comparison to the effect that would take place as a result of β_1-adrenergic activation alone. Although this may reduce the extent of blood pressure elevation, it does not reduce the arrhythmogenic potential of the electrophysiological processes discussed above. In fact, the participation of the parasympathetic nervous system to slow heart rate does so by release of its mediator, acetylcholine. Acetylcholine also shortens the refractory period of atrial tissues, which may be causally related to the induction of atrial fibrillation, atrial flutter, or paroxysmal supraventricular tachycardia [11]. Thus, direct sympathomimetic stimulation of β_1-adrenergic receptors, indirect effects through released norepinephrine, ischemia, and compensatory acetylcholine release, may each be primary or may summate to induce cardiac arrhythmias.

As a result of the loss of availability of ephedra-containing products, the sympathomimetic amine, synephrine, appears to have become an important focus of the food supplement industry. Synephrine is chemically related to the well-known vasopressor drug, phenylephrine; phenylephrine is the *meta*-hydroxy-phenyl isomer of synephrine. As with phenylephrine, synephrine increases blood pressure through its α_1-adrenergic-mediated vasoconstrictor effect [13]. There are a number of supplements currently on the market that identify synephrine as a major ingredient of the product. Information about these products is readily available through searches for synephrine. Various claims are made as to the value of this constituent for weight loss and development of physical fitness. Several of these commercial products contain a fairly well-characterized extract of Citrus aurantium (bitter orange), which is called "ADVANTRA Z". ADVANTRA Z is supplied by Nutratech, Inc. [22]. The descriptions of patents for ADVANTRA Z indicate that, in addition to synephrine, it contains three other orally active sympathomimetic agents, N-methyltyramine, hordenine, and octopamine [13]. The Nutratech website suggests a daily intake of 100 to 120 mg of synephrine, in two to three divided servings.

The potential cardiovascular effects of the doses of synephrine present in ADVANTRA Z can be placed in perspective because synephrine is the active ingredient in the orally active vasopressor drug, oxedrine tartrate. A common trade name for this preparation in Europe is Sympatol [23]. Oxedrine tartrate is not marketed in the United States. Sympatol carries the clinical indication for the treatment of hypotension, that is, to *raise* blood pressure, with oral doses of 100 to 150 mg, up to three times a day. To compare doses of synephrine in ADVANTRA Z with clinical doses of Sympatol, it is necessary to correct for the fact that Sympatol is the racemic mixture of synephrine (the *l*-isomer has the predominant sympathomimetic activity) and that it is the tartrate salt. (The *l*-isomer of synephrine is the form in citrus extracts [24, 25].) Therefore, the molecular weight of synephrine tartrate is 484.5. Of this, the *l*-synephrine content is 167.2 or 34.5% of the dose.

Thus, the single oral dose range of 100 to 150 mg of Sympatol supplies 34.5 to 51.8 mg of *l*-synephrine. If 3 doses are given per day, the total daily dose would be 103.5 to 155.4 mg of synephrine. This overlaps the suggested intake for ADVANTRA Z of 100 to 120 mg of synephrine per day. As discussed above, in addition to the danger of stroke associated with drug-induced increases in blood pressure, compensatory responses to these increases, mediated through the parasympathetic nervous system, may be arrhythmogenic. Also, these concerns would be expected to be enhanced in conjunction with physical effort. The National Center for Complementary and Alternative Medicine, National Institutes of Health, has noted the potential risks for cardiovascular toxicity from consumption of extracts of Citrus aurantium [26].

In summary, sympathomimetic agents do not have a successful record for the treatment of weight loss. They apparently possess an unreasonable cardiovascular toxic potential that emerges when the drugs are applied to a broad spectrum of the population, many of whom may have unrecognized risk factors.

Disclosure

The author was compensated as a consultant to the US Food Administration for evaluation of sympathomimetic agents in food supplements.

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Review Article

The Role of Testosterone in the Etiology and Treatment of Obesity, the Metabolic Syndrome, and Diabetes Mellitus Type 2

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Obesity has become a major health problem. Testosterone plays a significant role in obesity, glucose homeostasis, and lipid metabolism. The metabolic syndrome is a clustering of risk factors predisposing to diabetes mellitus type 2, atherosclerosis, and cardiovascular morbidity and mortality. The main components of the syndrome are visceral obesity, insulin resistance, glucose intolerance, raised blood pressure and dyslipidemia (elevated triglycerides, low levels of high-density lipoprotein cholesterol), and a proinflammatory and thrombogenic state. Cross-sectional epidemiological studies have reported a direct correlation between plasma testosterone and insulin sensitivity, and low testosterone levels are associated with an increased risk of type 2 diabetes mellitus, dramatically illustrated by androgen deprivation in men with prostate carcinoma. Lower total testosterone and sex hormone-binding globulin (SHBG) predict a higher incidence of the metabolic syndrome. Administration of testosterone to hypogonadal men reverses part of the unfavorable risk profile for the development of diabetes and atherosclerosis.

1. Introduction

A major problem is the management of overweight. Obesity is a condition that is reaching epidemic proportions in both the developed and the developing world. In the United States, 63% of men and 55% of women are classified as overweight. Of these, 22% are deemed grossly overweight, with a body mass index above 30 kg/m^2, and the consequences of this rapid increase are serious [1]. Approximately 80% of obese adults suffer from at least one, and 40% from two or more of the diseases associated with obesity, such as type 2 diabetes, hypertension, cardiovascular disease, gallbladder disease, cancers, and diseases of the locomotor system, such as arthrosis [2].

This contribution will highlight the significance of testosterone in the development and treatment of obesity. The reality of life is that the practice of medicine is subdivided into medical specialties, each with its own perspective and problems. Obesity and particularly its sequels, such as diabetes mellitus, cardiovascular disease, and locomotor problems, are not primarily treated by endocrinologists. Even among endocrinologists the expertise on sex hormones, not to speak of testosterone, is often limited. This contribution will argue that testosterone has a significant role to play in the etiology and treatment of obesity and its sequels in the male.

2. Sex Differences in Fat Distribution

Adult men and women differ in their fat distribution; the regional distribution of body fat is a characteristic of masculinity and femininity [3]. In premenopausal women a larger proportion of fat is stored in peripheral fat depots such as breasts, hips, and thighs. Men tend to deposit excess fat in the abdominal regions (both subcutaneous and intra-abdominal or visceral fat depots) and generally have a larger visceral fat depot than (premenopausal) women [4]. As regional localization of body fat is considered a secondary sex characteristic, it is likely that sex steroids are involved in the male and female patterns of fat deposition.

This view is strengthened by the observation that variations in sex steroid levels in different phases of (reproductive) life parallel regional differences in fat storage and fat mobilization, until puberty boys and girls do not differ very much in the amount of body fat and its regional distribution. From puberty on words, differences become manifest [5, 6]. The ovarian production of estrogens and progesterone induce an increase in total body fat as well as selective fat deposition in the breast and gluteofemoral region. Pubertal boys show a strong increase in fat free mass while the amount of total body fat does not change very much [5]. Adolescent boys lose subcutaneous fat but accumulate fat in the abdominal region, which in most boys, is not very visible in that stage of development but clearly demonstrable with imaging techniques [7]. The sex steroid-induced regional distribution is not an all-or-none mechanism; it is a preferential accumulation of excess fat. Obese men and women still show their sex-specific fat accumulation but store their fat also in the "fat depots of the other sex". Not only the fat distribution differs between the sexes from puberty on, but also the dynamics of fat cell size and fat metabolism are different. The amount of fat in a certain depot is dependent on the number and size of the fat cells. Fat cells in the gluteal and femoral region are larger than in the abdominal region [8]. The activity of lipoprotein lipase, the enzyme responsible for accumulation of triglycerides in the fat cell, is higher in the gluteo-femoral region than in the abdominal area [9]. Conversely, lipolysis is regulated by hormone sensitive lipase, which in turn is regulated by several hormones and by the sympathetic nervous system. Catecholamines stimulate lipolysis via the β-adrenergic receptor while α2-adrenoreceptors inhibit lipolysis. Hormones affect the catecholamine receptors of the adipocytes. Testosterone stimulates the β-adrenergic receptor while estrogens/progesterone stimulate preferentially α2-adrenoreceptors [10]. Insulin stimulates fat accumulation. It is not an unreasonable speculation that the sex steroid-dependent fat distribution serves (or from this millennium on has served?) the different roles of men and women in reproduction and caring for their progeny. The visceral fat depot constitutes a quickly available source of calories and energy. By its close anatomical proximity to the liver, it delivers fatty acids through the portal system [11]. The latter may have served a useful function in evolution when there was a more pronounced labour division between the sexes suiting the needs of men in their manual labour and quick physical action.

3. The Paradoxical Relationships of Testosterone and Fat Distribution in Adulthood and Ageing

While the evidence that pubertal sex steroids induce a sex-specific fat distribution with preferential abdominal/visceral fat accumulation in males and preferential gluteo-femoral fat accumulation in females is quite solid, later in life a number of paradoxes occur in the relationship between sex steroids and fat distribution.

Acquired adult onset hypogonadism in men is associated with an amount of visceral fat which is not less and mostly more than in a comparison group of eugonadal men [12]. So, apparently while androgens induce visceral fat accumulation, once fat has been stored in the visceral depot it does not need continued androgen stimulation as opposed to maintenance of bone and muscle mass, which are lower in men with adult onset hypogonadism than in eugonadal controls [13]. Induction of androgen deficiency by administration of an LHRH agonist leads to an increase of fat mass [14]. Androgen deprivation treatment of men with prostate cancer increases fat mass, reduces insulin sensitivity, and impairs lipid profiles increasing cardiovascular risk [15, 16] or worsens metabolic control of men with diabetes mellitus considerably [17]. Correlation studies in large groups of subjects have shown that visceral fat increases with ageing. There is an inverse correlation between the amount of visceral fat and plasma insulin on the one hand and levels of testosterone and SHBG on the other [18, 19]. Correlation studies cannot unravel the cause and effect relationships between the correlates whether low testosterone induces visceral fat deposition or whether a large visceral fat depot leads to low testosterone levels. Prospective studies have confirmed that lower endogenous androgens predict central adiposity in men [20] and that these low testosterone levels are significantly inversely associated with levels of blood pressure, fasting plasma glucose, triglycerides, and body mass index but positively correlated with HDL-cholesterol [21]. A five-year follow-up study of Swedish men indicated that elevated plasma cortisol and low testosterone were prospectively associated with increased incidence of cardiovascular-related events and diabetes mellitus type 2 [20]. The fact that androgen deprivation of men with prostate cancer induces a worsening of elements of the metabolic syndrome reveals a role for testosterone in its etiology [15, 22].

4. The Metabolic Syndrome

A closer examination of obesity has revealed that a preferential accumulation of fat in the abdominal region is associated with an increased risk of noninsulin-dependent diabetes mellitus and cardiovascular disease, not only in obese subjects but even in non-obese subjects [23]. A large number of cross-sectional studies have established a relationship between abdominal obesity and cardiovascular risk factors such as hypertension, dyslipidaemia (elevated levels of cholesterol, of triglycerides, of low-density lipoproteins and low levels of high-density lipoproteins), impaired glucose tolerance with hyperinsulinaemia, a cluster known as the "insulin resistance syndrome" or "metabolic syndrome" [24–27]. The term metabolic syndrome is now preferred. There is a debate in the literature whether combining these components or conditions has an added diagnostic or prognostic value [28]. In recent years, three main definitions of the metabolic syndrome were used. These definitions overlapped but differ in the points of emphasis of the components. The definition of the National Cholesterol Education program places equal emphasis on the various components of the metabolic syndrome

[29]. The definition adopted by the WHO assigns greater value to insulin resistance as a required component of the metabolic derangements [30]. Increasingly, professional organizations have now proposed definitions. The International Diabetes Federation has drafted a singly unifying definition in 2005. The main emphasis in this definition is central obesity defined by waist circumference: waist circumference in Europids ≥94 cm and in Asians >90 cm and two or more of the following four factors: elevated triglycerides ≥1.7 mmol/L (≥150 mg/dL), reduced HDL-cholesterol <1.03 mmol/L (<40 mg/dL), elevated blood pressure systolic ≥130 mm Hg, diastolic ≥85 mm Hg (or treatment), and dysglycaemia (raised fasting plasma glucose ≥5.6 mmol/L (≥100 mg/dL) (or type 2 diabetes) [31], (http://www.idf.org/webdata/docs/MetSyndrome_FINAL.pdf). Recently, several scientific societies have arrived at a joint interim statement to harmonize the approach to the metabolic syndrome [32].

5. Testosterone in Men Suffering from the Metabolic Syndrome and Diabetes Mellitus

Numerous studies have found inverse associations between the severity of features of the metabolic syndrome and plasma testosterone [33–38]. For review see [39]. There is an inverse relationship between waist circumference, a reliable indicator of visceral obesity, and testosterone levels over all age groups [40].

The inverse relationship of testosterone and the metabolic syndrome is consistent across race and ethnic groups [41]. Similar to studies in men with the metabolic syndrome, there is in men an inverse relationship between testosterone levels and diabetes. For review: [42]. Men with diabetes have lower testosterone levels compared to men without a history of diabetes [43, 44], and there is an inverse association between testosterone levels and glycosylated hemoglobin [45]. This is no artifact due to medication with, for instance, statins [44]. A systematic review and meta-analysis of cross-sectional studies indicated that testosterone level was significantly lower in men with type 2 diabetes (mean difference, −76.6 ng/dL; 95% confidence interval [CI], −99.4 to −53.6) [46]. In men with low plasma testosterone, the likelihood of diabetes mellitus is increased. Prospective studies have shown that men with higher testosterone levels (range 449.6–605.2 ng/dL) had a 42% lower risk of type 2 diabetes (RR, 0.58; 95% CI, 0.39 to 0.87) [46]. In addition, several large prospective studies have shown that low testosterone levels predict development of type 2 diabetes in men. There is persuasive epidemiological evidence form several longitudinal population studies that low testosterone is an independent risk factor for the development of both the metabolic syndrome and type 2 diabetes in later life [43, 47] and their clinical sequels such as stroke or transient ischemic attacks [48]. The Massachusetts Male Aging Study (MMAS) [49] and the Multiple Risk Factor Intervention Trial (MRFIT) [50] have shown that low levels of total testosterone and SHBG (which is associated with insulin resistance) were both independent risk factors in middle-aged men who later developed diabetes. The Rancho-Bernardo Study based in California demonstrated a significant inverse correlation between baseline total testosterone with long-term (8-year follow-up) fasting glucose and insulin levels as well as glucose intolerance [51]. A Finnish study has shown that low testosterone and SHBG levels also predict the development of the metabolic syndrome as well as diabetes [18], recently confirmed by a German group [52]. Importantly, the MMAS has provided evidence that low testosterone is a risk factor for metabolic syndrome and diabetes in men who were not initially obese [19]. Recently the Third National Health and Nutrition survey (NHANES III) in a population of 1,413 men after adjustment for age, race/ethnicity, and adiposity showed that those men initially in the lowest tertile of either free or bioavailable (but not total testosterone) were approximately four times more likely to have prevalent diabetes compared to those in the third tertile [53]. These findings support those of the MMAS in that the risk is independent of adiposity [49] recently confirmed in Australia [54]. For review see [47, 55].

Interestingly, there is a significant difference in plasma testosterone levels between men with diabetes type 1 (who have normal levels) and type 2 (who have subnormal levels) [56]. This difference was attributed to the differences in circulating levels of insulin (low in type 1 and high in type 2). There is an inverse relationship between insulin levels and sex hormone-binding globulin (SHBG) and, consequently, plasma levels of total testosterone are lower in men with type 2 diabetes. This assumption is confirmed by the observation that men with type 1 diabetes with a high BMI show lower levels of testosterone. Androgen receptor CAG repeat polymorphism appears associated with serum testosterone levels, obesity, and serum leptin in men with type 2 diabetes [57].

6. The Vicious Circle of Low Testosterone and the Metabolic Syndrome

Adiposity with its associated hyperinsulinism suppresses sex hormone-binding globulin (SHBG) synthesis and therewith the levels of circulating testosterone [58, 59]. It also may affect the strength of luteinizing hormone (LH) signaling to the testis [60]. Further, insulin [61] and leptin [62] have a suppressive effect on testicular steroidogenesis [45]. So, there are reasons to believe that adiposity is a significant factor in lowering circulating levels of testosterone, even occurring in men under the age of 40 years [63]. For review see [64, 65].

While it is clear that disease, and in the context of this contribution, in particular the metabolic syndrome suppresses circulating testosterone levels, it has also been documented that low testosterone induces the metabolic syndrome [18, 49]. Even in the absence of late-stage consequences such as diabetes and cardiovascular disease, subtle derangements in sex hormones are present in the metabolic syndrome and may contribute to its pathogenesis [66].

The role of testosterone is dramatically demonstrated by findings in men with prostate cancer who undergo

androgen ablation therapy [22, 67], particularly in the longer-term [68]. Another study showed convincingly that acute androgen deprivation reduces insulin sensitivity in young men [69] and strongly impairs glycemic control of men with diabetes mellitus [70].

7. Can the Age-Related Decline of Testosterone Be Prevented or Reversed?

As indicated above the age-related changes in neuroendocrine functioning leading to a diminished efficacy of LH stimulation of the Leydig cell and impairments of the steroidogenic process of testosterone synthesis are probably inherent factors in the age-related decline of circulating testosterone levels [58, 71]. But increasingly there is insight that disease significantly contributes to the age-related decline of testosterone [72]. Changes in lifestyle (diet/exercise) might partially prevent or redress the decline of androgen levels with aging [73–75] and should be encouraged.

8. Effects of Testosterone Administration on Fat Tissue and Lipid Metabolism

Sex steroid hormones are involved in the metabolism, accumulation, and distribution of adipose tissues. It is now known that estrogen receptors, progesterone receptors, and androgen receptors exist in adipose tissues, so their actions could be direct. Sex steroid hormones carry out their function in adipose tissues by both genomic and nongenomic mechanisms. Activation of the cAMP cascade by sex steroid hormones would activate hormone-sensitive lipase leading to lipolysis in adipose tissues. In the phosphoinositide cascade, diacylglycerol and inositol 1,4,5-trisphosphate are formed as second messengers ultimately causing the activation of protein kinase C [76]. Their activation appears to be involved in the control of preadipocyte proliferation and differentiation. The role of testosterone in regulating lineage determination in mesenchymal pluripotent cells by promoting their commitment to the myogenic lineage and inhibiting their differentiation into the adipogenic lineage through an androgen receptor-mediated pathway has been convincingly demonstrated [77]. In a clinical study, it could be shown that testosterone inhibits triglyceride uptake and lipoprotein lipase activity and causes a more rapid turnover of triglycerides in the subcutaneous abdominal adipose tissue and less so in femoral fat and, maybe, mobilizes lipids from the visceral fat depot. In this study, testosterone administration restoring testosterone levels to midnormal values with a duration of 8-9 months led to a decrease of the visceral fat mass, a decrease of fasting glucose and lipid levels and an improvement of insulin sensitivity; in addition, a decrease in diastolic blood pressure was observed [76].

A meta-analysis of randomized controlled trials evaluating the effects of testosterone (T) administration to middle-aged and ageing men on body composition showed a reduction of 1.6 kg (CI: 2.5–0.6) of total body fat, corresponding to −6.2% (CI: 9.2–3.3) variation of initial body fat, an increase in fat-free mass of 1.6 kg (CI: 0.6–2.6), corresponding to +2.7% (CI: 1.1–4.4) increase over baseline and no change in body weight. In a placebo-controlled study using long-acting testosterone indecanoate injections, the reduction of fat mass was 5.3 kg with an increase of lean mass of 4.2 kg [78]. Testosterone also reduced total cholesterol by 0.23 mmol/L (CI: −0.37 to −0.10), especially in men with lower baseline T concentrations, with no change in low density lipoprotein (LDL-) cholesterol. A significant reduction of high density lipoprotein (HDL-) cholesterol was found only in studies with higher mean T-values at baseline or when androgens were nonaromatizable (−0.085 mmol/l, CI: −0.017 to −0.003) [79]. This underlines the necessity to use the chemically unmodified molecule of testosterone for treatment.

9. Testosterone Administration to Men with the Metabolic Syndrome and Diabetes Mellitus

So, it is clear now that low testosterone levels are a factor in the etiology of common ailments of elderly men such as the metabolic syndrome and its associated diseases such as diabetes mellitus and atherosclerotic disease. The question arises then whether testosterone treatment has a role to play in the treatment of the metabolic syndrome and its sequels such as diabetes mellitus type 2 and cardiovascular disease. There is increasing evidence of a beneficial effect of testosterone treatment on visceral fat and other elements of the metabolic syndrome [80]. Changes in visceral fat appeared to be a function of changes in serum total testosterone [81]. The beneficial effects of androgens on (visceral) fat have been confirmed in other studies [82, 83]. A study investigating the effects of normalization of circulating testosterone levels in men with subnormal testosterone levels receiving treatment with parenteral testosterone undecanoate found favorable effects on body composition (waist circumference) [84]. In another study, 32 hypogonadal (plasma testosterone <12 nmol/L) men with the metabolic syndrome, with newly diagnosed type 2 diabetes mellitus were single-blindly randomized to diet and exercise alone ($n = 16$) or to diet and exercise in combination with testosterone gel 50 mg once daily ($n = 16$) and treated for 52 weeks. No glucose-lowering agents were administered prior to or during the study period. Addition of testosterone significantly further improved these measures compared to diet and exercise alone on glycaemic control, waist circumference, and other parameters of the metabolic syndrome [75].

Testosterone substitution in hypogonadal men improves insulin sensitivity [85]. Furthermore, testosterone reduces insulin levels and insulin resistance in men with obesity. A study in hypogonadal men with type 2 diabetes has shown that testosterone replacement also improves glycaemic control although this study was nonblinded [86]. In a recent Korean study, glucose levels were significantly reduced after 24 weeks of testosterone treatment in men with baseline glucose ≥110 mg/dL while there was no change in men with baseline glucose <110 mg/dL [87]. By contrast, two studies replacing testosterone in men with diabetes type 2

and hypogonadism found little or no effect on glycaemic control [88, 89], but a more recent study analyzing the effects of testosterone administration to 24 hypogonadal men (10 treated with insulin) over the age of 30 years with type 2 diabetes found that testosterone replacement therapy reduced insulin resistance (as measured by homeostatic model index) and improved glycaemic control in hypogonadal men with type 2 diabetes. So, while the evidence for powerful effects of normalization of circulating levels of testosterone on glucose homeostasis so far is limited, there are studies to prove that administration of testosterone may have favorable effects on glycaemic control and the metabolic sequels of diabetes mellitus.

10. New Perspectives on Testosterone

In recent times, the understanding and thinking about the (patho)physiological functions of testosterone have undergone a revolutionary development. The traditional assumption was that hypogonadism in men usually resulted in loss of libido and potency which could be restored by androgen administration. While the significance of testosterone for male reproductive/sexual functioning has been obvious to most physicians, they now need to familiarize themselves with the insight that testosterone is a key player in glucose homeostasis and lipid metabolism.

Physicians will have to make a change of their mindset that testosterone, rather being a dangerous companion to a man's life, bringing joy but exacting its toll, is a vital hormone for men's health, from early prenatal development to the end of a man's life. Earlier it has been questioned whether testosterone has an essential role to play in male physiology. Recent epidemiological studies have found that low testosterone levels are a predictor of mortality in elderly men [90–94]. Obviously, epidemiological studies cannot unravel cause relationships, but the evidence is convincing that the decline in testosterone levels with aging is accounted for rather by (age-related) disease than the calendar age of men [95, 96]. Intervention studies provide potential answers to the causality of the relationship. It is no exaggeration to say that in modern medicine and endocrinology testosterone is no longer a marginal hormone. Neither is it a lifestyle hormone for those men seeking eternal youth. Its deficiency leads to a serious deterioration of the health of men expressing itself in the metabolic syndrome and its sequels: diabetes mellitus type 2 and atherosclerotic disease, osteoporosis and sarcopenia, all strongly limiting physical independence in old age and accelerating morbidity and mortality.

11. Measuring Serum Testosterone

There is no generally accepted cutoff value of plasma testosterone for defining androgen deficiency, and in the absence of convincing evidence for an altered androgen requirement in elderly men, Arbitrarily, the normal range of testosterone and free testosterone (fT) levels in young males is considered also valid for elderly men. Then the normal levels of total testosterone are between 12 and 35 nmol/L. It is of note that the determination of reference values of laboratory parameters is based on statistical analysis of a population of subjects [97]. For the clinician workable criteria are the following: the lower limit of normal of total testosterone is 12 nmol/L and of fT 250 pmol/L. Most authors agree that plasma testosterone levels should be measured in early morning samples in view of the circadian rhythm of plasma testosterone, with shows its lowest values in the late afternoon. (Late) afternoon samples might present unduly low values and not be representative of a man's androgen status [58, 98]. The recommendation also agrees that a single measurement providing a low testosterone value is to be repeated, certain when that value would be enough reason to start testosterone administration. Small ailments, like a cold or other minor stressors, may temporarily suppress circulating testosterone.

It will not be rare to find rather ambiguous, borderline normal/abnormal levels of plasma total testosterone in elderly men, even in those men with clinical symptoms of androgen deficiency. In these cases, assessment of bioavailable or free testosterone might be an asset. Bioavailable testosterone can be measured in the laboratory using the ammoniumsulphate precipitation technique. The gold standard for free testosterone measurement still is the dialysis method, although a mass spectrometry-based assessment of free testosterone in ultrafiltrates was recently proposed as a candidate reference method (for review: [58]). However, both ammoniumsulphate precipitation and the dialysis technique are nonautomated, time-consuming, and expensive techniques and therefore not routinely available in the vast majority of laboratories. There has been a direct radioimmunoassay claiming to measure free testosterone but this assay has been universally criticized because of lack of accuracy and should not be used [58]

The two most widely used equations for calculating bioavailable or free testosterone are those described by Vermeulen et al. [99] and Sodergard et al. [100]. The equations are largely identical apart from the association constants for the binding of testosterone to albumin and sex hormone-binding globulin.

At least two algorithms have been placed on the internet as so-called bioavailable testosterone calculators (http://www.issam.ch/ and http://www.get-back-on-track.com/en/tools/kalkulator.php and http://www.him-link/.com/) making these algorithms readily available for distant users. It is redundant to measure/calculate biovailable/free testosterone if plasma total testosterone appeared to be in the truly hypogonadal (<6 nmol/L)or in the truly eugonadal range (>15 nmol/L).

12. Safety Concerns

Traditionally, the majority of physicians associate the administration of testosterone, particularly to elderly men, with a serious risk of inducing malignancies of the prostate or exacerbating voiding problems of the elderly male.

Several follow-up studies of men receiving testosterone treatment [101–103] have failed to demonstrate an exacerbation of voiding symptoms due to benign prostatic

hyperplasia. Complications such as urinary retention in therapy groups did not occur at higher rates than in controls receiving placebo.

The occurrence of prostate cancer after testosterone administration to (elderly) men has been reported [104–108] but its causality has not been established. Aging, typically, increases the risk of developing prostate cancer. By contrast, a variety of studies, using various designs and testosterone formulations, over periods ranging from several months to 15 years, in men with a wide range of ages, have not revealed an increased risk of prostate cancer [109–116] see for review [117, 118]. A meta-analysis found that testosterone treatment in older men compared to placebo was not associated with a significantly higher risk of detection of prostate cancer, although the frequency of prostate biopsies was much higher in the testosterone-treated group than in the placebo group [103]. Presently, it is believed that testosterone can be administered to men whose prostate cancer has been radically cured [119].

The above applies also to the assessment of safety of testosterone administration to elderly men. There is no convincing evidence that testosterone is a main factor in the development of prostate cancer in elderly men [120], and guidelines for monitoring have been developed which, if rigorously applied, render testosterone administration to elderly men, an acceptably safe therapy in men without a prior history of prostate carcinoma or without evidence of harboring a prostate carcinoma [121, 122]. There are now at least three publications demonstrating a lack of prostate carcinoma recurrence with testosterone therapy after definitive prostate carcinoma treatment. Two articles have reported no PSA recurrence in a total of 17 men, following radical prostatectomy in men with undetectable PSA [123, 124]. A third study reported that no cancer recurrence was noted in 31 hypogonadal men treated with brachytherapy with a follow-up of approximately 5 years [125]. These small studies suggest that normalization of testosterone in men who have shown no signs of recurrence of prostate cancer after treatment, testosterone replacement could be beneficial.

There is a consensus now that administration of testosterone to elderly men is a responsible practice provided certain guidelines of professional bodies are followed with regard to testosterone administration to elderly men [121, 126].

13. Conclusion

In recent times, the understanding and thinking about the (patho)physiological functions of testosterone have undergone a revolutionary development. While the significance of testosterone for male reproductive/sexual functioning has always been obvious to physicians, they now need to familiarize themselves with the insight that testosterone is a key player in glucose homeostasis, lipid metabolism, and cardiovascular pathology. Physicians will have to change their mind-set and accept that testosterone is a vital hormone for many aspects of men's health. The long-held belief that testosterone has adverse effects on cardiovascular disease explaining the male preponderance in cardiovascular morbidity and mortality appears not to be supported by rigorous scientific testing. Recent epidemiological studies have found that low testosterone levels are a predictor of mortality in elderly men [90–92, 94].

Obviously, epidemiological studies cannot unravel cause relationships. Intervention studies provide potential answers to the causality of the relationship. It is no exaggeration to say that in modern medicine and endocrinology testosterone is no longer a marginal hormone. Neither is it a life-style hormone for those men seeking eternal youth. Its deficiency leads to a serious deterioration of the health of men expressing itself in the metabolic syndrome and its sequels: diabetes mellitus type 2 and atherosclerotic disease accelerating morbidity and mortality. Intervention studies in men with diabetes mellitus are limited in number but hold promise. Normalization of testosterone levels may improve insulin sensitivity and have favorable effects on visceral adiposity and lipid profiles. The fear that testosterone administration to elderly men increases the risk of prostate malignancies is not justified. It only requires prudence in clinical management.

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Review Article

An Evidence-Based Review of Fat Modifying Supplemental Weight Loss Products

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Objective. To review the literature on fat modifying dietary supplements commonly used for weight loss. *Methods*. Recently published randomized, placebo-controlled trials were identified in PubMed, MEDLINE, *International Pharmaceutical Abstracts*, Cochrane Database, and Google Scholar using the search terms dietary supplement, herbal, weight loss, obesity, and individual supplement names. *Discussion*. Data for conjugated linoleic acid (CLA), *Garcinia cambogia*, chitosan, pyruvate, *Irvingia gabonensis*, and chia seed for weight loss were identified. CLA, chitosan, pyruvate, and *Irvingia gabonensis* appeared to be effective in weight loss via fat modifying mechanisms. However, the data on the use of these products is limited. *Conclusion*. Many obese people use dietary supplements for weight loss. To date, there is little clinical evidence to support their use. More data is necessary to determine the efficacy and safety of these supplements. Healthcare providers should assist patients in weighing the risks and benefits of dietary supplement use for weight loss.

1. Introduction

The prevalence of obesity has continued to increase over the last several years in the United States. Per the National Health and Nutrition Examination Survey (NHANES) for the 2007-2008 year, the prevalence of obesity, defined as a body mass index (BMI) $\geq 30\,kg/m^2$, among adults was greater than 30% and those who were overweight or obese (BMI $\geq 25\,kg/m^2$) was almost 70% for both men and women. The trend over the past 20 years has shown an increase in the prevalence of obesity of six to seven percent every 10 years [1]. In addition, health care costs are approximately 42% higher for obese patients when compared to normal-weight patients [2].

Dietary supplements for weight loss are marketed to offer patients improved success that is faster and easier than calorie reduction and increased exercise. Despite concerns with efficacy and safety, these products continue to be an appealing alternative or adjunct to weight management [3, 4]. A national survey published in 2008 found that 33.9% of adults who have made a weight loss attempt had used a dietary supplement to do so. It was also found that the use was more common among women, younger adults, minorities, and those with less education and lower incomes [5]. Reasons why patients may opt for dietary supplements include the perception that they are "natural" and perhaps safer than prescription medications. In addition, patients often do not perceive a need to seek the assistance of a healthcare professional with these alternative therapies, and they also may be an alternative to previously failed attempts with conventional approaches [6].

Despite widespread use, there is still limited data on the safety and efficacy of the products currently on the market. Because dietary supplements are viewed as food and not drugs, they are not regulated by the Food and Drug Administration (FDA). Instead, under the Dietary Supplement Health and Education Act (DSHEA), dietary supplements can be marketed without evidence to support efficacy and safety. If a dietary supplement appears to be

unsafe after being marketed, the FDA can then decide whether or not to have the product removed from the market. This was the case for the weight loss supplement ephedra which was removed from the market in 2004 after reports of serious health risks [5]. The literature published in the arena of weight loss continues to be plagued by concerns such as: small studies, inconsistency with participant body weight (BMI), variation in length of studies, use of exercise, and a variety of products at differing dosages.

Several mechanisms are proposed to differentiate how these products work. These include products that claim to be: fat blockers, lipotropics or fat busters, thermogenic or energy modifiers, and products that can change carbohydrate metabolism, water elimination, or the feeling of satiety or fullness. The purpose of this paper is to review the literature on dietary supplements currently being marketed and promoted for weight loss via the mechanisms of altered fat absorption, fat metabolism and/or the storage of fat.

2. Methods

Published articles and abstracts were identified using PubMed, MEDLINE, *International Pharmaceutical Abstracts,* Cochrane Database, and Google Scholar with the search terms dietary supplement, herbal, weight loss, obesity, and by using individual supplement names. The primary emphasis was on pertinent articles based on human trials involving overweight subjects that were performed in a randomized and placebo-controlled process. Additional articles were identified from the references of the retrieved literature. Only studies that tested single dietary supplement products were included in this paper. Studies which included multiple products were not included due to inconclusive evidence of the effectiveness of individual products.

2.1. Fat Modifying Supplemental Weight Loss Products. Table 1 summarizes the data for the following fat modifying supplemental weight loss products.

2.1.1. Conjugated Linoleic Acid. Conjugated linoleic acid (CLA) is a naturally occurring fatty acid that is found in beef and dairy products [7]. Studies conducted in animals have shown that CLA is effective in reducing body fat mass, increasing insulin sensitivity, decreasing plasma glucose levels, is anticarcinogenic, and may have positive effects on atherosclerosis [8]. Conjugated linoleic acid has been indicated for the use in cancer, diabetes, hypertension, and hypercholesterolemia, as well as weight loss and body fat reduction. In regards to weight loss, CLA is believed to work by promoting apoptosis in adipose tissue. Many animal studies have shown CLA to be effective in weight loss and body fat reduction. This information has led to an increased interest as to whether or not CLA would have the same effects in humans [7].

Blankson et al. [9] performed a randomized, double-blind, placebo-controlled trial in which 52 patients (35 women and 17 men) with a BMI between 25 to 35 kg/m^2 were randomized to receive CLA 1.7, 3.4, 5.1, or 6.8 grams per day or placebo (9 grams of olive oil) for 12 weeks. At the end of 12 weeks, the results demonstrated a decrease in body fat mass (BFM) of 1.73 kg in the group receiving CLA 3.4 grams ($P \leq .05$) and a decrease of 1.3 kg in the group receiving 6.8 grams ($P \leq .05$). There were no statistical differences seen in body mass, BMI, or lean body mass (LBM). The most common adverse effects reported were gastrointestinal effects [9].

In a double-blind, placebo-controlled study performed by Risérus et al. [10], 24 men with an average baseline BMI of 32 kg/m^2 were randomized to receive either CLA 4.2 grams per day or placebo for 4 weeks. Along with other weight related endpoints, this study also evaluated the effects of CLA on sagittal abdominal diameter (SAD) as abdominal obesity has been linked with the metabolic syndrome. At the end of the study period, it was found that there was a significant decrease in SAD (−0.57 cm, $P = .04$). However, there was no difference seen in body weight or BMI. There were no adverse events reported [10].

A randomized, double-blind, placebo-controlled study by Smedman and Vessby [11] evaluated the effects of CLA 4.2 grams per day versus placebo for 12 weeks on percent body fat, body weight, BMI, and SAD in 50 patients (25 men and 25 women) with an average BMI of 25 kg/m^2. The results showed a 3.8% decrease in body fat in those receiving CLA ($P = .05$). There was no change seen in body weight, BMI, and SAD. CLA was well tolerated by all participants [11].

CLA is marketed as either a triacylglycerol or free fatty acid (FFA). Gaullier et al. [8] decided to not only evaluate the effectiveness of CLA on weight loss, but to see if either CLA-triacylglycerol or CLA-FFA is more efficacious than the other. This was a double-blind, placebo-controlled trial in which 180 patients (31 men and 149 women) with an average BMI of 28 kg/m^2 were randomized to receive 4.5 grams of olive oil (placebo), 4.5 grams 80% CLA-FFA, or 4.5 grams 76% CLA-triacylglycerol for 12 months. The results demonstrated a significant decrease in BFM in both the CLA-FFA and CLA-triacylglycerol groups compared to placebo (−1.7 and −2.4, resp.; $P < .05$). In the CLA-triacylglycerol group, there was also a significant decrease in body weight (−1.8 kg versus 0.2 kg; $P < .05$) and BMI (−0.6 kg/m^2 versus 1.8 kg/m^2; $P < .05$) when compared with placebo. The CLA-FFA group demonstrated an increase in LBM (2.0 kg versus 0; $P < .05$). All adverse events reported were rated as "mild" or "moderate" with the most common being gastrointestinal side effects [8].

Of the 180 participants of this study, 134 continued on in an open-label study for another 12 months. All participants remained in their original treatment arm, but all were treated with 4.5 grams daily of CLA-triacylglycerol for the remaining 12 months. While there was no additional decrease in body weight or BFM, this study did demonstrate that participants were able to maintain their weight loss. While two patients were observed to have an increase in aspartate amino transferase (ASAT), these levels returned to normal once CLA was discontinued ($P = .002$, CLA-FFA; $P = .009$, CLA-triacylglycerol). Overall, this study demonstrated that CLA use is safe over 24 months and may be beneficial in initial weight loss and may help with

Table 1: Summary data of fat modifying supplemental weight loss products.

Dietary Supplement	Trial	Average BMI (kg/m^2)	Treatment	Length of Treatment	Results
Conjugated linoleic acid	Blankson et al. [9]	25–35	CLA 1.7, 3.4, 5.1, or 6.8 grams/day or placebo	12 weeks	↓ BFM with CLA 3.4 grams (−1.73 kg, $P = .05$) and 6.8 grams (−1.3 kg, $P = .02$). No differences in body weight or BMI
	Risérus et al. [10]	32	CLA 4.2 grams/day or placebo	4 weeks	↓ SAD in CLA group (−0.57 cm, $P = .04$). No difference in body weight or BMI
	Smedman and Vessby [11]	25	CLA 4.2 grams/day or placebo	12 weeks	↓ % body fat in CLA group (−3.8%, $P = .05$). No change in body weight or BMI
	Gaullier et al. [8]	28	4.5 grams/day of CLA-FFA, 4.5 grams/day of CLA-triacylglycerol, or placebo	12 months	↓ BFM with CLA-triacylglycerol and CLA-FFA groups (−1.7 and −2.4 kg, resp.; $P < .05$) ↓ Body weight with CLA-triacylglycerol (−1.8 kg, $P < .05$) ↓ BMI with CLA-triacylglycerol (−0.6 kg/m^2, $P < .05$) ↑ LBM with CLA-FFA (2.0 kg, $P < .05$)
	Gaullier et al. [12]	28	4.5 grams/day of CLA-FFA, 4.5 grams/day of CLA-triacylglycerol, or placebo	24 months	No additional ↓ body weight or BFM
Garcinia cambogia (Hydroxycitric acid)	Heymsfield et al. [13]	32	*Garcinia cambogia* 3000 mg/day (1500 mg of HCA) or placebo	12 weeks	Both groups demonstrated weight loss and decrease in percent body fat; there was no difference between the groups
	Mattes and Bormann [14]	NR	*Garcinia cambogia* 2.4 g/day (1.2 g of HCA) or placebo		↓ 3.7 kg with *Garcinia cambogia* versus ↓ 2.4 kg with placebo
Chitosan	Pittler et al. [15]	26	Chitosan 1 gram, or placebo twice daily	28 days	No difference between groups in regards to body weight or BMI
	Schiller et al. [16]	32	Chitosan 1500 mg or placebo twice daily	8 weeks	↓ Body weight of 1 kg with chitosan ($P < .005$) ↓ BMI of 0.3 kg/m^2 with chitosan ($P < .01$) ↑Body weight of 1.5 kg with placebo ($P < .001$) ↑ BMI of 0.6 kg/m^2 with placebo ($P < .01$)
	Ni Mhurchu et al. [17]	35–36	Chitosan 3 grams/day or placebo	24 weeks	Chitosan group lost 0.39 kg versus a weight gain of 0.17 kg in the placebo group ($P = .03$)
	Kaats et al. [18]	NR	Chitosan 3 grams/day with behavior modification; placebo with behavior modification; or minimal intervention control	60 days	↓ Body weight of 2.8 lbs. with chitosan versus placebo ($P = .03$) ↓ Percent body fat of 0.8% with chitosan versus placebo ($P = .003$) ↓ Fat mass of 2.6 lbs. with chitosan versus placebo ($P = .001$) ↑ BCI of 2.4 lbs. with chitosan versus placebo ($P = .002$)

Table 1: Continued.

Dietary Supplement	Trial	Average BMI (kg/m^2)	Treatment	Length of Treatment	Results
Pyruvate	Stanko et al. [19]	27.8–52.7	Pyruvate 30 grams/day and calcium pyruvate 16 grams/day, or placebo	21 days	↓ 0.22 kg with pyruvate versus ↓ 0.17 kg with placebo ($P < .05$) ↓ BMI 2.2 kg/m^2 with pyruvate versus ↓ 1.5 kg/m^2 with placebo ($P < .05$) ↓ Fat 7.3% with pyruvate versus ↓ 5.4% with placebo ($P < .05$)
	Kalman et al. [20]	>25	Pyruvate 6 grams/day, placebo or nothing (control)	6 weeks	↑ LBM 2.4% with pyruvate ($P = .001$) ↓ Fat mass 12.2% with pyruvate ($P < .001$), and ↓ Body fat 12.4% with pyruvate ($P < .001$)
	Kalman et al. [21]	>25	Pyruvate 6 grams/day, or placebo	6 weeks	↓ Body weight 1.6% with pyruvate ($P < .001$) ↓ Body fat 14% with pyruvate ($P < .001$) ↓ % body fat 11.7% with pyruvate ($P < .001$)
Irvingia gabonensis	Ngondi et al. [22]	NR	*Irvingia gabonensis* 350 mg/day, or placebo	4 weeks	↓ Body weight 5.6% with *Irvingia gabonensis* ($P < .0001$) ↓ Waist circumference 5.07% with *Irvingia gabonensis* ($P < .0001$) ↓ Hip circumference 3.42% with *Irvingia gabonensis* ($P < .0001$)
	Ngondi et al. [23]	26–40	*Irvingia gabonensis* 150 mg/day, or placebo	10 weeks	↓ Body weight 12.8 kg with *Irvingia gabonensis* ($P < .01$) ↓ Waist circumference 16.19 cm with *Irvingia gabonensis* ($P < .01$) ↓ % body fat 6.3% with *Irvingia gabonensis* ($P < .05$)
Chia seeds (*Salvia hispanica*)	Nieman et al. [24]	NR	Chia seeds 50 g/day, or placebo	12 weeks	No differences in body mass or body composition

BCI: Body Composition Improvement, BFM: Body Fat Mass, BMI: Body Mass Index, CLA: Conjugated Linoleic Acid, FFA: Free Fatty Acid, HCA: Hydroxycitric Acid, LBM: Lean Body Mass, NR: Not Reported, SAD: Sagittal Abdominal Diameter.

maintaining weight loss and reductions in BFM. The most commons adverse events reported were gastrointestinal [12].

Studies reviewed indicate that CLA appears to be safe with the most common adverse effects being gastrointestinal (GI). Overall, it appears as though that CLA helps to reduce BFM and SAD in patients, but minimal effect on BMI or body weight. In addition, CLA may be beneficial in helping to maintain changes in body composition such as reductions in BFM.

2.1.2. Garcinia cambogia (Hydroxycitric Acid). Hydroxycitric acid (HCA) is the active ingredient found in the fruit of the *Garcinia cambogia* plant [25]. It is believed that hydroxycitric acid aids in weight loss by inhibiting lipogenesis by inhibiting the adenosine triphosphate (ATP)-citrate-lyase enzyme which is responsible for converting citrate to acetyl-coenzyme A and ultimately fatty acid synthesis. It is also theorized that HCA may improve exercise endurance by increasing lipid oxidation and decreasing carbohydrate metabolism and stimulate appetite suppression [13, 25].

Heymsfield et al. [13] performed a randomized, double-blind, placebo-controlled trial to measure the effects of HCA on body weight change and fat mass. The 135 participants (19 men and 116 women) with an average BMI of 32 kg/m^2 were randomized to receive 3000 mg of *Garcinia cambogia* (1500 mg of HCA) per day or placebo along with a high fiber, low-calorie diet for 12 weeks. The placebo group lost 4.1 kg, and the HCA group lost 3.2 kg. While the results within each separate treatment arm was significant when compared to baseline, there were no differences between the groups ($P = .14$). In addition, the placebo group demonstrated a decrease in percent body fat mass of 2.16%, and the HCA group demonstrated a 1.44% decrease. Again, there were no differences between the groups ($P = .08$). The most commonly reported adverse events were headache, upper respiratory tract symptoms, and gastrointestinal symptoms.

However, there were no differences between the HCA group and placebo [13].

A double-blind, placebo-controlled parallel group study performed by Mattes and Bormann [14] enrolled 89 mildly overweight females to evaluate HCA on weight loss and appetite suppression. The participants were randomized to receive 2.4 grams of *Garcinia cambogia* (1.2 grams of HCA) or placebo per day, in addition to a low calorie diet. At the end of 12 weeks, it was noted that the HCA group lost significantly more weight than the placebo group (3.7 ± 3.1 kg versus 2.4 ± 2.9 kg). There were no changes on appetitive variables [14, 26].

While HCA appears to be well tolerated, there is limited data with regards to its efficacy. The data that is available, however, does not demonstrate significant weight loss. Therefore, *Garcinia cambogia* or HCA is not recommended at this time.

2.1.3. Chitosan. Chitosan is a form of chitin that comes from the shells of crustaceans such as shrimp, lobster, and crab. Several *in vitro* studies have shown that chitosan binds dietary fats and bile acids. Because of this proposed mechanism of action, it is theorized that chitosan may be useful for weight control, as well as for a treatment of hypercholesterolemia [15–18, 27, 28].

A randomized, double-blind placebo-controlled trial performed by Pittler et al. [15] examined the effects of chitosan on weight loss. Thirty-four patients (6 men and 28 women) with a BMI of approximately 26 kg/m^2 were randomized to receive one gram chitosan or placebo twice daily for 28 days. At the end of the study period, there was no difference in body weight or BMI between the two groups. Adverse effects reported with chitosan were minor. The most common complaint was constipation [15].

Schiller et al. [16] evaluated the use of rapidly soluble chitosan in weight loss and reducing body fat in 59 participants with an average BMI of 32 kg/m^2 consuming a high fat diet. In this double-blind, placebo-controlled trial, the participants were randomized to receive 1500 mg of chitosan or placebo twice a day with the largest meals of the day for eight weeks. At the end of the study period, patients in the chitosan group lost 1 kg ($P < .005$), and the BMI was significantly decreased by 0.3 kg/m^2 ($P < .01$). Patients in the placebo group gained 1.5 kg ($P < .001$), and the BMI was significantly higher by 0.6 kg/m^2 ($P < .01$). When treatment was compared to placebo, weight and BMI were significantly higher in the placebo group ($P < .0001$ and $P < .05$, resp.). The most common adverse effects reported were gastrointestinal, flatulence, increased stool bulkiness, bloating, nausea, and heartburn. This study demonstrated that chitosan may be an effective weight loss supplement [16].

Ni Mhurchu et al. [17] evaluated the effects of chitosan on 250 patients (44 men and 206 women) with a BMI 35 to 36 kg/m^2. This double-blind, placebo-controlled trial randomized patients to receive three grams of chitosan per day or placebo in addition to receiving standardized dietary and lifestyle advice. The trial was conducted over 24 weeks. At the end of the study period, the chitosan group lost more weight than placebo (-0.39 versus $+0.17$ kg, $P = .03$). There were ten serious adverse events, four of which occurred in the chitosan group. These included three hospitalizations and one cancer incidence. Thirty-six participants in the chitosan group reported some minor adverse events which were primarily gastrointestinal related [17].

In a randomized, double-blind, placebo-controlled trial by Kaats et al. [18], 150 overweight adults were randomized to three study groups: three grams of chitosan per day and a behavior modification program, placebo and a behavior modification program, or a minimum intervention control group. The trial was conducted over 60 days. At the end of the study period, participants in the chitosan group demonstrated a significant reduction in weight compared to control (-2.8 versus $+0.8$ pounds, $P < .001$) and a decrease in fat mass compared to control ($P = .006$). When compared to placebo, the chitosan group demonstrated a decrease in weight (-2.8 versus -0.6 pounds, $P = .03$), a decrease in percent fat ($-.08\%$ versus $+0.4\%$, $P = .003$), a decrease in fat mass (-2.6 versus $+0.6$ pounds, $P = .001$), and an increase in body composition improvement (BCI) ($+2.4$ versus -1.9 pounds, $P = .002$) [18].

Chitosan is well tolerated with the most common adverse effects being gastrointestinal. Based on the above studies, it appears as though chitosan may be effective to help aid weight loss. Because of limited data thus far, chitosan cannot be recommended at this time. Chitosan, however should be avoided in individuals with a shellfish allergy [27].

2.1.4. Pyruvate. Pyruvate is a three-carbon compound that is a byproduct of glucose metabolism. It is unclear how pyruvate works to promote weight loss, but in rats a lower respiratory exchange ratio has been demonstrated indicating that there was increased utilization of fat and an elevation in resting metabolic rate [21, 29].

Stanko et al. and Kalman et al. both performed several studies evaluating the use of pyruvate in weight loss [19–21]. The study performed by Stanko et al. [19] was a double-blind, placebo-controlled trial which evaluated body composition with a low energy diet and supplementation with pyruvate. Fourteen women with a BMI 27.8 to 52.7 kg/m^2 were placed on a low energy diet and then randomly assigned to receive either 30 grams of pyruvate plus 16 grams of calcium pyruvate per day or placebo for 21 days. The pyruvate group lost 0.22 kg compared to 0.17 kg in the placebo group ($P < .05$), and there was also a decrease in BMI of 2.2 kg/m^2 compared to 1.5 kg/m^2 in the placebo group ($P < .05$). In addition, the pyruvate group lost 7.3% fat versus 5.4% in the placebo group ($P < .05$) [19].

In the first study by Kalman et al. [20] a randomized, double-masked, placebo-controlled trial was performed in which participants received six grams/day of pyruvate, placebo, or nothing (control group) along with diet and exercise counseling. The 51 participants (25 men, 26 women) enrolled had a BMI greater than 25 kg/m^2. At the end of six weeks, fat mass decreased significantly (-12.2%; $P < .001$), percent body fat decreased significantly (-12.4%; $P < .001$), and lean body mass increased ($+2.4\%$; $P = .001$) in the pyruvate group when compared to baseline. The placebo and

control groups did not demonstrate any significant changes in fat mass, percent body mass, and lean body mass [20].

Kalman et al. [21] performed another six week, double-blind, placebo-controlled trial. Twenty-six subjects (10 men, 16 women) with a BMI greater than 25 kg/m^2 were randomly assigned to receive six grams of pyruvate per day or placebo. At the end of the trial, there was a 1.6% decrease in body weight ($P < .001$), a 14% decrease in body fat ($P < .001$), and an 11.7% decrease in percent body fat ($P < .001$) in the pyruvate group. There were no significant changes in the placebo group [21].

In studies published thus far, pyruvate has demonstrated that it may be beneficial for weight loss. In addition, it tends to be well tolerated with minimal adverse effects. The most common adverse effect is gastrointestinal upset [29]. However, the trials conducted so far have had small sample sizes and have only been performed for short periods of time. Although it appears to be safe, there is no data on the long term use of pyruvate.

2.1.5. Irvingia Gabonensis. *Irvingia gabonensis* is a mango-like fruit that comes from the deciduous forest tree found in West Africa [22, 30]. It is theorized that *Irvingia gabonensis* works by inhibiting adipogenesis by down-regulating peroxisome proliferator-activated receptor gamma (PPAR-gamma) which is responsible for the differentiation of adipocytes. In addition, it also observed that adiponectin levels increase and leptin levels decrease in patients given *Irvingia gabonensis*. *Irvingia gabonensis* has also been used to treat hypercholesterolemia [30].

The first trial was performed by Ngondi et al. in 2005. This was a randomized, double-blind, placebo-controlled trial that enrolled 40 obese subjects in Cameroon. Subjects were randomly assigned to receive 350 mg of *Irvingia gabonensis* seed extract or placebo for 4 weeks. At the end of the 4 weeks, the *Irvingia gabonensis* group was observed to have a decrease in body weight of 5.6 ± 2.7% ($P < .0001$), a decrease in waist circumference of 5.07 ± 3.18% ($P < .0001$), and a decrease in hip circumference of 3.42 ± 2.12% ($P < .0001$). The placebo group observed a decrease in body weight of 1.32 ± 0.41%. There was no change in percent body fat for both the treatment and placebo groups [22].

A second article by Ngondi et al. [23] also examined the effects of *Irvingia gabonensis* on weight loss. This was a double-blind, placebo-controlled trial in which 102 natives of Cameroon with a BMI of 26 to 40 kg/m^2 were randomized to receive 150 mg of *Irvingia gabonensis* or placebo daily for ten weeks. At the end of the study period, there was a significant difference between the treatment group and placebo for body weight (−12.8 kg versus −0.7 kg; $P < .01$), waist circumference (−16.19 cm versus −5.3 cm; $P < .01$), and percent body fat (−6.3% versus −1.99%; $P < .05$) [23].

Although the current data looks encouraging, to date there is limited data on the use if *Irvingia gabonensis* in weight loss. It appears to be safe and well tolerated as the most common adverse effects are headache, flatulence, and difficulty sleeping [30]. Due to the limited data, *Irvingia gabonensis* cannot be recommended at this time.

2.1.6. Chia Seed (Salvia hispanica). Chia seed, or *Salvia hispanica*, is a sprout that has high concentrations of omega-3-fatty acids, alpha-linoleic acid, and fiber [24, 31]. It has been hypothesized that these components of the seeds would not only help with diseases such as hypercholesterolemia or diabetes, but that it may also be beneficial in weight loss. Nieman et al. [24] performed a single-blinded, trial in which 76 overweight/obese participants were randomized to receive 50 grams of chia seed daily or placebo. At the end of 12 weeks, it was noted that there were differences pre- and poststudy on body mass or body composition [24]. While considered safe in the short term, there is limited data to suggest the use of chia seeds for weight loss.

3. Conclusion

Because the prevalence of obesity in the United States is significant, many people turn to the use of supplemental products as an assist with weight loss efforts. While there are several dietary supplements being marketed for the use in weight loss via several different mechanisms of action, there is very little clinical evidence to support their use. Conjugated linoleic acid, pyruvate, and *Irvingia gabonensis* have shown some potential benefit for weight loss. However, more data is necessary to draw any definitive conclusions on the use of dietary supplements for weight loss. Continued research is needed in this area to aid health care providers as well as the public in general. Health care providers should be aware of the weight loss products available to their patients and assist patients in determining the risks and benefits of supplement use for weight loss.

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