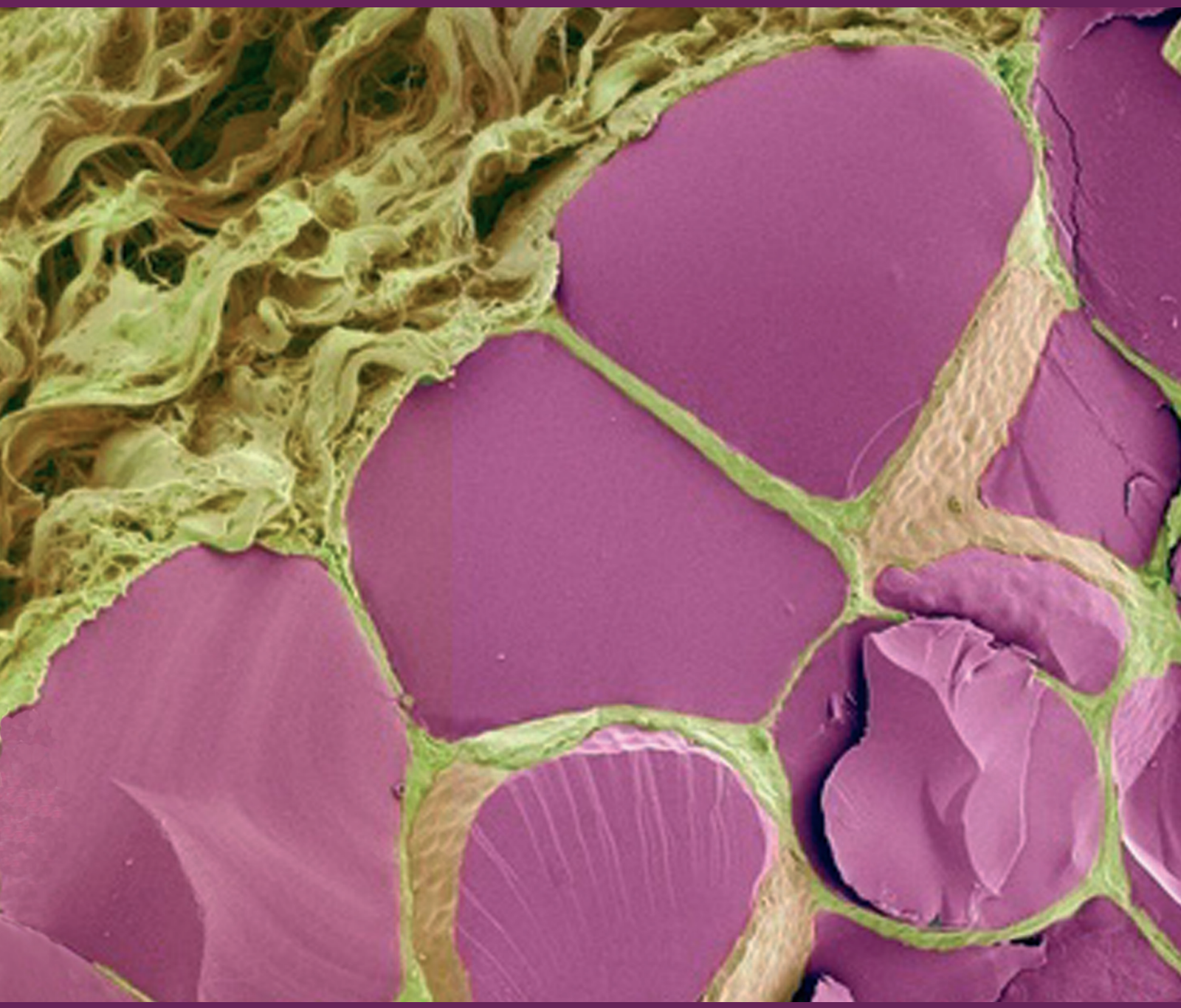
International Journal of Endocrinology

Obesity and the Body Weight Set Point Regulation

Guest Editors: Yi-Hao Yu, Joseph R. Vasselli, Liping Zhao, and Ralph Peterli



Obesity and the Body Weight Set Point Regulation

International Journal of Endocrinology

Obesity and the Body Weight Set Point Regulation

Guest Editors: Yi-Hao Yu, Joseph R. Vasselli, Liping Zhao, and Ralph Peterli

Editorial Board

Contents

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 175080, 10 pages
http://dx.doi.org/10.1155/2014/175080

Research Article

Modulation of the Activities of Catalase, Cu-Zn, Mn Superoxide Dismutase, and Glutathione Peroxidase in Adipocyte from Ovariectomised Female Rats with Metabolic Syndrome

Rebeca Cambray Guerra,[1] Alejandra Zuñiga-Muñoz,[1] Verónica Guarner Lans,[2] Eulises Díaz-Díaz,[3] Carlos Alberto Tena Betancourt,[4] and Israel Pérez-Torres[1]

[1] *Departments of Pathology, Instituto Nacional de Cardiología "Ignacio Chávez", Juan Badiano 1, Sección XVI, Tlalpan, 14080 México, DF, Mexico*
[2] *Departments of Physiology, Instituto Nacional de Cardiología "Ignacio Chávez", Juan Badiano 1, Sección XVI, Tlalpan, 14080 México, DF, Mexico*
[3] *Department of Reproductive Biology, Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", Vasco de Quiroga 15, Sección XVI, Tlalpan, 14000 México, DF, Mexico*
[4] *Departments of Vivarium, Instituto Nacional de Cardiología "Ignacio Chávez", Juan Badiano 1, Sección XVI, Tlalpan, 14080 México, DF, Mexico*

Correspondence should be addressed to Israel Pérez-Torres; pertorisr@yahoo.com.mx

Received 25 November 2013; Accepted 17 April 2014; Published 29 May 2014

Academic Editor: Joseph R. Vasselli

The aim of this study was to evaluate the association between estrogen removal, antioxidant enzymes, and oxidative stress generated by obesity in a MS female rat model. Thirty two female Wistar rats were divided into 4 groups: Control (C), MS, MS ovariectomized (Ovx), and MS Ovx plus estradiol (E_2). MS was induced by administering 30% sucrose to drinking water for 24 weeks. After sacrifice, intra-abdominal fat was dissected; adipocytes were isolated and lipid peroxidation, non-enzymatic antioxidant capacity, and the activities of Cu-Zn and Mn superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were determined. There were no significant differences in the activities of Cu-Zn, Mn SOD, CAT, and GPx between the C and MS groups, but in the MS Ovx group there was a statistically significant decrease in the activities of these enzymes when compared to MS and MS Ovx+E_2. The increased lipid peroxidation and nonenzymatic antioxidant capacity found in MS Ovx was significantly decreased when compared to MS and MS Ovx+E_2. In conclusion, the removal of E_2 by ovariectomy decreases the activity of the antioxidant enzymes in the intra-abdominal tissue of MS female rats; this is reflected by increased lipid peroxidation and decreased nonenzymatic antioxidant capacity.

1. Introduction

The metabolic syndrome (MS) is defined as a cluster of metabolic alterations [1], which includes the following diagnostic criteria: hypertension, diabetes, insulin resistance, dyslipidemia, and obesity [2]. Obesity, as component of MS, is considered a public health problem because of its magnitude and importance. Overweight and obesity are the fifth leading risk factor for death in the world [3]. The accumulation of abnormal or excessive fat stored in adipose tissue is the result of a chronic imbalance between energy intake and energy expenditure [2–4]. Adipose tissue, which was previously regarded as a tissue with few metabolic functions and considered only as passive reservoir, is now known to be metabolically active [5]. Several studies have suggested that obesity is associated with increased free radical concentrations [6], which can cause oxidative stress in the endoplasmic reticulum of the adipocyte [7]. An increase in oxygen consumption by mitochondria of the adipocyte [8] leads to excess processing of free fatty acids [9–11], which are produced by the hydrolysis of triglycerides in adipose tissue [12]. An excess of adipose tissue is also a source of inflammatory

cytokines such as IL-1, IL-6, and TNF-α, and obesity is considered as a chronic inflammatory state. These cytokines are a potent stimulus for the production of reactive oxygen species [13]. Moreover, the mammalian cells are equipped with enzymatic antioxidant defense mechanisms among which are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) enzyme, and the nonenzymatic systems such as vitamins A, C, and E, among others [14, 15]. The antioxidant enzymes contribute to eliminate radicals such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), preventing the formation of the very active species O_2^- and radical hydroxyl (HO^-) which are damaging to cells.

Sexual dimorphism is involved in the clinical manifestations of MS [16] and this disease tends to occur more often in postmenopausal than in premenopausal women [17–19]. Additionally, increased oxidative stress after menopause is associated with loss of endogenous estrogen synthesis [20]. Protection by female sex hormones is attributed to the well-demonstrated antioxidant properties of estrogen *in vivo*; estrogen decreases the occurrence of cardiovascular diseases in postmenopausal women [21], and *in vitro* estradiol acts as a molecule with antioxidant activity decreasing lipid peroxidation in rat liver microsomes [22]. GPx activity in female rat liver is increased by 60% when compared to Ovx female rats [23]. Behl et al. postulated that the antioxidant activity of estradiol in neuronal cells depends on the presence of the hydroxyl group (OH) at the C-3 position of the phenolic ring of the molecule [24], and another study showed that E_2 can inhibit the oxidation cascades through the hydroxyl group of phenolic ring A [25]. Other antioxidant properties of estrogen action are exerted on glutathione (GSH). Cellular protection against oxidative stress has been demonstrated in neural cells, through the synergistic activity of estrogens and GSH [25]. Other studies show that postmenopausal women have a higher incidence of abdominal adiposity, associated with an increase in systemic levels of inflammatory cytokines, which suggests that estrogen can modulate body fat and systemic inflammation [26]. Stubbins et al. recently demonstrated that estrogen protected females against inflammation and oxidative stress when compared to males when studying intact female mice adipocytes, Ovx plus estradiol mice, and males subjected to a high-fat diet for 10 weeks [26]. It has been demonstrated that in kidney homogenates of intact rats with MS the activity of the enzymes CAT and SOD was significantly increased when compared to the MS Ovx group. These results suggest that female rats are protected against the estrogen prooxidant effects in the renal system induced by the high consumption of sucrose in the diet, but the protective effect decreases after ovariectomy [27].

On the other hand, Reaven and Ho [28] developed MS in rats by the administration of high-sucrose or fructose diets, which induce hypertriglyceridemia, hypertension, hyperinsulinemia, and insulin resistance and increase intra-abdominal fat tissue. In our laboratory we have developed a variant MS rat model by chronic administration of 30% sucrose in the drinking water for a period of 24 weeks.

Excess adipose tissue generates oxidative stress and estrogens have antioxidant properties; however, studies of the antioxidant capacity of estrogens in adipocytes are scarce. Therefore, the aim of this study was to evaluate the association between estrogen removal in MS female rat model and levels of antioxidant enzymes and oxidative stress generated by obesity.

2. Material and Methods

2.1. Animals. Experiments in animals were approved by the Laboratory Animal Care Committee of our institution and were conducted in compliance with the Guide for the Care and Use of Laboratory Animals of NIH. Weanling female rats weighing 100 ± 10 g, $n = 8$ per group. The groups were control (C), 30% sucrose-fed (MS), MS ovariectomized (Ovx), and MS Ovx + estradiol (E_2). The animals were housed in ad hoc plastic boxes and were subjected to 12-hour light/obscurity cycles and environmental temperature between 18 and 26°C. They were fed commercial rodent pellets (23% of crude protein, 4.5% of crude fat, 8% of ashes, and 2.5% of added minerals; PMI Nutrition International, Inc., LabDiet 5008, Richmond, IN USA) ad libitum. At the end of the experimental period of 24 weeks, the rats were weighed and their blood pressure (BP) was measured by the tail-cuff method [27]; after overnight fasting, the animals were subjected to euthanasia with a guillotine and their blood was collected in vacutainer tubes. The samples were centrifuged for 20 min at 936 g and 4°C, in order to collect the serum in aliquots of 400 μL and store it at −70°C.

2.2. Ovariectomy. Surgical ovariectomy was performed at 1 month of age. This was performed under anaesthesia (pentobarbital sodium 63 mg/Kg of body weight). The abdominal and pelvic area of the back was depilated, cleaned with soap, and disinfected with ethanol. A longitudinal incision of 1.5 cm was made, the skin was separated from the muscle, and a second incision of 0.5 cm was made in the muscle on both sides of the first, to exteriorize the ovaries. The Fallopian tubes were ligated and cut below the ligature. After the extirpation, the incision was sutured [27].

2.3. Hormonal Treatment. Estradiol valerate (Primogyn, Schering, Mexico; 1 mg/Kg body weight) was injected i.m. every 3 days, during the experimental period.

2.4. Measurement of Serum Sex Hormones. Serum estradiol was measured using the Diagnostic Products Corporation kit (Los Angeles, CA) and determination of some rat biochemical variables, such as glucose, cholesterol, triglycerides, and insulin, was determinate using commercially obtained kits. The HOMA index of resistance to insulin was calculated.

2.5. Isolation of Adipocytes. White adipocytes were isolated by collagenase digestion as described by Rodbell [29] with the following modifications: 4 g of adipose tissue was removed and transferred into Krebs buffer (containing 2% bovine serum albumin (BSA), 118 mM NaCl, 24 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 4.7 mM KCl, 2.5 mM Ca_2Cl, and 4.5 mM D-glucose at pH 7.35). Adipose tissue pieces were minced and digested with collagenase II (Sigma) and at 37°C for 90 min in a shaking water bath; the fat

Table 1: General and biochemical characteristics.

Variables	C	SM	SM Ovx	SM Ovx+E_2
Body mass (g)	272.5 ± 6.2	305.4 ± 2.0**	377.4 ± 2.6††	296.2 ± 3.2
Intra-abdominal fat (g)	4.8 ± 0.5	7.1 ± 0.4*	13.3 ± 0.7††	6.0 ± 0.1
Systolic blood pressure (mm/Hg)	116.6 ± 2.6	125.3 ± 3.1	146.1 ± 2.2††	124.5 ± 1.8
Cholesterol (mg/dL^{-1})	68.2 ± 3.3	69.2 ± 5.0	69.7 ± 2.8	70.0 ± 3.6
Triglycerides (mg/dL^{-1})	57.2 ± 5.8	96.2 ± 10.0**	105.5 ± 5.9	108.7 ± 9.1
Glucose (mmol/dL^{-1})	6.4 ± 0.2	6.3 ± 0.3	7.3 ± 0.4	6.3 ± 0.2
Insulin (μUI/mL^{-1})	2.7 ± 0.7	7.6 ± 0.9**	11.6 ± 1.5†	6.1 ± 1.2
HOMA	0.5 ± 0.1	2.5 ± 0.4*	3.9 ± 0.6†	1.8 ± 0.3
Leptin (ng/mL)	3.2 ± 0.5	2.8 ± 0.4	1.3 ± 0.1†	2.1 ± 0.2
Estradiol (pg/mL)	24.8 ± 3.2	29.1 ± 5.8	9.2 ± 1.1†	26.8 ± 3.6

Data are means ± SE; $n = 8$ each group. Statistically significant at C versus MS $^{*}P < 0.01$ and $^{**}P < 0.001$; $^{\dagger}P < 0.01$ and $^{\dagger\dagger}P < 0.001$ MS versus MS Ovx. C: control; MS: metabolic syndrome; MS Ovx: metabolic syndrome ovariectomized; MS Ovx+E_2: metabolic syndrome ovariectomized plus estradiol.

cell suspension thus obtained was filtered through a 250 μm nylon mesh and centrifuged for 1 min at 300 g. The adipocytes collected from the top phase were washed with 10 ml of Krebs buffer without BSA three times, then resuspended in 900μl buffer sucrose containing (1mM EDTA, 10 mM TRIS and 250 mM sucrose) and 100 μL of protease inhibitors (1 mM PMSF, 2 mM pepstatin, 2 mM leupeptin, and 0.1% aprotinin) and homogenized; the sample was frozen at −30°C. Total proteins were determined by the method of Bradford [30].

2.6. Evaluation of Antioxidant Enzymatic System. The measurement of the activity of antioxidant enzymes was carried out by electrophoresis in 10% polyacrylamide native gels. To determine the activity of CAT, the gel was washed with distilled water during 5 minutes; this procedure was repeated three times; then it was incubated with a mixture of 1% $K_3Fe(CN)_6$ and 1% of $FeCl_3$ $6H_2O$ for 10 minutes in the dark and then washed with distilled water to stop the reaction [27].

To determine the activity of SOD, the gel was washed with distilled water during 5 minutes; this procedure was repeated three times; then it was incubated with 2.45 mM nitro blue tetrazolium (NBT) for 20 minutes; then the NBT solution was discarded and the gel was incubated in a solution of 28 mM EDTA, 0.028 mM riboflavin, and 36 mM phosphate buffer, pH 7.8. After 15 minutes of incubation in the dark, the NBT stain for O_2 was developed by exposure to UV light for another 10 minutes [27]. The gels were analyzed by densitometry with an image Sigma Scan Pro 5 Analyzer.

The activity of enzyme GPX was measured by spectrophotometry: 1 mg of protein from the adipocyte homogenate was suspended in 1.6 mL of 50 mM phosphate buffer (pH 7.0), with added 0.2 mM NADPH, 1 mM GHS, and 1 UI/mL glutathione reductase. The mixture was incubated for 5 minutes at room temperature; then 100 μL of 0.25 mM H_2O_2 was added and the reading was taken immediately at 340 nm (initial reading) and again after 5 minutes (final reading) [31].

2.7. Evaluation of Antioxidant Capacity of the Nonenzymatic System. For the precipitation of the proteins in the samples, 100 μL of 10% $ZnSO_4$ and 100 μL of 0.5 N NaOH were added to one mg of protein from the adipocyte homogenate and centrifuged at 7155 ×g; the supernatant was suspended in 1.5 mL of reaction mixture (300 mM acetate buffer pH 3.6, 20 mM hexahydrate of ferric chloride, 10 mM of 2, 4, 6 Tris-2-pyridil-s-triazine dissolved in 40 mM chlorhydric acid (HCl) were added in a relation of 10 : 1 : 1 v/v, resp.); the mixture was shaken vigorously in vortex for 5 sec. Then it was incubated at 37°C for 15 minutes in the dark. The absorbance was measured at 593 nm. The calibration curve was obtained using μmol Trolox equivalent [31].

2.8. Lipid Peroxidation (TBARS). TBARS, a marker of damage by free radicals, was measured by a standard method. To 1 mg of protein from the adipocyte homogenate, 50 μL methanol with 4% BHT plus phosphate buffer pH 7.4 was added. The mixture was shaken vigorously in a vortex for 5 seconds and then incubated in a water bath at 37°C for 30 minutes. This was followed by the addition of 1.5 mL of 0.8 M thiobarbituric acid and then incubated in a water bath at boiling temperature for 1 hour. After this time and to stop the reaction, the samples were placed on ice; 1 mL 5% KCL was added to each sample as well as 5 mL n-butanol; they were shaken in a vortex for 30 seconds and centrifuged at 2000 rpm., at room temperature for 2 minutes. Then the n-butanol phase was extracted and the absorbance was measured at 532 nm. The calibration curve was obtained using tetraethoxypropane as standard [27].

2.9. Statistical Analysis. Statistical analysis and graphics were performed with a SigmaPlot 11 program. The data are presented as the mean ± SEM. Statistical significance was determined by one-way ANOVA test, followed by the post hoc Tukey test. Differences were considered statistically significant at $P < 0.05$.

3. Results

3.1. General Characteristics. Table 1 shows some general and biochemical characteristics of the experimental animals. The body mass, intra-abdominal fat, triglycerides, insulin, and

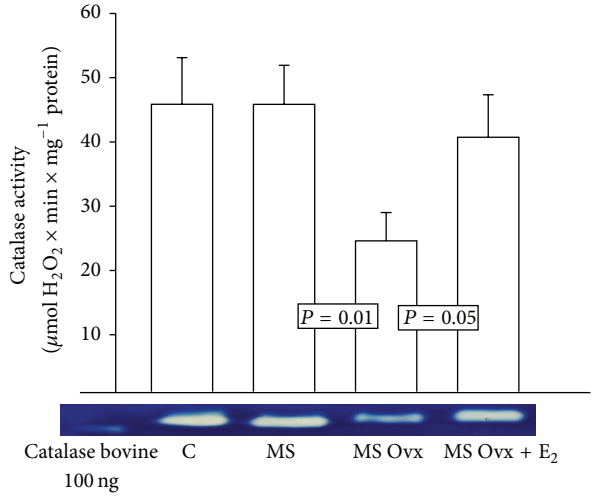


Figure 1: Effect of the estrogen removal and estradiol replacement on catalase activity in adipocyte homogenate. Native-gel electrophoresis with 10% polyacrylamide. CAT: catalase, C: control, MS: metabolic syndrome, MS Ovx: metabolic syndrome ovariectomized, and MS Ovx+E_2: metabolic syndrome ovariectomized plus estradiol. Data are means ± SE; n = 8 in each group.

HOMA index were significantly elevated in MS in comparison with C ($P < 0.001$), and the MS Ovx increased it in comparison to MS intact ($P < 0.01$). Cholesterol and glucose remained at normal levels in all groups. In MS Ovx+E_2 rats hormonal treatment did not modify the variables in comparison with MS intact rats.

3.2. Antioxidant Enzymes. There was no difference in the activity of CAT in adipocyte homogenate between female C, MS intact, or MS Ovx+E_2. However, a significant decrease in CAT activity was observed in MS Ovx when compared with MS intact ($P = 0.01$) (Figure 1). The treatment with estradiol induced a significant increase in MS Ovx+E_2 versus MS Ovx ($P = 0.05$).

There were no differences in Mn SOD activity except between MS intact and MS Ovx, the latter being reduced ($P = 0.05$) (Figure 2), but treatment with estradiol significantly increased it in MS Ovx+E_2 versus MS Ovx ($P = 0.05$). Figure 3 shows the Cu-Zn SOD activity. There were no significant differences between groups C, MS intact, or MS Ovx+E_2. However, a significant decrease in this enzyme activity was observed in MS Ovx when compared with MS intact ($P = 0.03$) (Figure 1). The treatment with estradiol induced a significant increase in MS Ovx+E_2 versus MS Ovx ($P = 0.01$).

Figure 4 shows the activity of the GPx enzyme in C and MS intact groups; there are no significant changes; however, the MS Ovx group showed lower activity than the MS intact rat group ($P = 0.04$). The treatment with estradiol in MS Ovx+E_2 rats group tended to increase the activity but the change was not statistically significant.

3.3. Lipid Peroxidation (TBARS). The lipid peroxidation index in adipocyte homogenates showed no changes between

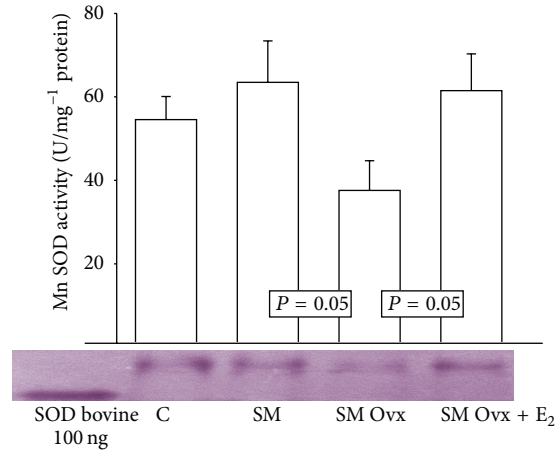


Figure 2: Effect of the estrogen removal and estradiol replacement on Mn SOD activity in adipocyte homogenate. Mn SOD: superoxide dismutase manganese. Native-gel electrophoresis with 10% polyacrylamide. Data are means ± SE; n = 8 in each group.

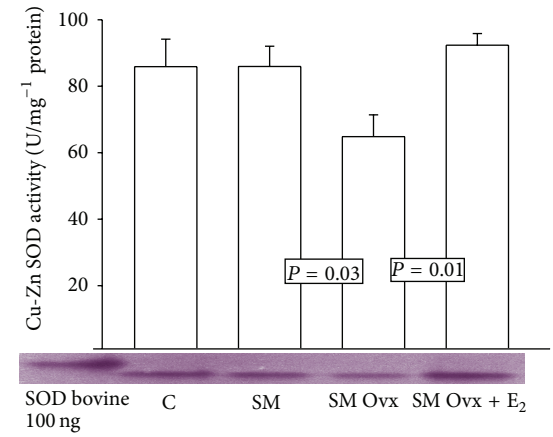


Figure 3: Cu-Zn SOD activity in adipocyte homogenate of experimental groups. Cu-Zn SOD: superoxide dismutase copper-zinc. Data are means ± SE; n = 8 in each group.

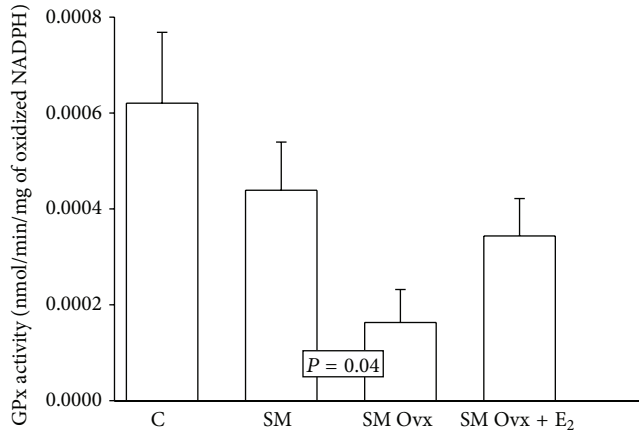


Figure 4: Effect of the ovariectomy and estradiol replacement on glutathione peroxidase activity in the adipocyte homogenate. Data are means ± SE; n = 8 each group.

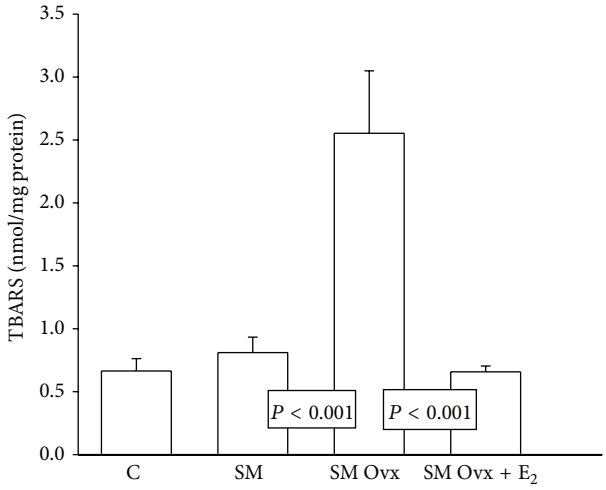


Figure 5: Lipid peroxidation was measured in adipocyte homogenate. See Table 1 legend for abbreviations. Values are means ± SE; $n = 8$ each group.

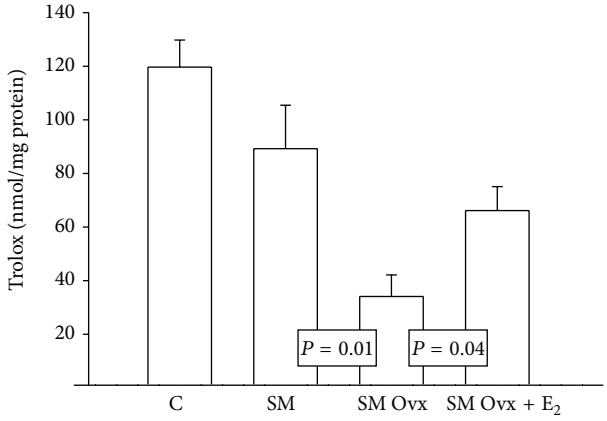


Figure 6: Effects of the ovariectomy and estradiol replacement upon the antioxidant capacity of the nonenzymatic system. See Table 1 legend for abbreviations. Values are means ± SE; $n = 8$ each group.

C and MS intact. Ovariectomy increased lipid peroxidation in the MS group in comparison to MS intact and MS Ovx+E_2 rats groups ($P < 0.001$). The hormonal treatment did not produce significant changes in MS Ovx+E_2 when compared to MS intact group (Figure 5).

3.4. Antioxidant Capacity of the Nonenzymatic System. Figure 6 shows that there were no statistically significant changes in the antioxidant capacity of the nonenzymatic system in the C and MS intact group but ovariectomy decreased the antioxidant capacity in the MS Ovx group versus MS intact group ($P = 0.01$). In the MS Ovx+E_2 group there were significant increases in the antioxidant capacity of the nonenzymatic system in comparison with MS Ovx ($P = 0.04$).

4. Discussion

Obesity is a component of MS and is the consequence result of a positive energy balance resulting from the interaction of several factors, including feeding, reduced physical activity, and genetic components. There is enough literature showing that in diseased states, such as MS and obesity, there is increased systemic oxidative stress [32–34]. Moreover, the reduction of the synthesis endogenous estrogen is associated with the onset of MS development. The aim of this study was to evaluate the association between estrogen removal and antioxidant enzymes and oxidative stress generated by obesity in a MS female rat model.

4.1. Body Weight and Intra-Abdominal Fat. The results showed that body weight in the MS group was significantly higher than in the C group; however, the group with the highest increase in weight was the MS Ovx group; these results are similar to those obtained by Stubbins et al. who showed that after 10 weeks of consuming high-fat diet male mice had significantly higher body weight than intact female mice. Ovx females show similar changes to male mice with respect to changes in body weight, but when supplemented with estradiol, changes in body weight were minimal and similar to intact females [26]. Accumulation of intra-abdominal adipose tissue is considered as a risk factor for the development of MS [35–37]. Intra-abdominal fat is more expandable than subcutaneous adipose tissue; it is metabolically active and secretes substances directly to the portal circulation such as inflammatory cytokines and free fatty acids which are associated with insulin resistance, hypertension, and cardiometabolic risk [38]. Estrogens are important regulators of adipose tissue deposition in humans, rodents, and other species [39]. Our results show that MS Ovx group had a higher amount of intra-abdominal fat when compared to MS intact and MS Ovx+E_2 groups. These results suggest that intra-abdominal fat increases in the MS Ovx group and this is probably due to the absence of estradiol. In premenopausal women it has been reported that fat tissue is located primarily in subcutaneous deposits; however, at menopause, there is redistribution to visceral adiposity, which is sensitive to estrogen therapy [40]. It has been described that the intra-abdominal adipose tissue expresses α and β receptors but that α receptor expression is predominant; Meyer et al. have reported that female mice lacking α estrogen receptor develop central obesity [40]. Likewise, Brown et al. mention that estrogens can regulate energy intake through direct action of α estrogen receptor or through indirect action decreasing orexigenic peptides; therefore the absence of estrogens may promote hyperphagia, although some authors report that ovariectomy is not necessarily accompanied by increased food intake [35]. Another study, in a female mice model subjected to ovariectomy and in which there was no decrease in energy expenditure or concomitant changes in energy intake and adipocyte hypertrophy, showed that Ovx female mice replaced with estradiol were protected from adipocyte hypertrophy [36]. Estrogen may directly inhibit the deposition of adipose tissue by reducing lipogenesis through decreased mRNA, activity,

and expression of lipoprotein lipase, an enzyme that regulates the storage of triglycerides in the adipocytes [39]. It has been described that ovariectomy can increase the activity of lipoprotein lipase and lipid deposition in adipocytes, but the administration of physiological estrogen doses reverses this condition. Our results show that the MS Ovx rats had increased body weight and intra-abdominal adipose tissue when compared to the MS intact and MS Ovx+E_2 rats. In addition, estrogens may also affect lipid deposition through the hormone-sensitive lipase and increased fatty acid oxidation, diminishing the likelihood of lipotoxicity [41]. It has been reported that in postmenopausal women the adipocyte hypertrophy and lipolytic activity are high, which may explain why postmenopausal women show high levels systemic of FFA [26, 42].

4.2. Systolic Blood Pressure. The MS Ovx group showed a significant increase in SBP when compared to the other groups. This result is consistent with basic and clinical research, in which it was demonstrated that the SBP is elevated in premenopausal women compared to postmenopausal women [43, 44]. In addition, it is known that nitric oxide, a potent vasodilator, participates in the regulation of SBP [27]; nitric oxide metabolism is better preserved in females than in males, partly as a result of the action of estrogen [44]. The results show that, in the C groups and MS and MS Ovx+E_2 groups, SBP was decreased when compared to MS Ovx group. Another study showed that estradiol replacement in MS Ovx female rats with MS is associated with an increased activity of nitric oxide synthase, endothelium-dependent vasodilation, and decreased blood pressure [45].

4.3. Hypertriglyceridemia. Diets high in carbohydrates, such as fructose, sucrose, or both, induce hypertriglyceridemia and reduction in antioxidant reserves [45]. The results show that triglycerides are increased in MS groups and decreased when compared to the C group and are similar to those obtained by Pettersson et al. who induced MS in female rats by administering a high-fat diet (60%) over a period of 14 weeks and found a significant increase in triglycerides when compared to the C group [46]. Another study showed that hypertriglyceridemia, resulting from the intake of high carbohydrate diet in rats, was associated with hyperinsulinemia, increased SBP, and insulin resistance [47]. Moreover, decreased levels of ovarian hormones associated with menopause or ovariectomy have been related to a decrease in glucose uptake by insulin [48]. Our results show that the serum insulin levels were increased in the MS group when compared to the C group, but the group MS Ovx showed the highest values; therefore, it appears that estrogen may have protective effect against the development of hyperinsulinemia.

4.4. Insulin Resistance. In addition, alterations in lipid metabolism and fat body distribution coupled with estrogen deficiency have been postulated as a causative factor contributing to the increased prevalence of insulin resistance in postmenopausal when compared to premenopausal women [48]. Furthermore, as previously mentioned, the MS Ovx group was the one that had the highest rate of insulin resistance when compared to the MS Ovx+E_2. Saglam et al. showed that hormone replacement therapy increased insulin peripheral action in postmenopausal women [49], and, in another study, Abbas and Elsamanoudy found that estrogen exerted effects upon insulin resistance; these authors found that the estrogen administration in Ovx rats significantly decreased the plasma glucose, insulin concentration, and HOMA index when compared with C [48]. Insulin binding to its receptors on the cell membrane is required to cause the hormonal actions. Therefore, the structure and functional integrity of the cell membrane influence the properties of the insulin receptor. Cell membrane properties, particularly its fluidity, depend upon the fatty acid composition. In hyperinsulinemic states, increased saturated fatty acids lead to a decrease in the affinity and number of insulin receptors, which may cause insulin resistance associated with hyperinsulinemia [47]. Estrogens can improve insulin action by increasing receptor specific binding to insulin. Evidence suggests that estrogen can increase the action of insulin in adipocytes via activation of transcription factors (protein-1 activator than response to cAMP) which are orchestrated by insulin [35, 48]. Moreover, a high concentration of H_2O_2 (100 μM) decreases insulin receptor affinity [50]. Our results show that lipoperoxidation index in MS Ovx group was higher when compared to the other experimental groups; this suggests that the elimination of estradiol through ovariectomy increases reactive oxygen species that can oxidize polyunsaturated fatty acids of cell membrane favoring insulin resistance and oxidative stress.

4.5. Leptin. Leptin, an adipokine secreted by adipose tissue [51], is directly proportional to the fat content [52]. The results show that the ovariectomy reduces serum leptin concentration in comparison to MS intact female rats and this was restored by estradiol replacement. These results suggest that estradiol may modulate leptin secreted by adipocytes. To support the above, it has been described that leptin levels are increased in women when compared to men, partly as result of inhibition of androgen and estrogen stimulation [51]. Leptin action is mediated via a specific receptor Ob-Rb, located mainly in the hypothalamus; estrogen may modulate the catabolic action of leptin in the brain and it has been described that estrogens are associated with increased leptin sensitivity [35]. A study by Alonso et al. demonstrated that estradiol can directly modulate the expression of Ob-Rb receptor in adipose tissue and skeletal muscle [53]. Another study in Ovx rats demonstrated that leptin mRNA expression in adipose tissue was associated with decreased plasma leptin concentration when compared to rats treated with estradiol. The mechanism responsible for the effect of estrogen on leptin concentrations in plasma remains unclear, but it is postulated that estrogen may have a direct action on the leptin gene in the adipocytes [53].

4.6. Antioxidant Enzymes. Investigations on the differences in gender related antioxidant reserves include studies in humans and in several animal species in both normal populations and pathological conditions and different organs.

Several studies indicate that females have higher antioxidant potential, given by the enzymatic and nonenzymatic activity [54]. In addition, the activity of the antioxidant enzymes SOD, Cat, and GPx plays an important role in obesity associated with MS. The results show that the activity of the Cu-Zn SOD in the MS Ovx group was significantly decreased when compared to the MS intact and MS Ovx+E_2 group; concerning the activity of Mn SOD, the results showed the same tendency. This suggests that estradiol may modulate the activity of both SOD isoforms. It has been described that estrogens may regulate the nuclear transcription factor, Nrf-2 pathway, which controls the expression and induction of genes that are encoded for phase II antioxidant enzymes, including SOD isoforms [55]. Moreover, our results are similar to those of a study conducted by Kumar et al., in which the antioxidant effect of estradiol in liver fractions of rat females of 3, 12, and 24 months of age was evaluated which showed that the treatment with estrogens normalized the decrease of the SOD activity induced by aging and menopause [56]. Another study in kidney homogenate of MS female rat, which evaluated the activity of the Cu-Zn and Mn SOD, with estradiol replacement, showed an increase in the activity of these enzymes in the control rats in comparison to MS rats; the activity decreased after ovariectomy and treatment with E_2 restored it [27]. Furthermore, Baños et al. showed that SOD activity in the heart of MS male rats was decreased while oxidative stress was increased but these changes were not present in female rats [54]. Busserolles et al. demonstrated that the SOD activity was decreased in the heart of male rats that were fed with sucrose for two weeks, when compared to female rats [57]. Moreover, it has been shown that fructose or sucrose may inactivate CAT *in vitro* [58]. However, our results on CAT activity in adipocyte homogenates showed no significant changes between C, MS, and MS Ovx+E_2 groups but showed significant difference when comparing the MS intact and Ovx+E_2 group with the MS Ovx group. These results are similar to those of other studies which showed significant decrease in CAT activity in the MS Ovx female rats when compared to MS female intact and replaced with estradiol [27]. Therefore, the results suggest that E_2 can promote an increase in CAT activity. Furthermore, Pajović and Saicić mention that females had lower oxidative stress in the brain and increased activity of CAT in comparison with males [59]. Regarding the activity of GPx, the results show that the control group showed no significant difference when compared to the MS group, but there was significant decrease in the activity of this enzyme in the MS Ovx group compared to MS intact group. Baltgalvis et al. demonstrated in murine skeletal muscle that genes encoded for type 3 GPx expressions are sensitive to estradiol and regulated via α receptors [60]. In addition, another study showed that the activity of GPx was significantly increased in premenopausal women and it decreased after menopause [61]. However, another study showed no significant difference in the activity of this enzyme in the brain of male and female mice, while in the liver the enzyme activity was significantly higher in females than in males [59]. Supporting the above, *in vitro* studies have shown that damage induced in myocardial cells by free radicals was stopped by nuclear translocation of Nrf-2 and that this effect was promoted by pretreatment with estrogen, since it controls and induces expression of genes encoded for GPx [55]. Likewise, other investigations have shown synergistic interaction between estrogens and GSH in neuronal protection against oxidative stress [25].

4.7. Nonenzymatic Antioxidant Capacity. The nonenzymatic antioxidant capacity in adipocyte homogenates showed no significant differences when comparing the C group with the MS group, but the MS Ovx group showed a significant decrease in comparison with MS intact and MS Ovx+E_2. This result suggests that the E_2 have antioxidant properties that allow the increase in nonenzymatic antioxidant capacity in the adipocytes of the MS rats. Another study, which determined the protection of estradiol upon oxidative stress in visceral tissue in a murine model subjected to high-fat diet, showed that male mice and Ovx females have a significant increase in γH2AX, a biomarker for oxidative stress, in adipocyte core compared to intact and Ovx females with estradiol replacement. The researchers concluded that estradiol may protect from the development of oxidative stress in adipose tissue [26].

4.8. Lipid Peroxidation (TBARS). With respect to lipid peroxidation, the results show no significant difference in the C group compared to the MS groups, but in the MS Ovx group there was a significant increase when compared to MS and MS Ovx+E_2 group, which had similar lipoperoxidation values. Moreover, a study conducted by Taskiran and Evren demonstrated that H_2O_2 induced lipid peroxidation in cell cultures of adipose tissue and that it was attenuated with pretreatment with different concentrations of estradiol; the maximum effect was observed at 10 nM [62]. Signorelli et al. demonstrated that oxidative stress damage, measured by the concentrations of 4-hydroxynonenal, a waste product of the oxidation of lipids, was significantly increased in postmenopausal women when compared to premenopausal women, which suggest that estrogen may protect against lipid peroxidation [61]. The effect of estrogen decreasing the rate of lipid peroxidation can be explained by the key structure of the phenolic ring of estradiol which renders the molecule with antioxidant protection [63]. Wang et al. proposed that cell membranes are one of the primary targets of the antioxidant effects of estrogen. Antioxidant actions of estrogens on cell membranes are independent of estrogen receptor and the phenolic ring structure may play an important role in this effect [25]. In addition, another study postulated that the antioxidant capacity of E_2 in neuronal cells depends on the presence of the hydroxyl group at the C-3 position of the phenolic ring of the molecule [24]. This mechanism has also been previously demonstrated by Jellinck and Bradlow who postulated that E_2 could inhibit oxidative cascades through the hydroxyl group of the A phenolic ring [25, 64].

5. Conclusions

In conclusion, the results suggest that removal of E_2 by ovariectomy decreases the activity of the antioxidant enzymes in the intra-abdominal tissue of MS female rats; this is

reflected in increased lipid peroxidation and decreased nonenzymatic antioxidant capacity. Replacement with E_2 can protect MS female rats from increases in body weight, intra-abdominal fat accumulation, and hypertension. It can also improve insulin sensitivity by decreasing insulin resistance and sensitivity to leptin. The oxidative stress and obesity present in MS ovariectomized female rats may be attenuated by hormonal replacement therapy; however, more studies are still needed on the antioxidant capacity of E_2 in metabolic syndrome.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] P. Anagnostis, "Metabolic syndrome in the Mediterranean region: current status," *Indian Journal Endocrinology Metabolism*, vol. 16, no. 1, pp. 72–80, 2012.

[2] P. Singla, A. Bardoloi, and A. Parkash, "Metabolic effects of obesity: a review," *World Journal Diabetes*, vol. 1, no. 3, pp. 76–88, 2010.

[3] C. S. Fox, J. M. Massaro, U. Hoffmann et al., "Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the framingham heart study," *Circulation*, vol. 116, no. 1, pp. 39–48, 2007.

[4] B. Xie, M. J. Waters, and H. J. Schirra, "Investigating potential mechanisms of obesity by metabolomics," *Journal of Biomedicine and Biotechnology*, vol. 2012, Article ID 805683, 10 pages, 2012.

[5] E. E. Kershaw and J. S. Flier, "Adipose tissue as an endocrine organ," *The Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 6, pp. 2548–2556, 2004.

[6] B. P. Yu, "Cellular defenses against damage from reactive oxygen species," *Physiological Reviews*, vol. 74, no. 1, pp. 139–162, 1994.

[7] Y. B. Tripathi and V. Pandey, "Obesity and Endoplasmic Reticulum (ER) stresses," *Frontiers in Immunology*, vol. 3, no. 240, pp. 1–9, 2012.

[8] S. de Ferranti and D. Mozaffarian, "The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences," *Clinical Chemistry*, vol. 54, no. 6, pp. 945–955, 2008.

[9] H. K. Vincent, S. K. Powers, A. J. Dirks, and P. J. Scarpace, "Mechanism for obesity-induced increase in myocardial lipid peroxidation," *International Journal of Obesity*, vol. 25, no. 3, pp. 378–388, 2001.

[10] H. K. Vincent and A. G. Taylor, "Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans," *International Journal of Obesity*, vol. 30, no. 3, pp. 400–418, 2006.

[11] L. A. Brown, C. J. Kerr, P. Whiting, N. Finer, J. McEneny, and T. Ashton, "Oxidant stress in healthy normal-weight, overweight and obese individuals," *Obesity*, vol. 17, no. 3, pp. 460–466, 2009.

[12] A. Fernández-Sánchez, E. Madrigal-Santillán, M. Bautista et al., "Inflammation, oxidative stress and obesity," *International Journal Mollecular Science*, vol. 12, pp. 3117–3132, 2011.

[13] J. V. Higdon and B. Frei, "Obesity and oxidative stress: a direct link to CVD?" *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 3, pp. 365–367, 2003.

[14] Y.-S. Ho, J.-L. Magnenat, M. Gargano, and J. Cao, "The nature of antioxidant defense mechanisms: a lesson from transgenic studies," *Environmental Health Perspectives*, vol. 106, no. 5, pp. 1219–1228, 1998.

[15] E. C. Gomes, A. N. Silva, and M. R. de Oliveira, "Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species," *Oxidative Medicine and Cell Longevity*, vol. 2012, Article ID 756132, 12 pages, 2012.

[16] I. Pérez and G. Baños, "Sex hormones receptors in metabolic syndrome, sex hormones: development, regulation and disorders," *Nova Science Publishers*, vol. 1, no. 1, pp. 53–74, 2011.

[17] V. Regitz-Zagrosek, E. Lehmkuhl, and S. Mahmoodzadeh, "Gender aspects of the role of the metabolic syndrome as a risk factor for cardiovascular disease," *Gender Medicine*, vol. 4, no. 2, pp. S162–S177, 2007.

[18] J. Hong, R. E. Stubbins, R. R. Smith, A. E. Harvey, and N. P. Núñez, "Differential susceptibility to obesity between male, female and ovariectomized female mice," *Nutrition Journal*, vol. 8, no. 1, article 11, 2009.

[19] P. E. Gustafsson, M. Persson, and A. Hammarstrom, "Life course origins of the metabolic syndrome in middle-aged women and men: the role of socioeconomic status and metabolic risk factors in adolescence and early adulthood," *Annals of Epidemiology*, vol. 21, no. 2, pp. 103–110, 2011.

[20] B. L. Crist, D. L. Alekel, L. M. Ritland, L. N. Hanson, U. Genschel, and M. B. Reddy, "Association of oxidative stress, iron, and centralized fat mass in healthy postmenopausal women," *Journal of Women's Health*, vol. 18, no. 6, pp. 795–801, 2009.

[21] R. A. Lobo, "Cardiovascular implications of estrogen replacement therapy," *Obstetrics and Gynecology*, vol. 75, no. 4, pp. 185–255, 1990.

[22] M. B. Ruiz-Larrea, M. J. Garrido, and M. Lacort, "Estradiol-induced effects on glutathione metabolism in rat hepatocytes," *The Journal of Biochemistry*, vol. 113, no. 5, pp. 563–567, 1993.

[23] R. E. Pinto and W. Bartley, "The nature of the sex-linked differences in glutathione peroxidase activity and aerobic oxidation of glutathione in male and female rat liver," *Biochemical Journal*, vol. 115, no. 3, pp. 449–456, 1969.

[24] C. Behl, T. Skutella, F. Lezoualc'h et al., "Neuroprotection against oxidative stress by estrogens structure activity relationship," *Molecular Pharmacololy*, vol. 51, no. 4, pp. 535–547, 1997.

[25] X. Wang, J. A. Dykens, E. Perez et al., "Neuroprotective effects of 17β-estradiol and nonfeminizing estrogens against H_2O_2 toxicity in human neuroblastoma SK-N-SH cells," *Molecular Pharmacology*, vol. 70, no. 1, pp. 395–404, 2006.

[26] R. E. Stubbins, K. Najjar, V. B. Holcomb, J. Hong, and N. P. Núñez, "Oestrogen alters adipocyte biology and protects female mice from adipocyte inflammation and insulin resistance," *Diabetes, Obesity and Metabolism*, vol. 14, no. 1, pp. 58–66, 2012.

[27] I. Pérez-Torres, P. Roque, M. El Hafidi, E. Diaz-Diaz, and G. Baños, "Association of renal damage and oxidative stress in a rat model of metabolic syndrome. Influence of gender," *Free Radical Research*, vol. 43, no. 8, pp. 761–771, 2009.

[28] G. M. Reaven and H. Ho, "Sugar-induced hypertension in Sprague-Dawley rats," *American Journal of Hypertension*, vol. 4, no. 7, pp. 610–614, 1991.

[29] M. Rodbell, "Metabolism of isolated fat cells," *The Journal of Biological Chemistry*, vol. 239, pp. 375–380, 1964.

[30] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle

of protein dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[31] A. M. Zúñiga-Muñoz, V. Guarner, A. Díaz-Cruz et al., "Modulation of oxidative stress in fatty liver of rat with metabolic syndrome by hibiscus sabdariffa," *Immunology, Endocrine and Metabolic Agents in Medicinal Chemistry*, vol. 13, no. 3, pp. 196–205, 2013.

[32] I. Savini, W. V. Catani, D. Gasperi, and L. Avigliano, "Obesity-associated oxidative stress: strategies finalized to improve redox state," *International Journal of Molecular Sciences*, vol. 14, no. 5, pp. 10497–10538, 2013.

[33] L. Vávrová, J. Kodydková, M. Zeman et al., "Altered activities of antioxidant enzymes in patients with metabolic syndrome," *Obesity Facts*, vol. 6, no. 1, pp. 39–47, 2013.

[34] R. Hutcheson and P. Rocic, "The metabolic syndrome, oxidative stress, environment, and cardiovascular disease: the great exploration," *Experimental Diabetes Research*, vol. 2012, Article ID 271028, 13 pages, 2012.

[35] L. M. Brown, L. Gent, K. Davis, and D. J. Clegg, "Metabolic impact of sex hormones on obesity," *Brain Research*, vol. 1350, pp. 77–85, 2010.

[36] N. H. Rogers, J. W. P. Li, K. J. Strissel, M. S. Obin, and A. S. Greenberg, "Reduced energy expenditure and increased inflammation are early events in the development of ovariectomy-induced obesity," *Endocrinology*, vol. 150, no. 5, pp. 2161–2168, 2009.

[37] S. L. Hocking, L. E. Wu, M. Guilhaus, D. J. Chisholm, and D. E. James, "Intrinsic depot-specific differences in the secretome of adipose tissue, preadipocytes, and adipose tissue-derived microvascular endothelial cells," *Diabetes*, vol. 59, no. 12, pp. 3008–3016, 2010.

[38] R. O'Rourke, "Inflammation in obesity-related disease," *Surgery*, vol. 145, no. 3, pp. 255–259, 2009.

[39] P. S. Cooke and A. Naaz, "Role of estrogens in adipocyte development and function," *Experimental Biology and Medicine*, vol. 229, no. 11, pp. 1127–1135, 2004.

[40] M. R. Meyer, D. J. Clegg, E. R. Prossnitz, and M. Barton, "Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors," *Acta Physiologica*, vol. 203, no. 1, pp. 259–269, 2011.

[41] S. E. Campbell, K. A. Mehan, R. J. Tunstall, M. A. Febbraio, and D. Cameron-Smith, "17β-estradiol upregulates the expression of peroxisome proliferator-activated receptor α and lipid oxidative genes in skeletal muscle," *Journal of Molecular Endocrinology*, vol. 31, no. 1, pp. 37–45, 2003.

[42] T. M. D'Eon, S. C. Souza, M. Aronovitz, M. S. Obin, S. K. Fried, and A. S. Greenberg, "Estrogen regulation of adiposity and fuel partitioning: evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways," *The Journal of Biological Chemistry*, vol. 280, no. 43, pp. 35983–35991, 2005.

[43] G. Baños, K. Carvajal, G. Cardoso, J. Zamora, and M. Franco, "Vascular reactivity and effect of serum in a rat model of hypertriglyceridemia and hypertension," *American Journal Hypertension*, vol. 10, no. 4, pp. 379–388, 1997.

[44] V. Guarner-Lans, M. E. Rubio-Ruiz, I. Pérez-Torres, and G. Baños de MacCarthy, "Relation of aging and sex hormones to metabolic syndrome and cardiovascular disease," *Experimental Gerontology*, vol. 46, no. 7, pp. 517–523, 2011.

[45] I. Pérez-Torres, M. El Hafidi, O. Infante, and G. Baños, "Effects of sex hormone levels on aortic vascular reactivity and variables associated with the metabolic syndrome in sucrose-fed female rats," *Canadian Journal of Physiology and Pharmacology*, vol. 86, no. 1-2, pp. 25–35, 2008.

[46] U. S. Pettersson, T. B. Waldén, P. O. Carlsson, L. Jansson, and M. Phillipson, "Female mice are protected against high-fat diet induced metabolic syndrome and increase the regulatory T cell population in adipose tissue," *PLoS ONE*, vol. 7, no. 9, pp. 1–10, 2012.

[47] I. Pérez-Torres, A. Zúñiga Muñoz, E. Díaz-Díaz, R. Martínez-Memije, and L. V. Guarner, "Modification of the liver fatty acids by Hibiscus sabdariffa Linnaeus (Malvaceae) infusion, its possible effect on vascular reactivity in a metabolic syndrome model," *Clinical and Experimental Hypertension*, vol. 36, no. 3, pp. 123–131, 2013.

[48] A. M. Abbas and A. Z. Elsamanoudy, "Effects of 17β-estradiol and antioxidant administration on oxidative stress and insulin resistance in ovariectomized rats," *Canadian Journal of Physiology and Pharmacology*, vol. 89, no. 7, pp. 497–504, 2011.

[49] K. Saglam, Z. Polat, M. I. Yilmaz, M. Gulec, and S. B. Akinci, "Effects of postmenopausal hormone replacement therapy on insulin resistance," *Endocrine*, vol. 18, no. 3, pp. 211–214, 2002.

[50] B. J. Deroo and K. S. Korach, "Estrogen receptors and human disease," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 561–570, 2006.

[51] M. Coelho, T. Oliveira, and R. Fernandes, "Biochemistry of adipose tissue: an endocrine organ," *Archives Medicinal Science*, vol. 9, no. 2, pp. 191–200, 2013.

[52] S.-C. Chu, Y.-C. Chou, J.-Y. Liu, C.-H. Chen, J.-C. Shyu, and F.-P. Chou, "Fluctuation of serum leptin level in rats after ovariectomy and the influence of estrogen supplement," *Life Sciences*, vol. 64, no. 24, pp. 2299–2306, 1999.

[53] A. Alonso, R. Fernández, M. Moreno, P. Ordóñez, F. Díaz, and C. González, "Leptin and its receptor are controlled by 17 beta-estradiol in peripheral tissues of ovariectomized rats," *Experimental Biology and Medicine*, vol. 232, no. 4, pp. 542–549, 2007.

[54] G. Baños, O. N. Medina-Campos, P. D. Maldonado et al., "Antioxidant enzymes in hypertensive and hypertriglyceridemic rats: effect of gender," *Clinical and Experimental Hypertension*, vol. 27, no. 1, pp. 45–57, 2005.

[55] J. Yu, Y. Zhao, B. Li, L. Sun, and H. Huo, "17 β-estradiol regulates the expression of antioxidant enzymes in myocardial cells by increasing Nrf2 translocation," *Journal of Biochemical Molecular Toxicology*, vol. 26, no. 7, pp. 264–269, 2012.

[56] P. Kumar, R. K. Kale, and N. Z. Baquer, "Estradiol modulates membrane-linked ATP ases, antioxidant enzymes, membrane fluidity, lipid peroxidation, and lipofuscin in aged rat liver," *Journal of Aging Research*, vol. 2011, Article ID 580245, 8 pages, 2011.

[57] J. Busserolles, A. Mazur, E. Gueux, E. Rock, and Y. Rayssiguier, "Metabolic syndrome in the rat: females are protected against the pro-oxidant effect of a high sucrose diet," *Experimental Biology and Medicine*, vol. 227, no. 9, pp. 837–842, 2002.

[58] W. Zhao, P. S. Devamanoharan, and S. D. Varma, "Fructose induced deactivation of antioxidant enzymes: preventive effect of pyruvate," *Free Radical Research*, vol. 33, no. 1, pp. 23–30, 2000.

[59] S. B. Pajović and Z. S. Saicić, "Modulation of antioxidant enzyme activities by sexual steroid hormones," *Physiological Research*, vol. 57, no. 6, pp. 801–811, 2008.

[60] K. A. Baltgalvis, S. M. Greising, G. L. Warren, and D. A. Lowe, "Estrogen regulates estrogen receptors and antioxidant gene

expression in mouse skeletal muscle," *PLoS ONE*, vol. 5, no. 4, Article ID e10164, 2010.

[61] S. S. Signorelli, S. Neri, S. Sciacchitano et al., "Behaviour of some indicators of oxidative stress in postmenopausal and fertile women," *Maturitas*, vol. 53, no. 1, pp. 77–82, 2006.

[62] D. Taskiran and V. Evren, "Estradiol protects adipose tissue-derived stem cells against H_2O_2 induced toxicity," *Journal of Biochemical and Molecular Toxicology*, vol. 26, no. 8, pp. 301–307, 2012.

[63] T. E. Richardson, A. E. Yu, Y. Wen, S.-H. Yang, and J. W. Simpkins, "Estrogen prevents oxidative damage to the mitochondria in Friedreich's ataxia skin fibroblasts," *PLoS ONE*, vol. 7, no. 4, Article ID e34600, 2012.

[64] P. H. Jellinck and H. L. Bradlow, "Peroxidase-catalyzed displacement of tritium from regiospecifically labeled estradiol and 2-hydroxyestradiol," *Journal of Steroid Biochemistry*, vol. 35, no. 6, pp. 705–710, 1990.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 917813, 12 pages
http://dx.doi.org/10.1155/2014/917813

Review Article

Bariatric Endocrinology: Principles of Medical Practice

J. Michael Gonzalez-Campoy, Bruce Richardson, Conor Richardson, David Gonzalez-Cameron, Ayesha Ebrahim, Pamela Strobel, Tiphani Martinez, Beth Blaha, Maria Ransom, Jessica Quinonez-Weislow, Andrea Pierson, and Miguel Gonzalez Ahumada

Minnesota Center for Obesity, Metabolism and Endocrinology (MNCOME), 1185 Town Centre Drive, Suite 220, Eagan, MN 55123, USA

Correspondence should be addressed to J. Michael Gonzalez-Campoy; drmike@mncome.com

Received 15 November 2013; Revised 19 March 2014; Accepted 11 April 2014; Published 12 May 2014

Academic Editor: Yi-Hao Yu

Obesity, is a chronic, biological, preventable, and treatable disease. The accumulation of fat mass causes physical changes (adiposity), metabolic and hormonal changes due to adipose tissue dysfunction (adiposopathy), and psychological changes. Bariatric endocrinology was conceived from the need to address the neuro-endocrinological derangements that are associated with adiposopathy, and from the need to broaden the scope of the management of its complications. In addition to the well-established metabolic complications of overweight and obesity, adiposopathy leads to hyperinsulinemia, hyperleptinemia, hypoadiponectinemia, dysregulation of gut peptides including GLP-1 and ghrelin, the development of an inflammatory milieu, and the strong risk of vascular disease. Therapy for adiposopathy hinges on effectively lowering the ratio of orexigenic to anorexigenic signals reaching the the hypothalamus and other relevant brain regions, favoring a lower caloric intake. Adiposopathy, overweight and obesity should be treated indefinitely with the specific aims to reduce fat mass for the adiposity complications, and to normalize adipose tissue function for the adiposopathic complications. This paper defines the principles of medical practice in bariatric endocrinology—the treatment of overweight and obesity as means to treat adiposopathy and its accompanying metabolic and hormonal derangements.

1. Introduction

Overweight and obesity are a continuum, and together they represent a chronic, biological, preventable, and treatable disease. Overweight and obesity are an epidemic affecting two thirds of the American population [1–3]. The accumulation of fat mass leads to physical changes (adiposity), metabolic changes due to adipose tissue dysfunction (adiposopathy), and psychological changes, each of which adversely affects health (Table 1) [4].

Obesity has long been held a risk factor for diabetes, hypertension, dyslipidemia, and premature cardiovascular disease [5–8]. The strong association between intra-abdominal, visceral fat and metabolic disorders led the National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, Adult Treatment Panel III (NCEP-ATP III) to include the measurement of waist circumference as a defining element of the dysmetabolic syndrome [9, 10]. In the NCEP-ATP III initial report, the five elements that defined dysmetabolic syndrome included a waist circumference (≥40 inches in men and ≥35 inches in women), high blood pressure (≥130/85), high fasting plasma glucose concentration (≥110 mg/dL), hypertriglyceridemia (≥150 mg/dL), and low plasma levels of high density lipoprotein cholesterol (HDL-C) (<40 mg/dL in men and <50 mg/dL in women). Three or more of these five diagnostic criteria give an individual the diagnosis of dysmetabolic syndrome, for which the International Classification of Diseases, 9th edition, introduced the code 277.7. Following this report, intra-abdominal or visceral fat became a treatment target in medical practice.

Adipose tissue dysfunction is etiological in the development of the metabolic and endocrine derangements that accompany overweight and obesity. This concept is now well established in the medical literature [8, 11–13]. What follows from acceptance of this premise is near-universal agreement

Table 1: Some complications of obesity by body system.

Cardiovascular
(i) Hypertension
(ii) Congestive heart failure
(iii) Cor pulmonale
(iv) Varicose veins
(v) Peripheral edema
(vi) Pulmonary embolism
(vii) Coronary artery disease
Endocrine (adiposopathy)
(i) The metabolic syndrome
(ii) Type 2 diabetes/hyperinsulinemia
(iii) Dyslipidemia
(iv) Polycystic ovarian syndrome/androgenicity
(v) Amenorrhea/infertility/menstrual disorders
(vi) Hyperleptinemia/hypoadiponectinemia
Gastrointestinal
(i) Gastroesophageal reflux disease (GERD)
(ii) Nonalcoholic fatty liver disease (NAFLD)
(iii) Cholelithiasis
(iv) Hernias
(v) Colon cancer
Genitourinary
(i) Urinary stress incontinence
(ii) Obesity-related glomerulopathy
(iii) Hypogonadism (male)
(iv) Breast and uterine cancer
(v) Pregnancy complications
Integument
(i) Striae distensae (stretch marks)
(ii) Stasis pigmentation of legs
(iii) Lymphedema
(iv) Cellulitis
(v) Intertrigo, carbuncles
(vi) Acanthosis nigricans/skin tags
Musculoskeletal
(i) Hyperuricemia and gout
(ii) Immobility
(iii) Osteoarthritis (knees, hips)
(iv) Low back pain
Neurologic
(i) Stroke
(ii) Idiopathic intracranial hypertension
(iii) Meralgia paresthetica
Psychological
(i) Depression/low self esteem
(ii) Body image disturbance
(iii) Social stigmatization
Respiratory
(i) Dyspnea
(ii) Obstructive sleep apnea
(iii) Hypoventilation syndrome
(iv) Pickwickian syndrome
(v) Asthma

that early weight loss is a desirable part of medical treatment, best implemented prior to the development of complications with irreversible health consequences. The observation that in some cases overweight and obesity are associated with better health outcomes, especially as it relates to vascular disease, the so-called "obesity paradox," has subtracted from the sentiment that overweight and obesity should be treated [14–19]. The obesity paradox is likely due to having better lean mass index and cardiovascular fitness in some people with overweight and obesity, and to differences in treatment. Patients with overweight and obesity that have had vascular events are treated more aggressively than thin patients [17–20]. The "obesity paradox" aside, the formal implementation of a long-standing weight management program that includes pharmacological intervention is still missing from medical practice. Even contemporary reviews by leaders in the field of obesity lack this important element of medical practice. The discussion of the management of overweight and obesity frequently jumps from lifestyle changes to bariatric surgery, and the lack of data on the long-term use of pharmacotherapy is the only mention given to this subject [21, 22].

The drivers for food intake are complex, and hedonic food control plays a major role for many people [23–25]. The hypothalamus is the major control center for energy balance and adipose tissue stores, but other brain centers also play a role [26–29]. Therefore, therapy for adiposopathy hinges on effectively lowering the ratio of orexigenic to anorexigenic signals reaching the the hypothalamus and other relevant brain regions, favoring a lower caloric intake.

Effective reductions of body fat (including intra-abdominal fat) are accomplished by an integrated, team approach to patient care [11]. Adiposopathy, overweight and obesity should be treated indefinitely with the specific aims to reduce fat mass for the adiposity complications, and to normalize adipose tissue function for the adiposopathic complications.

This paper defines the principles of medical practice in bariatric endocrinology—the treatment of overweight and obesity as means to treat adiposopathy and its accompanying metabolic and hormonal derangements.

2. Principle 1—Overweight and Obesity Are a Continuum and Together Represent a Chronic, Biological, Preventable, and Treatable Disease

Adipose tissue is distributed throughout the human body. Adipose tissue is metabolically active, both receiving and sending signals that help regulate metabolism [30–33]. Adiposity, the physical accumulation of fat mass through fat hypertrophy or fat hyperplasia, leads to many mechanical complications that need to be identified and treated, since they are obstacles to effective weight loss. Among these are degenerative osteoarthritis and obstructive sleep apnea [34–39]. The accumulation of fat mass is also strongly associated with psychiatric disease [40–45]. Additionally, psychotropic medications are associated with metabolic diseases (Table 2) [46–49]. Simply on the basis of physical and psychological complications, overweight and obesity meet the definition of a chronic disease.

Table 2: Second-generation antipsychotics and the risk of metabolic abnormalities [47, 49].

Drug	Weight gain	Risk for diabetes mellitus	Worsening lipid profile
Clozapine	+++	+	+
Olanzapine	+++	+	+
Risperidone	++	D	D
Quetiapine	++	D	D
Aripiprazole*	+/−	−	−
Ziprasidone*	+/−	−	−

+: increase effect; −: no effect; D: discrepant results; *newer drugs with limited data.

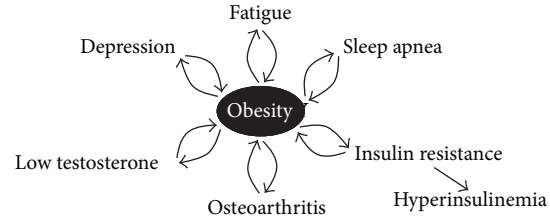


Figure 1: Complications of obesity that interfere with its treatment (Copyright © Minnesota Center for Obesity, Metabolism and Endocrinology, PA).

Overweight and obesity are commonly defined by the body mass index (BMI)—calculated by dividing body weight in kilograms by height in meters squared [50]. Commonly used BMI cutoff values to diagnose obesity have high specificity but low sensitivity to identify adiposity, as they fail to identify half of the people with excess body fat percentage [51]. Thus, newer approaches that seek to quantitate different fat pools and differentiate between lean and fat mass, including DXA body composition analysis, may have more clinical relevance [8, 52–57].

In clinical practice, body weight, waist circumference, and waist-to-hip ratio provide sufficient assessment of metabolic risk. These measurements are best interpreted as they evolve over time. When possible, the differentiation between lean and fat mass is desirable, especially to find individuals who have significant visceral fat mass, but whose BMI might not place them in an overweight or obesity category.

Adiposopathy is a complication of excess fat mass when adipose tissue becomes dysfunctional. Bariatric endocrinology takes into account the physical and psychological complications of overweight and obesity and the role they may play as obstacles to effective weight loss and metabolic health (Figure 1).

3. Principle 2—Every Patient Who Has Overweight or Obesity Should Be Initially Evaluated for Causes and Complications of Weight Gain, Including Adiposopathy

Adipose tissue is not just for the storage of energy. Rather, it is a very active endocrine and metabolic tissue, and it actively helps regulate metabolism. Like any other tissue, adipose tissue may change anatomically, and it may become dysfunctional. The concept that altered anatomy and function of adipose tissue cause metabolic diseases is now well engrained in the medical literature [58–61]. Cardiomyopathy, myopathy, encephalopathy, ophthalmopathy, retinopathy, enteropathy, nephropathy, neuropathy, and dermopathy define disease of specific tissues or organs. Similarly, adiposopathy defines disease of adipose tissue. To continue the analogy, in cardiomyopathy there is enlargement of cardiac myocytes (hypertrophy), which leads to clinical disease (cardiac outlet obstruction). And in adiposopathy there is enlargement of fat cells, leading to clinical disease. Adiposopathy is also anatomically manifested by visceral adiposity, growth of adipose tissue beyond its vascular supply leading to ischemia and inflammation, increased number of adipose tissue immune cells, and ectopic fat deposition (in other body tissues, i.e., fatty liver) [8, 13].

Adiposopathy is functionally manifested by: [12, 13]

(i) impaired adipogenesis with the subsequent development of adipocyte hypertrophy [64–66],

(ii) heterogeneous distribution of adipose tissue (e.g., visceral adiposity),

(iii) adipocyte lipolysis in excess of lipogenesis leading to increased free fatty acid release into the circulation,

(iv) pathogenic adipose tissue endocrine responses (e.g., hypoadiponectinemia and hyperleptinemia),

(v) pathogenic adipose tissue immune responses,

(vi) pathogenic "crosstalk" between adipose tissue and other organs [13, 67].

The adipose tissue changes of adiposopathy affect other tissues, changing homeostasis and leading to derangements of metabolism (e.g., insulin resistance with eventual hyperglycemia, hypogonadism, hepatosteatosis possibly leading to steatohepatitis, etc.) [61] and increased cardiovascular risk [8, 68, 69].

For all of the above reasons, in the evaluation and management of patients with overweight and obesity there must be a thorough evaluation looking for causes and complications of the disease, including a complete history and physical examination and laboratory testing [21, 70–72]. Table 3 lists medications that are commonly associated with weight gain, which should be substituted for as possible. Table 4 lists the laboratory findings that may be found in patients with adiposopathy.

4. Principle 3—Every Patient Who Has Overweight or Obesity Should Have Periodic Risk Restratification

Not all patients with an elevated BMI have adiposopathy and metabolic diseases. And not all patients who have adiposopathy and metabolic diseases have an elevated BMI

Table 3: Selected medications associated with increased adipose tissue mass.

Psychiatric/neurological
(i) Antipsychotic agents: phenothiazine, olanzapine, clozapine, risperidone
(ii) Mood stabilizers: lithium
(iii) Antidepressants: tricyclics, monoamine oxidase inhibitors, selective serotonin reuptake inhibitors (paroxetine hydrochloride), mirtazapine
(iv) Antiepileptic drugs: gabapentin, valproate sodium, carbamazepine
Steroid hormones
(i) Corticosteroids
(ii) Progestational steroids
Antidiabetes agents
Insulin, sulfonylureas, thiazolidinediones
Antihypertensive agents
Beta- and alpha-1 adrenergic receptor blockers
Antihistamines
Cyproheptadine hydrochloride
HIV protease inhibitors
Lipodystrophy (central obesity)

Table 4: Laboratory findings in patients with adiposopathy.

(i) Elevated leptin
(ii) Decreased adiponectin
(iii) Increasing leptin to adiponectin ratio over time
(iv) Increased tumor necrosis factor alpha
(v) Increased c-reactive protein
(vi) High triglycerides/low HDL cholesterol
(vii) Elevated free fatty acids
(viii) Hyperinsulinemia/hyperglycemia
(ix) Activation of renin-angiotensin-aldosterone
(x) Hypoandrogenemia in men
(xi) Hyperandrogenemia in women

(Table 5) [62]. For this reason, it is imperative that patients have a periodic re-stratification of their metabolic risk. In our practice we recommend that all patients who have overweight or obesity by their BMI have a yearly risk re-stratification with laboratory testing (Table 4).

5. Principle 4—There Are No Short-Term Solutions to a Chronic Medical Problem: Overweight and Obesity Should Be Treated with the Same Model of Chronic Disease Management That We Use for Other Chronic Diseases

No one would think of treating diabetes for just a few weeks or months. No one would think of stopping treatment if initial monotherapy for diabetes did not result in the expected reductions in hemoglobin A_{1c}. And no one would ever think of treating congestive heart failure, hypertension, epilepsy, asthma, multiple sclerosis, hypothyroidism, or any other chronic disease, for just a short while. Yet, overweight and obesity are not held in the same light. Historically, treatments for obesity were limited in duration. The genesis of this restriction in duration of treatment was the fear that centrally acting medications might have addictive potential or lead to tolerance. Because of these government agency restrictions, the prevailing attitude in the medical community has been that overweight and obesity are not treatable as a chronic disease.

The last five years have brought about a change in this attitude. Several leading organizations including the American Association of Clinical Endocrinologists [73], the National Lipid Association [8], the Obesity Society/American College of Cardiology/American Heart Association [21], the American Society of Bariatric Physicians [74], and the National Diabetes Education Program [75] have all issued statements calling for the treatment of obesity as a chronic disease. And the American Medical Association (AMA) finally recognized obesity as a chronic disease at the annual meeting of the House of Delegates in Chicago, in June 2013.

The approach to overweight and obesity should be exactly the same as the approach to any other chronic disease. Using diabetes as an example, the management of overweight and obesity over time should follow a parallel track (Table 6).

6. Principle 5—Effective Behavior Modification to Achieve a Negative Energy Balance Is the Primary Long-Term Goal of the Medical Treatment of Overweight and Obesity

The hypothalamus and other relevant brain regions regulate the frequency and volume of feedings [26–28, 76–79]. This is a highly complex process that takes input from throughout

Table 5: Incidence of metabolic disorders by BMI category.

Comorbidity	Overall population with comorbidity, %	Subjects in BMI ranges (kg/m^2), %					
		18.5–24.9	25.0–26.9	27.0–29.9	30.0–34.9	35.9–39.9	≥40
Diabetes*	8.9	4.2	5.7	10.1	12.1	16.4	27.2
Hypertension†	28.9	17.6	25.3	30.8	39.3	44	51.3
Dyslipidemia‡	52.9	38.2	53.1	62.2	68	67.5	62.5

*Includes previously diagnosed and undiagnosed diabetes mellitus. Diagnosed is based on self-report. Undiagnosed is defined using the criterion of fasting glucose >125 mg/dL.
†Elevated blood pressure (systolic ≥140 mm Hg, or diastolic ≥90 mm Hg), or taking antihypertensive medications.
‡Any of the following: total cholesterol ≥240 mg/dL, triglycerides >200 mg/dL, low-density lipoprotein ≥160 mg/dL, or high-density lipoprotein <40 mg/dL.
Adapted from: [62].

Table 6: Management model for diabetes and obesity: a successful model of care.

Diabetes mellitus	Overweight and obesity
Initial workup/risk assessment	Initial workup/risk assessment
Institute treatment	Institute treatment
Patient and family education	Patient and family education
Glucose self-monitoring	Step count and weekly weight
Quarterly office assessments: follow A1C, BMI, BP	Quarterly office assessments: follow BMI, BP, WC
Periodic screening for complications and risk restratification	Periodic screening for complications and risk restratification

A1C: glycosylated hemoglobin A1C; BMI: body mass index; BP: blood pressure; WC: waist circumference.
See [63].

the body, and carefully determines the balance of orexigenic and anorexigenic stimuli both in health and disease [80–87]. The primary long-term goal of the medical management of overweight and obesity is to reset the hypothalamus and other relevant brain regions to allow for satiety at a lower set point.

Behavior modification to achieve a lower caloric intake and an increased level of physical activity (caloric expenditure) should be implemented and maintained. Patients must learn how to achieve these goals. It is not enough to tell a patient that they should restrict calories and become more active. They must be taught *how* to achieve these goals as explicitly as possible.

Simple concepts are important to deliver in patient education and need to be reviewed repeatedly.

(i) It is possible to achieve fullness with fewer calories by increasing the volume of meals with foods that have a lower caloric density [11, 88, 89].

(ii) Drink water instead of high-calories beverages—a twist of lime makes it much more palatable.

(iii) Using a smaller diameter plate for meals makes portions smaller (i.e., the MyPlate campaign by the FDA) [11, 90–92].

(iv) It should be a goal to ingest 10 servings of fresh fruits or vegetables every day [11].

(v) Caloric expenditure is accomplished by moving body mass over distance or against gravity. It does not have to be done fast, or all at once. A two-minute walk every hour on the hour during the waking hours of the day is equivalent to a 30-minute walk [8].

(vi) Avoid the words "diet" and "exercise" and focus on healthy eating and meal planning and on physical activity that is achievable, realistic, sustainable, and incrementable.

(vii) Quantitate physical activity with tools such as a pedometer.

(viii) Involve your support structures at home and at work.

(ix) Push yourself to be physically active when you get hungry. Going up and down two flights of stairs instead of going to the kitchen for food will make it unlikely that hunger prevails.

All of these examples of behavior modification have the common goal of decreasing orexigenic stimuli, and increasing anorexigenic signals to the hypothalamus and other relevant brain regions. It takes time, but by achieving consistent lifestyle changes, the signals that reach the hypothalamus and other relevant brain regions will change to favor a lower set point for energy balance.

7. Principle 6—The Team Approach to Overweight and Obesity Should Be Offered to All Patients to Provide Nutrition Education and Physical Activity Coaching

Obesity lends itself to the model of chronic endocrine disease management that is commonly accepted for other chronic diseases, such as diabetes (Table 6) [63]. Implicit in this model is a comprehensive team approach to patient care with an emphasis on patient education [93–103]. Also implicit in this model of care is the existence of the infrastructure to make patients comfortable—wide chairs, large gowns, scales that accurately measure up to 800 pounds, large blood pressure cuffs, contingency plans that take into account very large

body mass (e.g., preparedness to handle a syncopal episode or a cardiac arrest of a very large person), and staff that has been trained to be understanding and supportive.

Central to patient education and support is the availability of materials to meet individual patient needs. Props and models, electronic media, print media, audiovisual resources, web-based education, and group classes are all viable means to provide education.

The health care team should include specialized clinicians, nurse educators, dietitians, behavioral counselors, mental health professionals, pulmonologists, physical medicine and rehabilitation consultants, physical therapists, pain specialists, dermatologists, gynecologists, oncologists, cardiologists, bariatric surgeons, and others.

8. Principle 7—Pharmacotherapy Should Be Used Indefinitely in the Management of Overweight and Obesity

"It is possible to frame a house with a bucket of nails and a hammer. But it is faster and better if one gets an air compressor and an air gun." This message resonates with patients when introducing the concept of pharmacotherapy for obesity. Pharmacotherapy significantly enhances the beneficial changes of better nutrition and increased physical activity.

Medications for obesity have a tainted past. When amphetamines were used as weight loss agents and their use became popular in the 1950s, it soon became evident that they are habit-forming, tolerance-building, and addictive. Subsequent centrally acting medications were all labelled as having the potential for addictiveness. Because of this concern, and because obesity was considered a condition and not a disease back then, medications for weight loss were approved only for short-term use.

Most recently, rimonabant, which was effective in achieving weight loss, was withdrawn from the market in 2009 because of an association with depression and suicides [104, 105]. Additionally, sibutramine was removed from the market in 2010 because it was not superior to placebo in decreasing vascular events in a population at risk [106–108], and because in the Sibutramine Cardiovascular Outcomes Trial (SCOUT) it was shown to have increased mortality in patients with established arteriosclerosis. These recent developments followed the association of fenfluramine use with valvular heart lesions in the 1990s, leading to an abrupt end to the use of fenfluramine with phentermine (fen-phen) and removal of fenfluramine and dexfenfluramine from the market.

The popular notion that pharmacotherapy for obesity carries a higher risk than benefit, and that the historical restrictions on length of treatment, are proof in practice of this risk, is pervasive in medicine. Indeed, it is pervasive in society. For example, Minnesota has a statute that makes it illegal for the state to pay for any obesity medications.

Thus, pharmacotherapy for obesity will need strong data to support its long-term use. In the meantime, a lack of such data is *not* a reason not to treat patients who have a serious, chronic disease.

The initial approach to overweight and obesity should always be to focus on lifestyle changes. The vast majority of patients will benefit from pharmacotherapy. Initially monotherapy with any one of a number of available agents is appropriate. Economics frequently dictates which agent will be used. Of all obesity medications, generic phentermine is the least expensive and is available, cash-out-of-pocket, for as little as $10.00 USD a month. Other medications for monotherapy will have incremental cost.

Of note, all medications currently approved for overweight and obesity in the United States are pregnancy category X, where the FDA considers the risks involved in use of the drug in pregnant women to clearly outweigh potential benefits. Paradoxically, pregnancy in some women may only be achieved with insulin sensitization and weight loss.

Phentermine, available since the 1950s, is indicated for short-term use, but it continues to be used as an "off-label" single agent for prolonged treatment of overweight and obesity. Phentermine is a trace amine-associated receptor 1 (TAAR1) agonist. TAAR1 is an amine-activated Gs and Gq protein-coupled receptor located at the neural presynaptic membrane [109]. Although the precise mechanism of action of phentermine on the hypothalamus and other relevant brain regions remains unknown, it causes an anorexigenic effect [110–113]. Phentermine also increases adrenergic tone, leading to adrenal release of catecholamines, which causes lipolysis. This adrenergic effect of phentermine mandates careful monitoring of the blood pressure. At clinically relevant doses, phentermine also releases serotonin and dopamine, but to a much lesser extent than norepinephrine [114]. Phentermine is not habit-forming, tolerance-building, or addictive. Discontinuation of phentermine leads to a loss of therapeutic effect and no other untoward effects [115]. Phentermine in combination with topiramate may be used as initial therapy for overweight and obesity and is discussed below.

It is important to note that neither phentermine nor any other currently available centrally acting agents take away a patient's appetite. Appetite is a survival mechanism that ensures a continuous energy supply for the organism's needs and is preserved with the use of phentermine and other weight loss drugs.

Orlistat is a pancreatic lipase inhibitor. By preventing fat break-down during digestion, orlistat causes fat malabsorption and weight loss. The potential side effects of this medication are mostly due to steatorrhea. Anecdotal reports of liver damage and acute oxalate nephropathy have been reported with orlistat use. Orlistat leads to significantly more weight loss than placebo, with the ensuing beneficial metabolic changes associated with it [116–118]. With orlistat treatment 35.5 to 54.8% of patients achieve ≥5% weight loss and 16.4 to 24.8% of patients achieve ≥10% weight loss after one year of treatment [119, 120]. Following 4 years of treatment with orlistat, 28% of the placebo patients and 45% of the orlistat patients lost ≥5% of their baseline body weight and 10% of the placebo patients and 21% of the orlistat patients lost ≥10% of their baseline body weight [116, 117].

Lorcaserin is a selective 5-hydroxytryptamine T_{2C} receptor agonist which promotes weight loss through satiety [121,

122]. The dose of lorcaserin is one 10 mg tablet twice daily. It was approved for the treatment of obesity in the United States in 2012. There are three registration trials for lorcaserin: Behavioral Modification and Lorcaserin for Overweight and Obesity Management (BLOOM) trial [123], Behavioral Modification and Lorcaserin Second Study for Obesity Management (BLOSSOM) trial [124], and the Behavioral Modification and Lorcaserin for Overweight and Obesity Management in Diabetes Mellitus (BLOOM-DM) trial [125]. In comparison to placebo, lorcaserin decreased waist circumference, blood pressure, total cholesterol, low-density lipoprotein-cholesterol and triglycerides. The most frequent adverse events reported with lorcaserin are headache, dizziness, and nausea [126]. Lorcaserin did not statistically affect heart rate, like phentermine does, or high-density lipoprotein-cholesterol [126].

Three other centrally acting drugs are currently FDA-approved for treatment of obesity: benzphetamine, phendimetrazine, and diethylpropion. All three are centrally acting medications that cause early satiety and have similar potential side effects to phentermine.

Bupropion, fluoxetine, and other antidepressants are sometimes useful in the management of obesity. Antidepressants have significant variability on weight. It remains unclear if the effect of antidepressants on weight is due to the medication itself or the treatment of the underlying depression. Some patients lose weight with resolution of depression, and some gain weight.

Other medications that are associated with weight loss include metformin, exenatide, liraglutide, pramlintide, canagliflozin, dapagliflozin, and topiramate monotherapy. None has an approved indication for weight loss.

9. Principle 8—Failure of Monotherapy to Achieve Effective Weight Loss Should Not Lead to Discontinuation of Treatment, but Rather to the Institution of Combination Therapy

Combination therapy for obesity has been an option for decades. Fen-phen, discussed above, helped thousands of patients lose weight and was a prime example of the benefit of combination therapy.

The US Food and Drug Administration requires that the labeling for obesity medications, including orlistat and lorcaserin, calls for discontinuation of these medication if 5% weight loss is not achieved after 12 weeks of treatment. The authors strongly disagree with this approach to the management of overweight, obesity, and adiposopathy. As is the case with diabetes mellitus, dyslipidemia, hypertension, heart failure, asthma, depression, and every other chronic disease, the initial agent used for weight loss monotherapy is unlikely to achieve a full therapeutic goal, even with dose titrations. For this reason, the authors strongly recommend that for overweight, obesity, and adiposopathy, as is the case with other chronic diseases, monotherapy should be continued, and combination therapy should be instituted when the treatment effect of one agent wanes or is insufficient to reach treatment goals. The 5% threshold set by the FDA is arbitrary, especially when one considers that lack of treatment might have led to continued weight gain. Compared to no treatment even those who do not reach the arbitrary 5% threshold benefit from pharmacotherapy. Of note, until the data is generated, prolonged use of older medications, and combinations of medications, other than phentermine-topiramate, which is already in the market, will have to be "off label."

Currently, the only combination product that is available in the United States market is Qsymia, a combination of phentermine with topiramate approved for treatment of obesity in 2012. There are three registration trials: EQUIP [127], EQUATE [128], and CONQUER [129]. Qsymia causes significant weight loss, reduction in waist circumference and improvements in glycemia, lipids, inflammatory markers, and blood pressure. The recommended initial dose is 3.75 mg/23 mg (phentermine 3.75 mg/topiramate 23 mg extended release) daily for 14 days and then increased to 7.5 mg/46 mg daily. If 3% weight loss is not achieved after 12 weeks on the 7.5 mg/46 mg dose, escalation to the maximum dose of 15 mg/92 mg is recommended. The 7.5 mg/46 mg dose should not be exceeded for patients with moderate or severe renal impairment or patients with moderate hepatic impairment. Adverse events of Qsymia included paresthesias, dry mouth, constipation, dysgeusia, and insomnia. Because the topiramate component has teratogenic potential, the combination product carries a black box warning. The doses of both phentermine and topiramate in the extended release combination tablet are lower than those approved for the individual components. Thus, the reported adverse events with phentermine-topiramate CR are manageable, and discontinuation rates are lower than placebo.

10. Principle 9—Bariatric Surgery Should Only Be Considered after Failure of a Formal Medical Management Program for Overweight and Obesity

This paper does not deal with the surgical management of overweight and obesity. Consideration of referral for bariatric surgery must always take place only after patients have had the benefit of the team approach to weight management, including behavior modification and pharmacotherapy. In our hands, most patients meet the goal of 5–10% body weight loss when we approach overweight and obesity like we approach any other chronic diseases. Overweight or obesity refractory to medical management does warrant consideration of bariatric surgery (Figure 2) [8].

11. Principle 10—A Major Goal of Treatment Should Be to Return Adipose Tissue Function toward Normal in Patients with Overweight and Obesity

The primary goal in the management of overweight and obesity is to correct their complications. A parallel goal

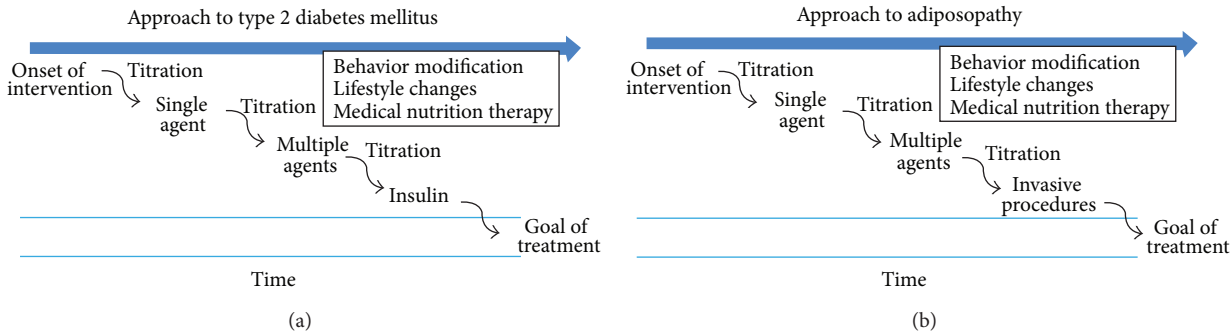


Figure 2: Models of chronic disease management as a practical future approach to treatment of adiposity and adiposopathy [8].

is to return adipose tissue function to normal through effective weight loss. Any weight loss will improve the fat-mass (adiposity) related complications. When weight loss is achieved, it is usually a global loss of fat mass. Thus, fat depots like intra-abdominal and visceral fat are also effectively reduced in most cases [8, 12]. In some individuals, however, visceral fat mass is preserved, and the metabolic disorders associated with it persist [69, 130–135]. For this reason, ongoing monitoring is necessary for all patients. Resolution of laboratory abnormalities or signs and symptoms due to adiposopathy (e.g., resumption of regular menstrual flows in women with polycystic ovarian syndrome) should be the desired outcome, not just the loss of pounds of body weight.

12. Conclusion

This paper defines the principles of medical practice in bariatric endocrinology—the treatment of overweight and obesity as a means to treat adiposopathy and its accompanying metabolic and hormonal derangements. Overweight and obesity are a continuum and together represent a chronic, biological preventable and treatable disease. The goal of treatment is not only to decrease fat mass, but also to return adipose tissue dysfunction to normal. The principles of chronic disease management that apply to other chronic diseases also apply to the management of overweight, obesity, and adiposopathy.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] A. Feneberg and P. Malfertheiner, "Epidemic trends of obesity with impact on metabolism and digestive diseases," *Digestive Diseases*, vol. 30, no. 2, pp. 143–147, 2012.

[2] Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults—The Evidence Report, "National Institutes of Health," *Obesity Research*, vol. 6, no. 2, pp. 51S–209S, 1998.

[3] Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary, "Expert panel on the identification, evaluation, and treatment of overweight in adults," *The American Journal of Clinical Nutrition*, vol. 68, pp. 899–917, 1998.

[4] S. R. Smith, J. C. Lovejoy, F. Greenway et al., "Contributions of total body fat, abdominal subcutaneous adipose tissue compartments, and visceral adipose tissue to the metabolic complications of obesity," *Metabolism: Clinical and Experimental*, vol. 50, no. 4, pp. 425–435, 2001.

[5] NIH Health implications of obesity, "National institutes of health consensus development conference statement," *Annals of Internal Medicine*, vol. 103, pp. 1073–1077, 1985.

[6] A. H. Mokdad, E. S. Ford, B. A. Bowman et al., "Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001," *Journal of the American Medical Association*, vol. 289, no. 1, pp. 76–79, 2003.

[7] O. Tochikubo, E. Miyajima, K. Okabe, K. Imai, and M. Ishii, "Improvement of multiple coronary risk factors in obese hypertensives by reduction of intra-abdominal visceral fat," *Japanese Heart Journal*, vol. 35, no. 6, pp. 715–725, 1994.

[8] H. E. Bays, P. P. Toth, P. M. Kris-Etherton et al., "Obesity, adiposity, and dyslipidemia: a consensus statement from the National Lipid Association," *Journal of Clinical Lipidology*, vol. 7, no. 4, pp. 304–383, 2013.

[9] National Cholesterol Education Program Expert Panel on Detection E, Treatment of High Blood Cholesterol in A, "Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report," *Circulation*, vol. 106, no. 25, pp. 3143–3421, 2002.

[10] J. I. Cleeman, "Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III)," *Journal of the American Medical Association*, vol. 285, no. 19, pp. 2486–2497, 2001.

[11] J. M. Gonzalez-Campoy, S. T. S. Jeor, K. Castorino et al., "Clinical practice guidelines for healthy eating for the prevention and treatment of metabolic and endocrine diseases in adults: cosponsored by the American Association of Clinical Endocrinologists/the American College of Endocrinology and the Obesity Society," *Endocrine Practice: Official Journal of the*

American College of Endocrinology and the American Association of Clinical Endocrinologists, vol. 19, supplement 3, pp. 1–82, 2013.

[12] H. E. Bays and J. M. Gonzalez-Campoy, "Adiposopathy," in *New-Opathies*, E. Friedberg, D. H. Castrillon, R. L. Galindo, and K. Wharton, Eds., pp. 105–168, 2012.

[13] H. E. Bays, J. M. González-Campoy, G. A. Bray et al., "Pathogenic potential of adipose tissue and metabolic consequences of adipocyte hypertrophy and increased visceral adiposity," *Expert Review of Cardiovascular Therapy*, vol. 6, no. 3, pp. 343–368, 2008.

[14] D. B. Diercks, M. T. Roe, J. Mulgund et al., "The obesity paradox in non-ST-segment elevation acute coronary syndromes: results from the Can Rapid risk stratification of Unstable angina patients Suppress ADverse outcomes with Early implementation of the American College of Cardiology/American Heart Association Guidelines Quality Improvement Initiative," *American Heart Journal*, vol. 152, no. 1, pp. 140–148, 2006.

[15] J.-P. Desprs, "Excess visceral adipose tissue/ectopic fat: the missing link in the obesity paradox?" *Journal of the American College of Cardiology*, vol. 57, no. 19, pp. 1887–1889, 2011.

[16] O. Ozeke, C. Ozer, M. Gungor, M. K. Celenk, H. Dincer, and G. Ilicin, "Chronic intermittent hypoxia caused by obstructive sleep apnea may play an important role in explaining the morbidity-mortality paradox of obesity," *Medical Hypotheses*, vol. 76, no. 1, pp. 61–63, 2011.

[17] M. Lainscak, S. von Haehling, W. Doehner, and S. D. Anker, "The obesity paradox in chronic disease: facts and numbers," *Journal of Cachexia, Sarcopenia and Muscle*, vol. 3, no. 1, pp. 1–4, 2012.

[18] C. J. Lavie, A. De Schutter, D. A. Patel, A. Romero-Corral, S. M. Artham, and R. V. Milani, "Body composition and survival in stable coronary heart disease: impact of lean mass index and body fat in the 'Obesity Paradox'," *Journal of the American College of Cardiology*, vol. 60, no. 15, pp. 1374–1380, 2012.

[19] P. A. McAuley, E. G. Artero, X. Sui et al., "The obesity paradox, cardiorespiratory fitness, and coronary heart disease," *Mayo Clinic Proceedings*, vol. 87, pp. 443–451, 2012.

[20] L. Schenkeveld, M. Magro, R. M. Oemrawsingh et al., "The influence of optimal medical treatment on the 'obesity paradox', body mass index and long-term mortality in patients treated with percutaneous coronary intervention: a prospective cohort study," *BMJ Open*, vol. 2, no. 1, Article ID 000535, 2012.

[21] M. D. Jensen, D. H. Ryan, C. M. Apovian et al., "AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association task force on practice guidelines and the obesity society," *Circulation*, 2013.

[22] J. I. Mechanick, R. F. Kushner, H. J. Sugerman et al., "Clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient—2013 update: cosponsored by American Association of Clinical Endocrinologists, the Obesity Society, and American Society for Metabolic & Bariatric Surgery," *Surgery for Obesity and Related Diseases*, vol. 9, pp. 159–191, 2013.

[23] P. Malaviya and C. M. Brendl, "Do hedonic motives moderate regulatory focus motives? Evidence from the framing of persuasive messages," *Journal of Personality and Social Psychology*, vol. 106, pp. 1–19, 2014.

[24] A. A. Witt, G. A. Raggio, M. L. Butryn, and M. R. Lowe, "Do hunger and exposure to food affect scores on a measure of hedonic hunger? An experimental study," *Appetite*, vol. 74, pp. 1–5, 2014.

[25] A. A. Witt and M. R. Lowe, "Hedonic hunger and binge eating among women with eating disorders," *International Journal of Eating Disorders*, vol. 47, no. 3, pp. 273–280, 2014.

[26] C. Zhan, J. Zhou, Q. Feng et al., "Acute and long-term suppression of feeding behavior by POMC neurons in the brainstem and hypothalamus, respectively," *The Journal of Neuroscience*, vol. 33, no. 8, pp. 3624–3632, 2013.

[27] J. H. Jennings, G. Rizzi, A. M. Stamatakis, R. L. Ung, and G. D. Stuber, "The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding," *Science*, vol. 341, no. 6153, pp. 1517–1521, 2013.

[28] S. J. Guyenet, M. E. Matsen, G. J. Morton, K. J. Kaiyala, and M. W. Schwartz, "Rapid glutamate release in the mediobasal hypothalamus accompanies feeding and is exaggerated by an obesogenic food," *Molecular Metabolism*, vol. 2, no. 2, pp. 116–122, 2013.

[29] T. R. Stratford and D. Wirtshafter, "Evidence that the nucleus accumbens shell, ventral pallidum, and lateral hypothalamus are components of a lateralized feeding circuit," *Behavioural Brain Research*, vol. 226, no. 2, pp. 548–554, 2012.

[30] S. A. Brown, B. Levi, C. Lequex, V. W. Wong, A. Mojallal, and M. T. Longaker, "Basic science review on adipose tissue for clinicians," *Plastic and Reconstructive Surgery*, vol. 126, no. 6, pp. 1936–1946, 2010.

[31] S. E. Wozniak, L. L. Gee, M. S. Wachtel, and E. E. Frezza, "Adipose tissue: The new endocrine organ? a review article," *Digestive Diseases and Sciences*, vol. 54, no. 9, pp. 1847–1856, 2009.

[32] A. Schäffler and C. Büchler, "Concise review: adipose tissue-derived stromal cells—basic and clinical implications for novel cell-based therapies," *Stem Cells*, vol. 25, no. 4, pp. 818–827, 2007.

[33] J. K. Sethi and A. J. Vidal-Puig, "Thematic review series: adipocyte Biology. Adipose tissue function and plasticity orchestrate nutritional adaptation," *Journal of Lipid Research*, vol. 48, no. 6, pp. 1253–1262, 2007.

[34] M. Lopata and E. Onal, "Mass loading, sleep apnea, and the pathogenesis of obesity hypoventilation," *American Review of Respiratory Disease*, vol. 126, no. 4, pp. 640–645, 1982.

[35] S. Rossner, "Snoring, sleep apnea and obesity," *Acta Medica Scandinavica*, vol. 221, no. 3, pp. 225–226, 1987.

[36] E. H. Wittels and S. Thompson, "Obstructive sleep apnea and obesity," *Otolaryngologic Clinics of North America*, vol. 23, no. 4, pp. 751–760, 1990.

[37] G. A. Bray, "Medical consequences of obesity," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 6, pp. 2583–2589, 2004.

[38] J. J. Anderson and D. T. Felson, "Factors associated with osteoarthritis of hte knee in the first National Health and Nutrition Examination Survey (HANES I). Evidence for an association with overweight, race, and physical demands of work," *American Journal of Epidemiology*, vol. 128, no. 1, pp. 179–189, 1988.

[39] M. A. Allman-Farinelli, R. J. Aitken, L. A. King, and A. E. Bauman, "Osteoarthritis—the forgotten obesity-related epidemic with worse to come," *Medical Journal of Australia*, vol. 188, no. 5, p. 317, 2008.

[40] R. O. Moreira, K. F. Marca, J. C. Appolinario, and W. F. Coutinho, "Increased waist circumference is associated with an increased prevalence of mood disorders and depressive

symptoms in obese women," *Eating and Weight Disorders*, vol. 12, no. 1, pp. 35–40, 2007.

[41] D. Arterburn, E. O. Westbrook, E. J. Ludman et al., "Relationship between obesity, depression, and disability in middle-aged women," *Obesity Research and Clinical Practice*, vol. 6, pp. e197–e206, 2012.

[42] G. E. Simon, D. Arterburn, P. Rohde et al., "Obesity, depression, and health services costs among middle-aged women," *Journal of General Internal Medicine*, vol. 26, no. 11, pp. 1284–1290, 2011.

[43] L. B. Palmese, P. C. DeGeorge, J. C. Ratliff et al., "Insomnia is frequent in schizophrenia and associated with night eating and obesity," *Schizophrenia Research*, vol. 133, no. 1–3, pp. 238–243, 2011.

[44] E. Incledon, M. Wake, and M. Hay, "Psychological predictors of adiposity: systematic review of longitudinal studies," *International Journal of Pediatric Obesity*, vol. 6, no. 2, pp. e1–e11, 2011.

[45] W. Greggersen, S. Rudolf, E. Fassbinder et al., "Major depression, borderline personality disorder, and visceral fat content in women," *European Archives of Psychiatry and Clinical Neuroscience*, vol. 261, no. 8, pp. 551–557, 2011.

[46] U. Zimmermann, T. Kraus, H. Himmerich, A. Schuld, and T. Pollmächer, "Epidemiology, implications and mechanisms underlying drug-induced weight gain in psychiatric patients," *Journal of Psychiatric Research*, vol. 37, no. 3, pp. 193–220, 2003.

[47] American Diabetes Association, American Psychiatric Association,, American Association of Clinical Endocrinologists, and North American Association for the Study of Obesity, "Consensus development conference on antipsychotic drugs and obesity and diabetes," *Diabetes Care*, vol. 27, no. 2, pp. 596–601, 2004.

[48] G. P. Reynolds and S. L. Kirk, "Metabolic side effects of antipsychotic drug treatment - pharmacological mechanisms," *Pharmacology and Therapeutics*, vol. 125, no. 1, pp. 169–179, 2010.

[49] L. V. Kessing, A. F. Thomsen, U. B. Mogensen, and P. K. Andersen, "Treatment with antipsychotics and the risk of diabetes in clinical practice," *British Journal of Psychiatry*, vol. 197, no. 4, pp. 266–271, 2010.

[50] NIH Clinical Guidelines on the indentification, evaluation, and treatment of overweight and obesity in adults, "The evidence report," in *U.S. Department of Health and Human Services; Public Health Service; National Institutes of Health*, National Heart, Lung, and Blood Institutes, 1998.

[51] D. O. Okorodudu, M. F. Jumean, V. M. Montori et al., "Diagnostic performance of body mass index to identify obesity as defined by body adiposity: a systematic review and meta-analysis," *International Journal of Obesity*, vol. 34, no. 5, pp. 791–799, 2010.

[52] A. Shuster, M. Patlas, J. H. Pinthus, and M. Mourtzakis, "The clinical importance of visceral adiposity: a critical review of methods for visceral adipose tissue analysis," *British Journal of Radiology*, vol. 85, no. 1009, pp. 1–10, 2012.

[53] A. Leppik, T. Jürimäe, and J. Jürimäe, "Influence of anthropometric parameters on the body composition measured by bioelectrical impedance analysis or DXA in children," *Acta Paediatrica, International Journal of Paediatrics*, vol. 93, no. 8, pp. 1036–1041, 2004.

[54] M. D. Van Loan, "Estimates of Fat-free mass (FFM) by densitometry, dual energy X-ray absorptiometry (DXA), and Bioimpedance spectroscopy (BIS)in Caucasian and Chinese-American women," *Applied Radiation and Isotopes*, vol. 49, no. 5-6, pp. 751–752, 1998.

[55] J. L. Clasey, C. Bouchard, C. D. Teates et al., "The use of anthropometric and dual-energy X-ray absorptiometry (DXA) measures to estimate total abdominal and abdominal visceral fat in men and women," *Obesity Research*, vol. 7, no. 3, pp. 256–264, 1999.

[56] M. A. Bredella, R. H. Ghomi, B. J. Thomas et al., "Comparison of DXA and CT in the assessment of body composition in premenopausal women with obesity and anorexia nervosa," *Obesity*, vol. 18, no. 11, pp. 2227–2233, 2010.

[57] J. LaForgia, J. Dollman, M. J. Dale, R. T. Withers, and A. M. Hill, "Validation of DXA body composition estimates in obese men and women," *Obesity*, vol. 17, no. 4, pp. 821–826, 2009.

[58] H. Bays, "Adiposopathy, metabolic syndrome, quantum physics, general relativity, chaos and the theory of everything," *Expert Review of Cardiovascular Therapy*, vol. 3, no. 3, pp. 393–404, 2005.

[59] H. Bays, N. Abate, and M. Chandalia, "Adiposopathy: sick fat causes high blood sugar, high blood pressure and dyslipidemia," *Future Cardiol*, vol. 1, no. 1, pp. 39–59, 2005.

[60] H. Bays, "Adiposopathy: role of adipocyte factors in a new paradigm," *Expert Review of Cardiovascular Therapy*, vol. 3, no. 2, pp. 187–189, 2005.

[61] H. E. Bays, J. M. González-Campoy, R. R. Henry et al., "Is adiposopathy (sick fat) an endocrine disease?" *International Journal of Clinical Practice*, vol. 62, no. 10, pp. 1474–1483, 2008.

[62] H. Bays and C. A. Dujovne, "Adiposopathy is a more rational treatment target for metabolic disease than obesity alone," *Current Atherosclerosis Reports*, vol. 8, no. 2, pp. 144–156, 2006.

[63] MMA. Minnesota Medical Association Obesity Task Force, "How to evaluate and treat obesity," *Minnesota Medicine*, vol. 88, pp. 40–46, 2005.

[64] N. Kubota, Y. Terauchi, H. Miki et al., "PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance," *Molecular Cell*, vol. 4, no. 4, pp. 597–609, 1999.

[65] P. Imbeault, S. Lemieux, D. Prud'homme et al., "Relationship of visceral adipose tissue to metabolic risk factors for coronary heart disease: is there a contribution of subcutaneous fat cell hypertrophy?" *Metabolism: Clinical and Experimental*, vol. 48, no. 3, pp. 355–362, 1999.

[66] T. J. Ryan and S. B. Curri, "Hypertrophy and atrophy of fat," *Clinics in Dermatology*, vol. 7, no. 4, pp. 93–106, 1989.

[67] J.-P. Després, "Inflammation and cardiovascular disease: is abdominal obesity the missing link?" *International Journal of Obesity*, vol. 27, no. 3, pp. S22–S24, 2003.

[68] M. Shields, M. S. Tremblay, S. C. Gorber, and I. Janssen, "Abdominal obesity and cardiovascular disease risk factors within body mass index categories," *Health Reports*, vol. 23, no. 2, pp. 7–15, 2012.

[69] M. A. Kamimura, J. J. Carrero, M. E. Canziani, R. Watanabe, M. M. Lemos, and L. Cuppari, "Visceral obesity assessed by computed tomography predicts cardiovascular events in chronic kidney disease patients," *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 23, no. 9, pp. 891–897, 2012.

[70] L. J. Aronne, D. S. Nelinson, and J. L. Lillo, "Obesity as a disease state: A new paradigm for diagnosis and treatment," *Clinical Cornerstone*, vol. 9, no. 4, pp. 9–29, 2009.

[71] V. A. Moyer, "Screening for and management of obesity in adults: U.S. preventive services task force recommendation statement," *Annals of Internal Medicine*, vol. 17, no. 5, pp. 1–32, 2012.

[72] R. F. Kushner and R. L. Weinsier, "Evaluation of the obese patient: practical considerations," *Medical Clinics of North America*, vol. 84, no. 2, pp. 387–399, 2000.

[73] A. J. Garber, M. J. Abrahamson, J. I. Barzilay et al., "AACE comprehensive diabetes management algorithm," *Endocrine Practice*, vol. 19, pp. 327–336, 2013.

[74] American Society of Bariatric Physicians Obesity Algorithm: Adult Adiposity Evaluation and Treatment, 2013.

[75] NDEP, "Guiding principles for diabetes care: for health care professionals," in *National Diabetes Education Program*, 2009.

[76] R. Gutierrez, M. K. Lobo, F. Zhang, and L. De Lecea, "Neural integration of reward, arousal, and feeding: recruitment of VTA, lateral hypothalamus, and ventral striatal neurons," *IUBMB Life*, vol. 63, no. 10, pp. 824–830, 2011.

[77] M. F. Wiater, S. Mukherjee, A.-J. Li et al., "Circadian integration of sleep-wake and feeding requires NPY receptorexpressing neurons in the mediobasal hypothalamus," *American Journal of Physiology, Regulatory Integrative and Comparative Physiology*, vol. 301, no. 5, pp. 1569–1583, 2011.

[78] K. W. Williams and J. K. Elmquist, "Lighting up the hypothalamus: coordinated control of feeding behavior," *Nature Neuroscience*, vol. 14, no. 3, pp. 277–278, 2011.

[79] D. Leroith, "Foreword. Control of feeding behavior and the peripheral metabolism by the hypothalamus," *Endocrinology and metabolism clinics of North America*, vol. 37, no. 4, pp. 11–13, 2008.

[80] L. M. Oyama, C. M. O. D. Nascimento, J. Carnier et al., "The role of anorexigenic and orexigenic neuropeptides and peripheral signals on quartiles of weight loss in obese adolescents," *Neuropeptides*, vol. 44, no. 6, pp. 467–474, 2010.

[81] J. Carnier, A. De Piano, P. De Lima Sanches et al., "The role of orexigenic and anorexigenic factors in an interdisciplinary weight loss therapy for obese adolescents with symptoms of eating disorders," *International Journal of Clinical Practice*, vol. 64, no. 6, pp. 784–790, 2010.

[82] N. A. King, P. P. Caudwell, M. Hopkins, J. R. Stubbs, E. Naslund, and J. E. Blundell, "Dual-process action of exercise on appetite control: increase in orexigenic drive but improvement in meal-induced satiety," *American Journal of Clinical Nutrition*, vol. 90, no. 4, pp. 921–927, 2009.

[83] S. C. Benoit, A. L. Tracy, J. F. Davis, D. Choi, and D. J. Clegg, "Novel functions of orexigenic hypothalamic peptides: from genes to behavior," *Nutrition*, vol. 24, no. 9, pp. 843–847, 2008.

[84] K. Diepvens, D. Häberer, and M. Westerterp-Plantenga, "Different proteins and biopeptides differently affect satiety and anorexigenic/orexigenic hormones in healthy humans," *International Journal of Obesity*, vol. 32, no. 3, pp. 510–518, 2008.

[85] J. Menyhért, G. Wittmann, E. Hrabovszky, É. Keller, Z. Liposits, and C. Fekete, "Interconnection between orexigenic neuropeptide Y- and anorexigenic α-melanocyte stimulating hormone-synthesizing neuronal systems of the human hypothalamus," *Brain Research*, vol. 1076, no. 1, pp. 101–105, 2006.

[86] R. H. Mak, W. Cheung, R. D. Cone, and D. L. Marks, "Orexigenic and anorexigenic mechanisms in the control of nutrition in chronic kidney disease," *Pediatric Nephrology*, vol. 20, no. 3, pp. 427–431, 2005.

[87] Y. Takimoto, A. Inui, H. Kumano, and T. Kuboki, "Orexigenic/anorexigenic signals in Bulimia nervosa," *Current Molecular Medicine*, vol. 3, no. 4, pp. 349–360, 2003.

[88] S. E. Shaffer and B. J. Tepper, "Effects of learned flavor cues on single meal and daily food intake in humans," *Physiology and Behavior*, vol. 55, no. 6, pp. 979–986, 1994.

[89] D. A. Levitsky, "The non-regulation of food intake in humans: hope for reversing the epidemic of obesity," *Physiology and Behavior*, vol. 86, no. 5, pp. 623–632, 2005.

[90] R. C. Post, J. Eder, S. Maniscalco, D. Johnson-Bailey, and S. Bard, "MyPlate is now reaching more consumers through social media," *Journal of the Academy of Nutrition and Dietetics*, vol. 113, no. 6, pp. 754–755, 2013.

[91] M. Ackley, "MyPlate—make it great!," *Ohio Nurses Review*, vol. 87, p. 4, 2012.

[92] R. Post, J. Haven, and S. Maniscalco, "Putting MyPlate to work for nutrition educators," *Journal of Nutrition Education and Behavior*, vol. 44, no. 2, pp. 98–99, 2012.

[93] J. McCaffree, "Position of the American Dietetic Association: integration of medical nutrition therapy and pharmacotherapy," *Journal of the American Dietetic Association*, vol. 103, no. 10, pp. 1363–1370, 2003.

[94] N. Durant and J. Cox, "Current treatment approaches to overweight in adolescents," *Current Opinion in Pediatrics*, vol. 17, no. 4, pp. 454–459, 2005.

[95] A. Frank, "A multidisciplinary approach to obesity management: the physician's role and team care alternatives," *Journal of the American Dietetic Association*, vol. 98, no. 10, pp. S44–S48, 1998.

[96] J. M. Ashley, S. T. S. Jeor, J. P. Schrage et al., "Weight control in the physician's office," *Archives of Internal Medicine*, vol. 161, no. 13, pp. 1599–1604, 2001.

[97] M. C. Battista, M. Labonte, J. Menard et al., "Dietitian-coached management in combination with annual endocrinologist follow up improves global metabolic and cardiovascular health in diabetic participants after 24 months," *Applied Physiology, Nutrition, and Metabolism*, vol. 37, no. 4, pp. 610–620, 2012.

[98] S. Cummings, E. S. Parham, and G. W. Strain, "Position of the American Dietetic Association: weight management," *Journal of the American Dietetic Association*, vol. 102, no. 8, pp. 1145–1155, 2002.

[99] A. R. Gabriel, "Shedding pounds for life. How a dietitian can help. In the war against weight gain, a registered dietitian can be your most important ally," *Diabetes forecast*, vol. 56, no. 4, pp. 93–94, 2003.

[100] L. A. Leiter, "A dietitian-led intervention reduced weight and waist circumference in obese patients with type 2 diabetes," *ACP Journal Club*, vol. 142, no. 1, p. 17, 2005.

[101] K. S. Rhodes, L. C. Bookstein, L. S. Aaronson, N. M. Mercer, and C. E. Orringer, "Intensive nutrition counseling enhances outcomes of national cholesterol education program dietary therapy," *Journal of the American Dietetic Association*, vol. 96, no. 10, pp. 1003–1010, 1996.

[102] T. L. Taillefer, "Nurses and dietitians collaborating to impact nutrition and diabetes mellitus management issues for patients with type 2 diabetes mellitus on hemodialysis," *Nephrology Nursing Journal: Journal of the American Nephrology Nurses' Association*, vol. 35, no. 5, pp. 503–505, 2008.

[103] F. K. Welty, M. M. Nasca, N. S. Lew, S. Gregoire, and Y. Ruan, "Effect of onsite dietitian counseling on weight loss and lipid levels in an outpatient physician office," *American Journal of Cardiology*, vol. 100, no. 1, pp. 73–75, 2007.

[104] K. Johansson, K. Neovius, S. M. DeSantis, S. Rössner, and M. Neovius, "Discontinuation due to adverse events in randomized trials of orlistat, sibutramine and rimonabant: a meta-analysis," *Obesity Reviews*, vol. 10, no. 5, p. 586, 2009.

[105] D. Taylor, "Withdrawal of rimonabant—walking the tightrope of 21st century pharmaceutical regulation?" *Current Drug Safety*, vol. 4, no. 1, pp. 2–4, 2009.

[106] S. Czernichow and D. Batty, "Editorial: Withdrawal of sibutramine for weight loss: where does this leave clinicians?" *Obesity Facts*, vol. 3, no. 3, pp. 155–156, 2010.

[107] G. Williams, "Withdrawal of sibutramine in Europe," *BMJ*, vol. 340, p. c824, 2010.

[108] A. Sayburn, "Withdrawal of sibutramine leaves European doctors with just one obesity drug," *BMJ*, vol. 340, p. c477, 2010.

[109] L. S. Barak, A. Salahpour, X. Zhang et al., "Pharmacological characterization of membrane-expressed human trace amine-associated receptor 1 (TAAR1) by a bioluminescence resonance energy transfer cAMP biosensor," *Molecular Pharmacology*, vol. 74, no. 3, pp. 585–594, 2008.

[110] J. Yelnosky, R. E. Panasevich, A. R. Borrelli, and R. B. Lawlor, "Pharmacology of phentermine," *Archives internationales de pharmacodynamie et de therapie*, vol. 178, no. 1, pp. 62–76, 1969.

[111] R. B. Lawlor, M. C. Trivedi, and J. Yelnosky, "A determination of the anorexigenic potential of dl-amphetamine, d-amphetamine, l-amphetamine and phentermine," *Archives internationales de pharmacodynamie et de therapie*, vol. 179, no. 2, pp. 401–407, 1969.

[112] K. W. Sehnert, "Development of phentermine, an appetite-control drug," *Clinical medicine*, vol. 70, pp. 400–403, 1963.

[113] B. A. Becker, "Pharmacologic activity of phentermine (Phenyl-t-butylamine)," *Toxicology and Applied Pharmacology*, vol. 3, no. 2, pp. 256–259, 1961.

[114] R. B. Rothman, M. H. Baumann, C. M. Dersch et al., "Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin," *Synapse*, vol. 39, pp. 32–41, 2001.

[115] E. J. Hendricks and F. L. Greenway, "A study of abrupt phentermine cessation in patients in a weight management program," *American Journal of Therapeutics*, vol. 18, no. 4, pp. 292–299, 2011.

[116] J. S. Torgerson, J. Hauptman, M. N. Boldrin, and L. Sjöström, "XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients," *Diabetes Care*, vol. 27, no. 3, p. 856, 2004.

[117] M. C. Mancini and A. Halpern, "Orlistat in the prevention of diabetes in the obese patient," *Vascular Health and Risk Management*, vol. 4, no. 2, pp. 325–336, 2008.

[118] Y. H. Zhou, X. Q. Ma, C. Wu et al., "Effect of anti-obesity drug on cardiovascular risk factors: a systematic review and meta-analysis of randomized controlled trials," *PLoS ONE*, vol. 7, no. 6, Article ID e39062, 2012.

[119] S. Henness and C. M. Perry, "Orlistat: a review of its use in the management of obesity," *Drugs*, vol. 66, no. 12, pp. 1625–1656, 2006.

[120] S. O'Meara, R. Riemsma, L. Shirran, L. Mather, and G. Ter Riet, "A systematic review of the clinical effectiveness of orlistat used for the management of obesity," *Obesity Reviews*, vol. 5, no. 1, pp. 51–68, 2004.

[121] C. K. Martin, L. M. Redman, J. Zhang et al., "Lorcaserin, a 5-HT2C receptor agonist, reduces body weight by decreasing energy intake without influencing energy expenditure," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 3, pp. 837–845, 2011.

[122] W. J. Thomsen, A. J. Grottick, F. Menzaghi et al., "Lorcaserin, a novel selective human 5-hydroxytryptamine2C agonist: in vitro and in vivo pharmacological characterization," *Journal of Pharmacology and Experimental Therapeutics*, vol. 325, no. 2, pp. 577–587, 2008.

[123] S. R. Smith, N. J. Weissman, C. M. Anderson et al., "Multicenter, placebo-controlled trial of lorcaserin for weight management," *The New England Journal of Medicine*, vol. 363, no. 3, pp. 245–256, 2010.

[124] M. C. Fidler, M. Sanchez, B. Raether et al., "A one-year randomized trial of lorcaserin for weight loss in obese and overweight adults: the BLOSSOM Trial," *Journal of Clinical Endocrinology and Metabolism*, vol. 96, no. 10, pp. 3067–3077, 2011.

[125] P. M. O'Neil, S. R. Smith, N. J. Weissman et al., "Randomized placebo-controlled clinical trial of lorcaserin for weight loss in type 2 diabetes mellitus: the BLOOM-DM study," *Obesity*, vol. 20, pp. 1426–1436, 2012.

[126] E. W. Chan, Y. He, C. S. Chui, A. Y. Wong, W. C. Lau, and I. C. Wong, "Efficacy and safety of lorcaserin in obese adults: a meta-analysis of 1-year randomized controlled trials (RCTs) and narrative review on short-term RCTs," *Obesity Reviews*, vol. 14, no. 5, pp. 383–392, 2013.

[127] D. B. Allison, K. M. Gadde, W. T. Garvey et al., "Controlled-release phentermine/topiramate in severely obese adults: a randomized controlled trial (EQUIP)," *Obesity*, vol. 20, no. 2, pp. 330–342, 2012.

[128] H. Bays, "Phentermine, topiramate and their combination for the treatment of adiposopathy ('sick fat) and metabolic disease," *Expert Review of Cardiovascular Therapy*, vol. 8, no. 12, pp. 1777–1801, 2010.

[129] K. M. Gadde, D. B. Allison, D. H. Ryan et al., "Effects of low-dose, controlled-release, phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial," *The Lancet*, vol. 377, no. 9774, pp. 1341–1352, 2011.

[130] T. Saito, M. Murata, T. Otani, H. Tamemoto, M. Kawakami, and S.-E. Ishikawa, "Association of subcutaneous and visceral fat mass with serum concentrations of adipokines in subjects with type 2 diabetes mellitus," *Endocrine Journal*, vol. 59, no. 1, pp. 39–45, 2012.

[131] A. Michaud, R. Drolet, S. Noël, G. Paris, and A. Tchernof, "Visceral fat accumulation is an indicator of adipose tissue macrophage infiltration in women," *Metabolism: Clinical and Experimental*, vol. 61, no. 5, pp. 689–698, 2012.

[132] X. Li, M. Katashima, T. Yasumasu, and K. J. Li, "Visceral fat area, waist circumference and metabolic risk factors in abdominally obese Chinese adults," *Biomedical and Environmental Sciences*, vol. 25, pp. 141–148, 2012.

[133] J. Lee, D.-S. Chung, J.-H. Kang, and B.-Y. Yu, "Comparison of visceral fat and liver fat as risk factors of metabolic syndrome," *Journal of Korean Medical Science*, vol. 27, no. 2, pp. 184–189, 2012.

[134] F. Item and D. Konrad, "Visceral fat and metabolic inflammation: the portal theory revisited," *Obesity Reviews*, vol. 13, supplement 2, pp. 30–39, 2012.

[135] C. S. Hung, C. Y. Yang, H. J. Hsieh, J. N. Wei, W. Y. Ma, and H. Y. Li, "BMI correlates better to visceral fat and insulin sensitivity than BAI," *Obesity*, vol. 20, no. 6, p. 1141, 2012.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 730827, 7 pages
http://dx.doi.org/10.1155/2014/730827

Review Article

Visceral Adiposity Index: An Indicator of Adipose Tissue Dysfunction

Marco Calogero Amato and Carla Giordano

Section of Cardio-Respiratory and Endocrine-Metabolic Diseases, Biomedical Department of Internal and Specialist Medicine (DIBIMIS), University of Palermo, Piazza delle Cliniche 2, 90127 Palermo, Italy

Correspondence should be addressed to Marco Calogero Amato; marcoamato7@gmail.com

Received 12 December 2013; Accepted 29 March 2014; Published 14 April 2014

Academic Editor: Yi-Hao Yu

The Visceral Adiposity Index (VAI) has recently proven to be an indicator of adipose distribution and function that indirectly expresses cardiometabolic risk. In addition, VAI has been proposed as a useful tool for early detection of a condition of cardiometabolic risk before it develops into an overt metabolic syndrome. The application of the VAI in particular populations of patients (women with polycystic ovary syndrome, patients with acromegaly, patients with NAFLD/NASH, patients with HCV hepatitis, patients with type 2 diabetes, and general population) has produced interesting results, which have led to the hypothesis that the VAI could be considered a marker of adipose tissue dysfunction. Unfortunately, in some cases, on the same patient population, there is conflicting evidence. We think that this could be mainly due to a lack of knowledge of the application limits of the index, on the part of various authors, and to having applied the VAI in non-Caucasian populations. Future prospective studies could certainly better define the possible usefulness of the VAI as a predictor of cardiometabolic risk.

1. Introduction

The BMI is wrongly considered a satisfactory predictor of the percentage of body fat, and it is known that it shows a curvilinear and not a linear association with the body fat percentage in both men and women [1].

Beyond the criticisms recently leveled [2] towards the normalization of weight by the square of the height (normalization suggested in the 19th century by Adolphe Quetelet), many factors affect the relationship between BMI and body fat percentage, such as gender, race, high muscle mass (e.g., subjects who practice body building), and changes in hydration status (in particular subjects having retention of extracellular fluids may lead to significant mistakes in interpretation about BMI). In older people, significant changes also occur in both BMI numerator and denominator.

Also, in 2005 in JAMA Katherine Flegal published one of the first studies [3] to analyze in detail the correlation between BMI and all causes of mortality on a large series. This study, based on National Health and Nutrition Examination Survey (NHANES) data, showed that BMI is not a good predictor of mortality risk. A subsequent meta-analysis published in *Lancet* in 2006 [4], evaluating 40 epidemiological studies for a mean followup of 3.8 years, confirmed Flegal's data. These data led in 2006 to an editorial [5] published in Lancet, provocatively titled "Should we continue to use BMI as a cardiovascular risk factor?" which states that "the BMI may now be withdrawn permanently as a clinical or epidemiological tool for evaluation of cardiovascular risk in both primary and secondary prevention."

This is a condition which deep-down does not appear desecrating to those who are concerned with prevention of cardiovascular disease: in fact the BMI does not appear in any of the versions of the algorithm of Framingham, one of the strongest tools for defining coronary and cardiovascular risk. Indeed, in the Framingham study it was assumed that there could be "metabolically healthy obese subjects."

An important contribution to evaluation of the influence of obesity on cardiovascular risk is the Interheart Study [6], which shows inconvertible evidence that abdominal obesity makes a higher contribution than BMI to the probability of these events. The following year, focusing attention on the

relation between obesity and heart attack risk, Yusuf et al. published a study [7] defining the results more accurately, showing that the association between abdominal adiposity and coronary heart disease risk is highly significant in all geographical areas in which Interheart Study data were collected.

These data incontrovertibly seem to support the usefulness of the evaluation of waist circumference (WC) in cardiovascular risk stratification, having been considered the most valid index of regional distribution of adipose tissue. Measurement of body circumferences, although it is a valid method, requires an accurate performance method in order to provide reproducible information. Several studies have shown that WC is strongly related to visceral fat and abdominal adiposity, more than BMI and waist/hip ratio.

The only limitation of WC is inaccurate distinction between visceral and subcutaneous adipose tissue in the abdominal region.

2. A New Method for Evaluation of Adipose Distribution and Function (Visceral Adiposity Index)

The Visceral Adiposity Index (VAI) is an empirical-mathematical model, gender-specific, based on simple anthropometric (BMI and WC) and functional parameters (triglycerides (TG) and HDL cholesterol (HDL)), and indicative of fat distribution and function [8]. It is an empirical-mathematical model that does not originate from theoretical assumptions, but from observation in a healthy normal/overweight population of a linear relationship between BMI and CV, from which a linear equation has been extrapolated (Figure 1).

At first a model of adipose distribution (MOAD) was created based on this linear equation (which shows a strong correlation with visceral fat mass determined by MRI. Subsequently MOAD was corrected for triglyceride and HDL cholesterol levels, determining the VAI:

$$\begin{aligned}\text{Females: VAI} &= \left(\frac{\text{WC}}{36.58 + (1.89 \times \text{BMI})}\right) \\ &\quad \times \left(\frac{\text{TG}}{0.81}\right) \times \left(\frac{1.52}{\text{HDL}}\right), \\ \text{Males: VAI} &= \left(\frac{\text{WC}}{39.68 + (1.88 \times \text{BMI})}\right) \\ &\quad \times \left(\frac{\text{TG}}{1.03}\right) \times \left(\frac{1.31}{\text{HDL}}\right),\end{aligned} \tag{1}$$

where WC is expressed in cm, BMI in K/m^2, TG in mmol/L, and HDL in mmol/L.

The VAI has shown a strong positive correlation with peripheral glucose utilization during euglycemic hyperinsulinemic clamp and seems to be independently associated with cardio- and cerebrovascular events [8]. In the last three years, it has been reported on more than 30 publications, in which the capability of the VAI to express a possible "adipose tissue dysfunction" and the cardiometabolic risk associated to it have been evaluated.

3. VAI in the General Population as a Marker of Cardiometabolic Risk

The main aim of our research field on the VAI has been to identify a simple clinical marker of adipose tissue dysfunction (indirectly reflecting cardiometabolic risk), before it develops into an overt metabolic syndrome and/or a cardiovascular complication. Unfortunately, today there are still no long-term prospective studies that allow us to evaluate the predictive power of the VAI regarding cardiovascular risk.

Our first study on the VAI [8], in a population of 1,498 Caucasian primary care patients, already showed that there was a strong independent association with both cardiovascular (odd ratio (95% CI): 2.45 (1.52–3.95)) and cerebrovascular events (odd ratio (95% CI): 1.63 (1.06–2.50)); in the same study, a receiver operating characteristic (ROC) analysis proved greater sensitivity and specificity of VAI, compared to its individual components (WC, BMI, HDL, and TG) with regard to cardiovascular and cerebrovascular events. In another ROC analysis on 1518 Peruvian adults, in which various measures of adiposity were evaluated, VAI, WC, and waist-height ratio (WHtR) were the best predictors of the individual components of the metabolic syndrome [9]. In particular, the VAI showed good predictive power regarding the visceral adiposity-related risk of type 2 diabetes [10–12] and hypertension [13]. Although in some studies the increase in VAI was significantly associated with a significant reduction in insulin sensitivity [8, 14, 15], VAI is not to be seen as an index of insulin sensitivity, but as an indicator of altered adipose function which is associated with insulin resistance.

Although the VAI was modelled on a Caucasian population, several studies confirm the validity of its use with other races. For example, in a large case-control study, a high VAI is associated with elevated risk of CHD in Chinese men and women [16]. Moreover, in a large cross-sectional study on 1,764 primary care patients, through an ROC analysis, appropriate stratified-for-age cut-offs were identified that were able to identify a supposed adipose tissue dysfunction [17] (Table 1). These cut-offs have been more or less confirmed in a recent study (data not yet published) in which adipose tissue dysfunction was directly investigated through a large panel of proinflammatory adipokines.

Despite this suggestive evidence, there are also conflicting data like those shown by an Iranian study, which concluded that using the "complex" VAI, instead of other simple anthropometric measures, may lead to loss of much information needed for predicting incident CVD [18]. Another Canadian study also indicated the nonsuperiority of the VAI, compared to BMI and WC, in predicting the visceral adipose tissue change in postmenopausal women during a low-calorie diet [19]. These data suggest the need for further prospective studies which take into account the greater variability in the years of the VAI, compared to other simple anthropometric measures.

Table 1: Age-stratified cut-off points of VAI for identification of adipose tissue dysfunction (ATD).

	ATD absent	Mild ATD	Moderate ADT	Severe ADT
Age < 30 years	≤2.52	2.53–2.58	2.59–2.73	>2.73
≥30 < 42 years	≤2.23	2.24–2.53	2.54–3.12	>3.12
≥42 < 52 years	≤1.92	1.93–2.16	2.17–2.77	>2.77
≥52 < 66 years	≤1.93	1.94–2.32	2.32–3.25	>3.25
≥66 years	≤2	2.01–2.41	2.42–3.17	>3.17

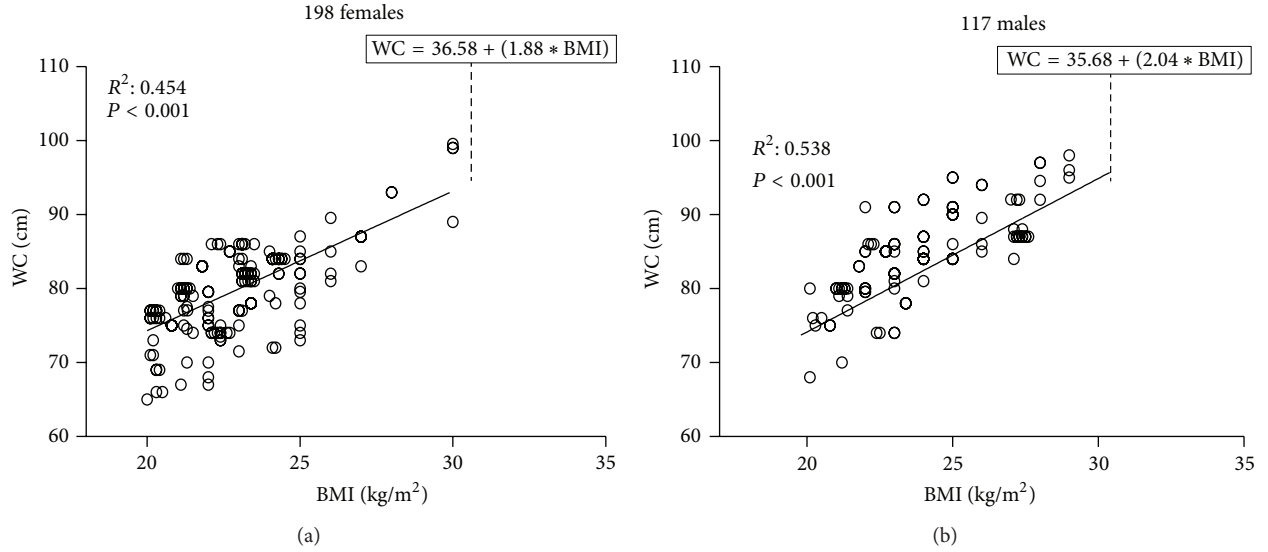


Figure 1: Linear relationship observed between BMI and WC in 315 primary care patients, with BMI between 20 and 30 Kg/m^2 and age 43.46 ± 14.30 years (range 19–83), selected because of absence of diabetes mellitus or FPG ≥ 5.6 mmol/L, high blood pressure, dyslipidemia, metabolic syndrome and cardiovascular disease (CVD). A model of adipose distribution (MOAD) was created based on this gender-specific linear equation. Taken from Amato et al. [8].

4. VAI in NAFLD and NASH

In the hepatological field, the VAI has been investigated in several studies in patients with NAFLD, with the main objective of identifying a clinical marker predictive of evolution towards necroinflammatory injury and fibrosis [20–25]. In this respect, there are contrasting results between the various studies, since according to some authors the VAI accurately predicted progressive liver histology more accurately than other validated noninvasive scores and identified patients with NAFLD at increased CVD risk [20, 24, 25], while according to other authors [21–23] the VAI is not more powerful than other anthropometric indices in discriminating steatosis from steatohepatitis. In our opinion, these discrepancies are attributable to differences between the patients enrolled, especially concerning the variables included in the VAI. This especially applies (as explained in the proper use and limits section) to the mean of triglyceride levels in the various populations [26]. Moreover, in the hepatological field, an interesting result was obtained from a study [27] on patients with chronic hepatitis C due to genotype 1. In these patients only older age, high VAI, and fibrosis were independently associated with moderate-severe necroinflammatory activity by a logistic regression analysis; a higher VAI also has a direct correlation with viral load. Probably, adipose tissue dysfunction (indirectly expressed by the VAI) by way of free fatty acid and proinflammatory cytokine secretion could directly participate in both liver steatosis and induction of inflammation. In this complex interplay between the liver and adipose tissue, HCV could have an important role. It is possible not only that adipose tissue could provide fatty substrates and a proinflammatory status, favouring HCV replication, but also that HCV could interfere with adipocyte function indirectly by increasing the inflammatory status and directly by colonizing adipocytes or immune cells infiltrating adipose tissue [28, 29].

5. VAI in Various Endocrine Diseases

It is now known that carbohydrate, lipid, and protein metabolism and the whole cardiovascular system respond to multiple endocrine signals, including those originating from the adipose endocrine organ. Therefore, there are several endocrine diseases that expose the patient to a significant cardiometabolic risk. This risk is unfortunately not easily identifiable through the classic systems of valuation (risk charts, Framingham risk score, etc.) based on classical cardiovascular risk factors. Nor, in this field, can one rely on the presence of obesity; it is now known that endocrinopathy may

increase cardiovascular risk even in lean subjects. Therefore, especially in young patients with endocrine disease, who have not yet developed an overt metabolic syndrome, the application of the VAI could give useful information.

5.1. VAI in the Polycystic Ovary Syndrome (PCOS). Insulin resistance and abdominal obesity are common but not universal features of PCOS, and they are not always associated with an increased BMI. Indeed, many studies have shown that both lean and obese women with PCOS have insulin resistance [30], though recently it has been shown that in PCOS there is an intrinsic IR that is further worsened by increasing BMI [31]. It is also known that obesity is not necessarily an expression of cardiometabolic risk, given that there exists "metabolically healthy obesity" in which the particular gynoid distribution of fat does not confer a cardiometabolic risk [32]. According to these findings, in women with PCOS, it is necessary to identify a simple clinical index able to distinguish metabolically healthy polycystic ovary syndrome (MH-PCOS) from metabolically unhealthy PCOS (MU-PCOS). In first study of ours [33] it was found that the oligomenorrhoic phenotypes of PCOS (applying the Rotterdam criteria) were characterized by a high VAI and a condition of cardiometabolic risk. Recently in young Korean women with PCOS, the VAI positively correlated with the visceral fat area (measured with computed tomography) and visceral-to-subcutaneous fat ratio and negatively correlated with the insulin-mediated glucose utilization (M value) during euglycemic hyperinsulinemic clamp [14]. Another recent study shows that in women with PCOS the VAI increases in relation to the severity of anovulation, insulin resistance, and inflammation [34]. Recently these findings have led us to verify whether it was possible to distinguish women with MH-PCOS from women with MU-PCOS through the use of simple diagnostic tools such as BMI, waist to hip ratio (WHR), the at-risk category suggested by Androgen Excess Society (AES) [35], and the VAI. Despite the risk according to AES, a BMI $> 27\,kg/m^2$ and a VAI > 1.675 have similar diagnostic value in detecting adverse metabolic profile in women with PCOS, a risk that according to AES and BMI $> 27\,kg/m^2$ criteria tends to overestimate the problem; therefore, given the simplicity of WC and BMI measurement and TG and HDL assessment, it has been suggested that the VAI could be an easy and useful tool for the assessment of MU-PCOS in daily clinical practice and in population studies [36].

5.2. VAI in Acromegaly. Untreated acromegalic patients have a decreased fat mass and increased lean body mass due to the lipolytic effect of GH [37, 38]. In such patients the subcutaneous adipose tissue is reduced, but the visceral adipose tissue secretes a number of cytokines that may cause adipocyte dysfunction and contribute to insulin resistance in the liver and skeletal muscle and adversely affect pancreatic β-cell function [39]. This pathophysiological condition can escape the dichotomous criteria of metabolic syndrome, especially because of the absence in many cases of an increased waist circumference. Although the VAI is a gender-specific index (separately modelled on healthy women and healthy men), in women with active acromegaly it is strongly associated with insulin resistance, adipose tissue dysfunction, and cardiometabolic risk, especially in the postmenopausal age [40]. In another study [41] the VAI also appears to be associated with disease activity, inversely with adiponectin levels, insulin sensitivity, and insulin secretion, and independently correlated with GH levels. These data suggest that the VAI could therefore be used as a useful tool for the assessment of cardiometabolic risk associated with active acromegaly, especially in postmenopausal acromegalic women.

5.3. VAI in Prolactinoma. Much is known about the effects of prolactinomas on the reproductive system, but few data are yet available regarding metabolism and adipose tissue function. However, in both men and women alterations have been observed in the distribution of adipose tissue, probably related to chronic hyperprolactinemia [42, 43]. In two recent studies it has been shown that in patients with prolactinoma cabergoline treatment is able to significantly reduce the VAI and improve metabolic profile and insulin sensitivity [44, 45]. In clinical practice, in young patients with prolactinoma without overt metabolic involvement, the VAI could be useful both at diagnosis and during the treatment followup, to prevent the metabolic complications of the disease.

5.4. VAI in Cushing Disease. The application of VAI in overt Cushing's syndrome, on the basis of the typical clinical and phenotypic alterations of the disease, is probably not very useful. The fact is that almost all patients with overt Cushing have a pathological increase in waist circumference, BMI, and triglycerides and lower HDL cholesterol. In these patients (who for the reasons indicated above present a high VAI), the use of the index adds nothing to simple application of the metabolic syndrome criteria. Furthermore, if on the one hand the VAI is probably an indicator of adipose dysfunction, on the other hand it is known that all patients with Cushing have visceral fat dysfunction. In our opinion, the only application field of the VAI in Cushing's syndrome is epidemiological studies with a high sample size, taking into account the well-known limits of the index. In a recent study, the VAI was tested in 140 patients with Cushing's syndrome [46]; in this study women with Cushing showed a significantly higher VAI (considering that in men with Cushing the VAI was also high, compared to values observed in the general population), consequent of the influence of cortisol excess on visceral adipose dysfunction. The increase in the VAI, specifically described in women with Cushing, confirms the loss of gender-related cardiovascular protection of the women when they develop an increase in visceral adipose tissue, as also observed in other endocrine diseases, such as acromegaly and prolactinoma [40, 44]. On the basis of these experiences, even if we consider unnecessary the use of the VAI in case-control studies on Cushing patients versus healthy populations, this index could show its usefulness in prospective studies which contemplate therapeutic outcomes.

6. Proper Use and Limits of Visceral Adiposity Index

The scientific evidence of the last three years on the use of VAI has allowed us to understand the usefulness of the index and especially also its limitations. These have been summarized in a recent letter to the journal NMCD [26]. The main aspect to consider is that the VAI is an indicator of early cardiometabolic risk in all borderline conditions in which overt metabolic syndrome is not present. This is explained by the fact that three of the variables making up the VAI (WC, TG, and HDL) are dichotomically expressed in the criteria for metabolic syndrome. Also, of the four variables that make up the VAI, triglycerides present the problem of the wide range of values found in the general population, and waist circumference the problem of the validity of measurement in subjects with morbid obesity and pendulous abdomen. For these reasons it has been recommended in an individual patient or in small sample studies the VAI should not be applied, above all in the presence of morbid obesity, pendulous abdomen, severe hypertriglyceridemia, and/or use of fibrates [26]. In this regard, we observed that, above values of serum triglycerides > 3.15 mmol/L (279 mg/dL), the impact of triglyceridemia on the VAI becomes very crucial: in these cases triglycerides alone can provide us with much more information than the VAI regarding the cardiometabolic risk associated with adipose dysfunction. The same can also be said about subjects with morbid obesity, in which even WC has no great diagnostic value. An example of incorrect application of the VAI is a recent study in a population with obstructive sleep apnoea, where the authors wanted to test the association with the severity of sleep apnoea [47]. In actual fact, in many patients with this problem morbid obesity and a metabolic syndrome occurred.

Another important aspect that deserves to be investigated concerns changes in the VAI with a low-calorie diet. Theoretically a healthy weight loss achieved with a mildly reduced calorie-balanced diet (which does not result in intense and rapid lipolysis), possibly accompanied by aerobic physical activity, should result in a reduction in the VAI. This aspect has not yet been studied. An original recent study found that Ramadan fasting in healthy adult men was associated with significant decreases in body weight, BMI, waist circumference to height ratio (WHtR), and Body Adiposity Index (BAI), but we found no significant changes in the VAI [48]. These data indicate that this particular form of fasting for a period of about a month, even if it determines weight loss, does not reduce the cardiometabolic risk.

Another important limitation to consider is the application of the VAI in non-Caucasian populations and in patients aged less than 16 years. The fact is that the numerical factors of the index arise from a mathematical modelling process on healthy Caucasian men and women, aged between 19 and 83 years. In this regard a study was recently published which evaluated the VAI in children [49]. The authors rightly conclude that the VAI should be extrapolated with caution in this age range. We will add more: the VAI "absolutely should not be applied in this age range," because the numerical factors that make up the formula of the VAI are derived by the linear equation linking the BMI and waist circumference in a healthy adult population and mean levels of triglycerides and HDL cholesterol in the same population (Online-Only Appendix in [8]).

Having made these considerations, we recommend the application of VAI in the following populations: healthy or apparently healthy population with BMI < 40 kg/m^2, patients with one or two of the 5 components of the metabolic syndrome, women with PCOS, and patients with endocrine disorders (e.g., acromegaly, adult GH deficiency, hypogonadism, hyperprolactinemia, or abnormal thyroid function).

7. Conclusion

An appropriate use of the VAI (which necessarily implies absolute knowledge of what we have already defined as limits of application) could help us in various clinical situations to assess the complex phenomenon of "adipose tissue dysfunction," especially in the absence of an overt metabolic syndrome. Unfortunately, today the ability of the VAI to express adipose tissue function is only to be seen as a hypothesis; indeed few studies exist that have evaluated the correlation between it and adipocytokine production [11, 25, 41]. However, in our recent study [50] we evaluated the correlations among various anthropometric indices (BMI, waist circumference (WC), hip circumference (HC), waist/hip ratio (WHR), BAI, and Visceral Adiposity Index (VAI)) and several adipocytokines (visfatin, resistin, leptin, soluble leptin receptors (sOB-R), adiponectin, ghrelin, adipsin, PAI-1, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) TNF-α, hs-CRP, IL-6, and IL-18) in patients with type 2 diabetes. Our data suggest that the VAI, among the most common indexes of adiposity assessment, would be an easy tool for clearly mirroring adipose tissue dysfunction and its associated cardiometabolic risk.

In conclusion, although still lacking prospective studies that can attribute a prognostic role to the VAI regarding cardiovascular risk, given the simplicity of WC and BMI measurement and triglycerides and HDL cholesterol assessment, we suggest that the VAI would be an easy tool for the evaluation of adipose tissue dysfunction and its associated cardiometabolic risk in various patient populations, mainly in the absence of an overt metabolic syndrome.

Conflict of Interests

The authors declare that no competing interests exist.

References

[1] D. Gallagher, S. B. Heymsfield, M. Heo, S. A. Jebb, P. R. Murgatroyd, and Y. Sakamoto, "Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index," *The American Journal of Clinical Nutrition*, vol. 72, no. 3, pp. 694–701, 2000.

[2] http://www.ox.ac.uk/media/science_blog/130116.html.

[3] K. M. Flegal, B. I. Graubard, D. F. Williamson, and M. H. Gail, "Excess deaths associated with underweight, overweight, and

obesity," *The Journal of the American Medical Association*, vol. 293, no. 15, pp. 1861–1867, 2005.

[4] A. Romero-Corral, V. M. Montori, V. K. Somers et al., "Association of bodyweight with total mortality and with cardiovascular events in coronary artery disease: a systematic review of cohort studies," *The Lancet*, vol. 368, no. 9536, pp. 666–678, 2006.

[5] M. G. Franzosi, "Should we continue to use BMI as a cardiovascular risk factor?" *The Lancet*, vol. 368, no. 9536, pp. 624–625, 2006.

[6] S. Yusuf, S. Hawken, S. Ounpuu et al., "Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study," *The Lancet*, vol. 364, no. 9438937, p. 952, 2004.

[7] S. Yusuf, S. Hawken, S. Ôunpuu et al., "Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case-control study," *The Lancet*, vol. 366, no. 9497, pp. 1640–1649, 2005.

[8] M. C. Amato, C. Giordano, M. Galia et al., "Visceral adiposity index: a reliable indicator of visceral fat function associated with cardiometabolic risk," *Diabetes Care*, vol. 33, no. 4, pp. 920–922, 2010.

[9] K. M. Knowles, L. L. Paiva, S. E. Sanchez et al., "Waist circumference, body mass index, and other measures of adiposity in predicting cardiovascular disease risk factors among peruvian adults," *International Journal of Hypertension*, vol. 2011, Article ID 931402, 10 pages, 2011.

[10] M. Bozorgmanesh, F. Hadaegh, and F. Azizi, "Predictive performance of the visceral adiposity index for a visceral adiposity-related risk: type 2 Diabetes," *Lipids in Health and Disease*, vol. 10, article 88, 2011.

[11] N. M. Al-Daghri, O. S. Al-Attas, M. S. Alokail et al., "Visceral adiposity index is highly associated with adiponectin values and glycaemic disturbances," *European Journal of Clinical Investigation*, vol. 43, no. 2, pp. 183–189, 2013.

[12] T. Du, X. Sun, R. Huo, and X. Yu, "Visceral adiposity index, hypertriglyceridemic waist and risk of diabetes: the China health and nutrition survey 2009," *International Journal of Obesity*, 2013.

[13] M. Stepien, A. Stepien, M. Banach et al., "New obesity indices and adipokines in normotensive patients and patients with hypertension: comparative pilot analysis," *Angiology*, vol. 65, no. 4, pp. 333–342, 2014.

[14] J. Y. Oh, Y. A. Sung, and H. J. Lee, "The visceral adiposity index as a predictor of insulin resistance in young women with polycystic ovary syndrome," *Obesity*, vol. 21, no. 8, pp. 1690–1694, 2013.

[15] M. Stepien, A. Stepien, R. N. Wlazel et al., "Predictors of insulin resistance in patients with obesity: a pilot study," *Angiology*, vol. 65, no. 1, pp. 22–30, 2014.

[16] X. Zhang, X. O. Shu, H. Li et al., "Visceral adiposity and risk of coronary heart disease in relatively lean Chinese adults," *International Journal of Cardiology*, vol. 168, no. 3, pp. 2141–2145, 2013.

[17] M. C. Amato, C. Giordano, M. Pitrone, and A. Galluzzo, "Cut-off points of the visceral adiposity index (VAI) identifying a visceral adipose dysfunction associated with cardiometabolic risk in a Caucasian Sicilian population," *Lipids in Health and Disease*, vol. 10, article 183, 2011.

[18] B. Mohammadreza, H. Farzad, K. Davoud, and A. Fereidoun, "Prognostic significance of the complex "visceral adiposity index" versus simple anthropometric measures: Tehran lipid and glucose study," *Cardiovascular Diabetology*, vol. 11, article 20, 2012.

[19] B. Elisha, V. Messier, A. Karelis et al., "The visceral adiposity index: relationship with cardiometabolic risk factors in obese and overweight postmenopausal women—a MONET group study," *Applied Physiology, Nutrition, and Metabolism*, vol. 38, no. 8, pp. 892–899, 2013.

[20] Y. Li, L. Liu, B. Wang, J. Wang, and D. Chen, "Simple steatosis is a more relevant source of serum inflammatory markers than omental adipose tissue," *Clinics and Research in Hepatology and Gastroenterology*, vol. 38, no. 1, pp. 46–54, 2014.

[21] Y. Li, L. Liu, B. Wang, and D. Chen, "Letter: is visceral adiposity index a predictor of liver histology in patients with non-alcoholic fatty liver disease?" *Alimentary Pharmacology and Therapeutics*, vol. 37, no. 5, p. 583, 2013.

[22] G. Musso, M. Cassader, F. de Michieli, F. Rosina, F. Orlandi, and R. Gambino, "Nonalcoholic steatohepatitis versus steatosis: adipose tissue insulin resistance and dysfunctional response to fat ingestion predict liver injury and altered glucose and lipoprotein metabolism," *Hepatology*, vol. 56, no. 3, pp. 933–942, 2012.

[23] R. Vongsuvanh, J. George, D. McLeod, and D. van der Poorten, "Visceral adiposity index is not a predictor of liver histology in patients with non-alcoholic fatty liver disease," *Journal of Hepatology*, vol. 57, no. 2, pp. 392–398, 2012.

[24] G. Musso, M. Cassader, and R. Gambino, "Diagnostic accuracy of adipose insulin resistance index and visceral adiposity index for progressive liver histology and cardiovascular risk in nonalcoholic fatty liver disease," *Hepatology*, vol. 56, no. 2, pp. 788–789.

[25] S. Petta, M. C. Amato, V. Di Marco et al., "Visceral adiposity index is associated with significant fibrosis in patients with non-alcoholic fatty liver disease," *Alimentary Pharmacology and Therapeutics*, vol. 35, no. 2, pp. 238–247, 2012.

[26] M. C. Amato and C. Giordano, "Clinical indications and proper use of visceral adiposity index," *Nutrition, Metabolism & Cardiovascular Diseases*, vol. 23, no. 8, pp. e31–e32, 2013.

[27] S. Petta, M. Amato, D. Cabibi et al., "Visceral adiposity index is associated with histological findings and high viral load in patients with chronic hepatitis C due to genotype 1," *Hepatology*, vol. 52, no. 5, pp. 1543–1552, 2010.

[28] J. R. Ticehurst, F. M. Hamzeh, and D. L. Thomas, "Factors affecting serum concentrations of hepatitis C virus (HCV) RNA in HCV genotype 1-infected patients with chronic hepatitis," *Journal of Clinical Microbiology*, vol. 45, no. 8, pp. 2426–2433, 2007.

[29] W. Chen, T. Wong, G. Tomlinson, M. Krahn, and E. J. Heathcote, "Prevalence and predictors of obesity among individuals with positive hepatitis C antibody in a tertiary referral clinic," *Journal of Hepatology*, vol. 49, no. 5, pp. 711–717, 2008.

[30] A. Dunaif, "Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis," *Endocrine Reviews*, vol. 18, no. 6, pp. 774–800, 1997.

[31] N. K. Stepto, S. Cassar, A. E. Joham et al., "Women with polycystic ovary syndrome have intrinsic insulin resistance on euglycaemic-hyperinsulaemic clamp," *Human Reproduction*, vol. 28, no. 3, pp. 777–784, 2013.

[32] J. P. Després, "What is "metabolically healthy obesity"? From epidemiology to pathophysiological insights," *The Journal of Clinical Endocrinology and Metabolism*, vol. 97, no. 7, pp. 2283–2285, 2012.

[33] M. C. Amato, M. Verghi, A. Galluzzo, and C. Giordano, "The oligomenorrhoic phenotypes of polycystic ovary syndrome are characterized by a high visceral adiposity index: a likely condition of cardiometabolic risk," *Human Reproduction*, vol. 26, no. 6, pp. 1486–1494, 2011.

[34] I. I. Androulakis, E. Kandaraki, C. Christakou et al., "Visceral adiposity index (VAI) is related to the severity of anovulation and other clinical features in women with polycystic ovary syndrome," *Clinical Endocrinology*, 2014.

[35] R. A. Wild, E. Carmina, E. Diamanti-Kandarakis et al., "Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the androgen excess and polycystic ovary syndrome (AE-PCOS) society," *The Journal of Clinical Endocrinology and Metabolism*, vol. 95, no. 5, pp. 2038–2049, 2010.

[36] M. C. Amato, V. Guarnotta, D. Forti, M. Donatelli, S. Dolcimascolo, and C. Giordano, "Metabolically healthy polycystic ovary syndrome (MH-PCOS) and metabolically unhealthy polycystic ovary syndrome (MU-PCOS): a comparative analysis of four simple methods useful for metabolic assessment," *Human Reproduction*, vol. 28, no. 7, pp. 1919–1928, 2013.

[37] L. Katznelson, "Alterations in body composition in acromegaly," *Pituitary*, vol. 12, no. 2, pp. 136–142, 2009.

[38] P. U. Freda, W. Shen, S. B. Heymsfield et al., "Lower visceral and subcutaneous but higher intermuscular adipose tissue depots in patients with growth hormone and insulin-like growth factor I excess due to acromegaly," *The Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 6, pp. 2334–2343, 2008.

[39] Y. F. Zhao, D. D. Feng, and C. Chen, "Contribution of adipocyte-derived factors to beta-cell dysfunction in diabetes," *International Journal of Biochemistry and Cell Biology*, vol. 38, no. 5-6, pp. 804–819, 2006.

[40] A. Ciresi, M. C. Amato, R. Pivonello et al., "The metabolic profile in active acromegaly is gender-specific," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 1, pp. E51–E59, 2013.

[41] A. Ciresi, M. C. Amato, G. Pizzolanti, and C. G. Galluzzo, "Visceral adiposity index is associated with insulin sensitivity and adipocytokine levels in newly diagnosed acromegalic patients," *The Journal of Clinical Endocrinology and Metabolism*, vol. 97, no. 8, pp. 2907–2915, 2012.

[42] E. C. O. Naliato, A. H. D. Violante, M. Gaccione et al., "Body fat in men with prolactinoma," *Journal of Endocrinological Investigation*, vol. 31, no. 11, pp. 985–990, 2008.

[43] E. C. O. Naliato, A. H. Violante, D. Caldas et al., "Body fat in nonobese women with prolactinoma treated with dopamine agonists," *Clinical Endocrinology*, vol. 67, no. 6, pp. 845–852, 2007.

[44] A. Ciresi, M. C. Amato, V. Guarnotta, F. Lo Castro, and C. Giordano, "Higher doses of cabergoline further improve metabolic parameters in patients with prolactinoma regardless of the degree of reduction in prolactin levels," *Clinical Endocrinology*, vol. 79, no. 6, pp. 845–852, 2013.

[45] R. S. Auriemma, L. Granieri, M. Galdiero et al., "Effect of chronic treatment with cabergoline on metabolic parameters in patients with prolactinomas," *Neuroendocrinology*, vol. 98, no. 4, pp. 299–310, 2013.

[46] C. Giordano, V. Guarnotta, R. Pivonello et al., "Is diabetes in cushing syndrome only a consequence of hypercortisolism?" *European Journal of Endocrinology*, vol. 170, no. 2, pp. 311–319, 2013.

[47] E. Mazzuca, S. Battaglia, O. Marrone et al., "Gender-specific anthropometric markers of adiposity, metabolic syndrome and visceral adiposity index (VAI) in patients with obstructive sleep apnea," *Journal of Sleep Research*, vol. 23, no. 1, pp. 13–21, 2014.

[48] A. Celik, E. Saricicek, V. Saricicek et al., "Effect of Ramadan fasting on serum concentration of apelin-13 and new obesity indices in healthy adult men," *Medical Science Monitor*, vol. 20, pp. 337–342, 2014.

[49] N. M. Al-Daghri, O. S. Al-Attas, M. Alokail et al., "Does visceral adiposity index signify early metabolic risk in children and adolescents? Association with insulin resistance, adipokines, and subclinical inflammation," *Pediatric Research*, vol. 75, no. 3, pp. 459–463, 2014.

[50] M. C. Amato, G. Pizzolanti, V. Torregrossa, G. Misiano, S. Milano, and C. Giordano, "Visceral adiposity index (VAI) is predictive of an altered adipokine profile in patients with type 2 diabetes," *PLoS ONE*, vol. 9, no. 3, Article ID e91969, 2014.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 120286, 8 pages
http://dx.doi.org/10.1155/2014/120286

Clinical Study

Recombinant Human Leptin Does Not Alter Gut Hormone Levels after Gastric Bypass but May Attenuate Sweet Cravings

Rushika Conroy,[1,2] Gerardo Febres,[3] Donald J. McMahon,[3] Michael O. Thorner,[4] Bruce D. Gaylinn,[4] Irene Conwell,[3] Louis Aronne,[5] and Judith Korner[3]

[1] *Division of Pediatric Endocrinology, Columbia University Medical Center, New York, NY 10032, USA*
[2] *Division of Pediatric Endocrinology, Baystate Medical Center, Springfield, MA 01199, USA*
[3] *Division of Endocrinology, Columbia University Medical Center, New York, NY 10032, USA*
[4] *Division of Medicine, University of Virginia Medical Center, Charlottesville, VA 22902, USA*
[5] *Division of Medicine, Cornell University Medical Center, New York, NY 10021, USA*

Correspondence should be addressed to Judith Korner; jk181@columbia.edu

Received 7 November 2013; Accepted 19 February 2014; Published 20 March 2014

Academic Editor: Yi-Hao Yu

Bariatric surgery improves glucose homeostasis and alters gut hormones partly independent of weight loss. Leptin plays a role in these processes; levels are decreased following bariatric surgery, creating a relative leptin insufficiency. We previously showed that leptin administration in a weight-reduced state after Roux-en-Y gastric bypass (RYGB) caused no further weight loss. Here, we discuss the impact of leptin administration on gut hormones, glucostasis, and appetite. Weight stable women after RYGB were randomized to receive placebo or recombinant human metreleptin (0.05 mg/kg twice daily). At weeks 0 and 16, a liquid meal challenge was performed. Glucose, insulin, C-peptide, GLP-1, PYY, glucagon, and ghrelin (total, acyl, and desacyl) were measured fasting and postprandially. Appetite was assessed using a visual analog scale. Mean post-op period was 53 ± 2.3 months; mean BMI was 34.6 ± 0.2 kg/m^2. At 16 weeks, there was no significant change in weight within or between groups. Fasting PYY was significantly different between groups and the leptin group had lower sweets craving at week 16 than the placebo group ($P < 0.05$). No other differences were observed. Leptin replacement does not alter gut hormones or glucostasis but may diminish sweet cravings compared to placebo in this population of post-RYGB women.

1. Introduction

Roux-en-Y gastric bypass (RYGB) surgery results in a reduction of approximately 38% of total body weight at one year that, unlike diet therapy alone, is mostly maintained over the long term [1]. In addition to weight reduction, improvement in glucose homeostasis has also been observed, which may be partly independent of reduced body weight. Unique alterations in circulating levels of gut hormones, such as ghrelin, peptide YY (PYY), and glucagon-like peptide 1 (GLP-1), also occur after RYGB that create an environment favoring decreased appetite, weight reduction, maintenance of a reduced body weight, and improved glucose tolerance. Ghrelin, an orexigenic hormone produced in cells of the oxyntic glands of the stomach, was found to decrease or remain the same following RYGB [2, 3], in contrast to the usual increase in ghrelin levels that occurs after diet or gastric banding. PYY is secreted by intestinal L cells in response to food intake, leading to a decrease in gastrointestinal motility and increased satiety. Postprandial PYY levels are markedly increased after RYGB [2, 4]. Circulating concentrations of GLP-1, also produced by L cells, are increased following RYGB, contributing directly to reduced appetite, increased satiety, and weight loss as well as increases in glucose-stimulated insulin release following food ingestion [4, 5].

Many individuals who have undergone RYGB experience a plateau in weight loss with a body mass index (BMI) still within the obese range [6]. Counterregulatory hormones may impede further loss despite the presence of excess of body fat [7]. Leptin is a critical afferent component of a regulatory loop linking fat mass to food intake and energy expenditure and has also been shown to play an important role in

glucose homeostasis through its effects on insulin as well as other mediators of glucose metabolism [8, 9]. Following weight loss, leptin levels decrease out of proportion to the amount of fat mass [10]. Leptin levels in those having lost weight following RYGB are less than levels in BMI-matched individuals who have not undergone weight loss [11], putting the former in a state of relative leptin insufficiency, which may be an important factor contributing to their inability to lose more weight.

Leptin is thought to modulate a number of hormones involved in appetite regulation and food metabolism, which are themselves altered by weight loss [12, 13]. While its relationship with some appetitive hormones is as yet unclear, animal studies have suggested that GLP-1 as well as PYY are increased following leptin administration [14–16], favoring appetite reduction and weight loss. Leptin and ghrelin have opposing actions and leptin administration in animal models has resulted in a reduction in ghrelin levels [17, 18]. Leptin administration has been shown to increase satiety and satiation in mouse models of obesity as well as in humans with leptin insufficiency [19–21], suggesting that it may affect the secretion and/or function of such hormones to promote weight reduction. Insulin sensitivity is improved following leptin administration [22], and leptin has been found to decrease glucagon levels in rat and mouse models of both type 1 and type 2 diabetes, contributing to the improvement in glycemic status [23, 24].

Leptin replacement therapy has been used in humans with congenital leptin deficiency and has resulted in weight loss when prescribed in physiologic doses. However, high pharmacologic levels of leptin are required to induce weight loss in otherwise healthy obese individuals; physiologic replacement of leptin has led to minimal to no weight loss [25–31]. In contrast, administration of physiologic replacement doses of leptin that restore circulating concentrations to preweight loss levels reverses many of the manifestations characteristic of the weight-reduced state, in some cases, irrespective of further weight loss [25–27]. Animal models of weight loss have suggested that leptin interacts with appetitive hormones in a manner that promotes further weight reduction [14, 15, 18]. Such interactions have yet to be studied in humans after RYGB.

We previously reported that, contrary to our expectation, leptin administration did not lead to further weight loss in women who had undergone weight loss after RYGB and whose leptin levels were lower than that predicted for a nonreduced individual with the same BMI [32]. This paper examines our secondary objective, which was to establish whether leptin administration in this weight-reduced state would be associated with changes in hormones involved in nutrient metabolism as well as in satiation. We hypothesized that leptin administration would lead to alteration in gut hormones that would promote further weight reduction and improve glucose homeostasis.

2. Materials and Methods

2.1. Study Subjects. Women between the ages of 25 and 65 years who were at least 18 months after RYGB, had a percent total weight loss from the highest presurgical weight to current weight between 18% and 45%, and had a current BMI of 28–50 kg/m^2 were invited to participate. Subjects were considered for enrollment if their plasma leptin level was less than the level predicted from the regression equation generated using leptin levels and BMI from a non-weight-reduced cohort of 55 women who had participated in previous studies from our group: (0.991 × BMI) − 3.37. [JK, unpublished data]. Exclusion criteria have been described elsewhere [32]. This study is in accordance with the guidelines of the Declaration of Helsinki and was approved by the Columbia University Institutional Review Board. All subjects provided written informed consent.

Thirty-five of the 69 subjects screened met enrollment criteria. Eight subjects failed to have at least one follow-up visit after randomization and were excluded from the analysis. Of the remaining 27 subjects, 22 completed the test meal at baseline and 16 weeks after treatment [32].

2.2. Protocol. The study protocol has been described previously [32]. Briefly, subjects who met criteria entered a 2 week single-blind placebo run-in period, after which they were randomized to receive either placebo or recombinant human metreleptin. Metreleptin, referred to as "leptin," and placebo were generously donated by Amylin Pharmaceuticals (San Diego, CA). The dose of leptin (0.05 mg/kg body weight self-administered via subcutaneous injection twice daily) was expected to raise maximum plasma leptin levels to high physiologic/low pharmacologic levels yet would not be expected to cause clinically significant weight loss in a person who had not undergone weight reduction [25].

At weeks 0 and 16, a meal challenge (Optifast; Novartis, Minneapolis MN; 474 mL, 320 Kcal, 50% carbohydrate, 35% protein, and 15% fat) was performed. PYY, insulin, glucose, insulin, C-peptide, and ghrelin (total, acyl, and desacyl) were measured in the fasted state as well as 15, 30, 60, 90, and 120 minutes after consumption of the liquid beverage, with the exception of total GLP-1 that was measured in the fasted state as well as 15 and 30 minutes postprandially. Appetite was assessed using a validated visual analog scale [33] with questions about a subjective feeling written below a 100 mm line anchored on either end with opposite descriptors (not at all, extremely). Subjects were asked to make a vertical mark across the line corresponding to their feelings. The answer was quantified by measuring the distance from the left end of the line to the mark. The VAS was administered in the fasted state as well as 60, 90, and 120 minutes after consumption of the liquid meal.

Venous blood samples were collected in EDTA tubes that were centrifuged for 15 minutes at 4°C and stored at −80°C until assayed in duplicate. Glucose, insulin, leptin, and total ghrelin were measured as described elsewhere [11]. Assays for total PYY and total GLP-1 were previously described as well [34]. C-peptide was measured with the Immulite Analyzer (Diagnostic Products Corp., Los Angeles, CA). Glucagon was measured by RIA as per manufacturer's instructions (Millipore Corporation, Billerica, MA). Blood samples for the measurement of acyl and desacyl-ghrelin were collected in

Table 1: Baseline characteristics of study participants.

Parameter	Placebo	Leptin	P value*
Age (y)	42.2 ± 2.8	51.4 ± 2.0	0.02
Pre-RYGB BMI (kg/m^2)	48.6 ± 1.9	47.1 ± 1.8	0.58
BMI at screen (kg/m^2)	35.0 ± 1.1	33.3 ± 1.4	0.35
Wt Loss (%)	30.2 ± 2.3	30.7 ± 2.1	0.88
Post-op period (mo)	44.2 ± 7.4	64.6 ± 8.6	0.09
Leptin (ng/mL)	27.1 ± 3.2	21.8 ± 2.5	0.20
Leptin/kg FM (ng/mL/kg)	0.70 ± 0.06[a]	0.66 ± 0.06[b]	0.61

Results are expressed as mean ± SEM. $n = 11$ subjects per group except as follows: [a]$n = 9$; [b]$n = 10$. *P value obtained by two-tailed independent t-test.

EDTA tubes containing AEBSF, were centrifuged, and then acidified with HCl prior to measurement by sandwich assays [35]. Plasma was diluted as necessary to obtain readings within the assay range.

2.3. Statistical Analysis. Data were evaluated for normality with the Kolmogorov-Smirnov test and none were found to require transformation. Raw score differences and percent change from baseline were calculated. For repeatedly measured postprandial samples, area-under-the-curve was calculated using the trapezoidal rule. Group differences at baseline were evaluated with independent t-tests for continuous measures. Group, time, and group by time interactions were estimated with linear mixed models for repeated measures as fixed effects, the value of the outcome at baseline entered as a continuous covariate, and a compound symmetry covariance structure for the autocorrelation of measures within subject. The covariance structure was selected prior to inferential testing from empirical evaluation of alternative structures. Model estimated mean and standard errors for differences between times within group and between groups at specific times were used for the specific comparisons. P values for differences are based on the method of simultaneous confidence intervals. Least squares regression was used to assess the association of weight loss to initial leptin levels, number of months between surgery and study entry, and percent weight loss from presurgical maximum weight. No adjustment for multiplicity was employed for the multiple endpoints assessed.

3. Results

Prior to RYGB, BMI was similar between groups (Table 1). Baseline characteristics, with the exception of age, were also similar at the time of this study. Duration of postoperative period was a mean of 53 ± 2.3 months for the study cohort. Mean percent weight loss from the highest preoperative weight to weight at time of screening visit was 30.7 ± 0.33%, with a range of 18.2–44.7%. The mean BMI for the study cohort at the time of screening was 34.6 ± 0.2 kg/m^2, with a range of 28.4–41.7 kg/m^2.

At 16 weeks there was no significant change in weight within or between groups (Table 2). As expected, leptin concentrations increased in the treated group. Group by time interaction testing revealed significant differences between leptin and placebo treated groups for fasting PYY and insulin AUC (Table 2); however, the latter difference was driven by one subject who had an unusually elevated postprandial insulin and C-peptide response in the placebo group at week 16 (Figure 1). Otherwise, there were no changes in glucostatic parameters that were different between the groups. Fasting ghrelin and the ghrelin response to the test meal were similar between groups (Figure 2). Similarly, acyl-ghrelin, desacyl-ghrelin, and the ratio of desacyl-to acyl-ghrelin in the fasted and postprandial state did not change with leptin treatment.

One question on the VAS scale showed a statistically significant difference between the leptin and placebo treated groups at week 16 ($P = 0.05$; Table 3). "How much do you crave something sweet right now?" The leptin treated group had a significantly lower rating for this question than did the placebo treated group.

4. Discussion

Our previously published report of this investigation showed that physiological leptin replacement in weight stable women who are in a state of relative leptin insufficiency after RYGB does not result in further weight loss [32]. Absence of an effect on body weight allowed for the unique opportunity to examine the role of leptin on gut hormone and glucose regulation independent of weight loss. Our findings indicate that leptin replacement does not lead to alterations in gut hormone physiology that would favor a further reduction in weight or improvement in glucose homeostasis compared to placebo in this population of obese women after RYGB.

Ghrelin is an orexigenic hormone that has an opposing effect to the anorexigenic properties of leptin. Leptin has been found to decrease ghrelin release from the stomach *in vitro* and suppress ghrelin secretion from wild type and *ob*/*ob* mouse models as well as diabetic rat models [17, 18, 36]. However, this effect was observed only transiently in adult rats [37]. Ghrelin O-acyl transferase (GOAT), the enzyme that octanoylates ghrelin from the desacylated to the acylated form, is increased by leptin *in vitro* in stomach cells, thus increasing expression of the more potent acylated form and promoting food intake [38]. Desacyl-ghrelin has been shown in some, but not all, studies to favor insulin sensitivity and lack of weight gain [18, 36, 38]. Glucose-stimulated insulin secretion was observed in rats following central desacyl-ghrelin administration but not following peripheral administration [39]. Our findings showed no changes in ghrelin levels after leptin administration, suggesting that modulation of ghrelin levels or acylation may not be dependent upon leptin in the weight-reduced state or that our subjects were resistant to the effects of leptin.

Leptin and PYY both play roles in appetite reduction yet it is unclear whether they work synergistically or independently toward this common goal. *Ad libitum* fed rats administered PYY followed by leptin had prolonged satiation compared to when administered PYY alone [40], suggesting that the two hormones work synergistically. Leptin administration to humans following diet-induced weight loss did not increase

Table 2: Changes in weight, glucostatic, and appetitive hormones.

	Placebo		Leptin		P^*
	Week 0	Week 16	Week 0	Week 16	
Wt (kg)	91.5 ± 4.9	91.1 ± 5.1	86.2 ± 3.8	86.4 ± 3.9	0.74
Leptin (ng/mL)	27.1 ± 3.2	29.8 ± 4.5	21.8 ± 2.5	223.4 ± 66[ab]	**0.01**
Glucose (mg/dL)	86.2 ± 1.8	84.4 ± 2.5	88.0 ± 1.7	88.0 ± 2.1	0.49
Insulin (μIU/mL)	3.0 ± 0.4	3.6 ± 0.6	4.2 ± 0.7	4.1 ± 0.8	0.34
Glucose AUC	10354 ± 599	11506 ± 294	11718 ± 338[b]	11814 ± 438	0.20
Insulin AUC	2990 ± 327	4693 ± 888[a]	4553 ± 455	4181 ± 561	**0.04**
HOMA-IR	0.63 ± 0.08	0.76 ± 0.13	0.90 ± 0.14	0.89 ± 0.19	0.40
CPEP (ng/mL)	1.31 ± 0.11	1.30 ± 0.15	1.42 ± 0.14	1.40 ± 0.17	0.94
CPEP AUC	8.5 ± 0.7	10.3 ± 1.4	10.9 ± 1.3	9.8 ± 1.2	0.07
GLP-1 (pg/mL)	11.5 ± 2.1	9.7 ± 2.2	6.8 ± 1.6	7.1 ± 1.4	0.54
GLP-1 AUC	1332 ± 166	1136 ± 84	1957 ± 395	1171 ± 198[a]	0.21
PYY (pg/mL)	69.1 ± 12.6	43.0 ± 10.6[a]	47.9 ± 10.4	58.5 ± 15.6	**0.04**
PYY AUC	19536 ± 2483	16968 ± 2793	19557 ± 3405	21619 ± 3380	0.15
Glucagon (pg/mL)	79 ± 7	75 ± 7	71 ± 7	82 ± 5	0.10
Glucagon AUC	197 ± 20	231 ± 20[a]	216 ± 15	251 ± 10[a]	0.96
Ghrelin (pg/mL)	379 ± 54	379 ± 53	343 ± 48	326 ± 43	0.42
Acyl-ghrelin (pg/mL)	34.1 ± 13.5	31.7 ± 9.9	18.8 ± 5.4	21.9 ± 4.4	0.59
Desacyl-ghrelin (pg/mL)	64.7 ± 16.3	68.4 ± 19.0	41.4 ± 0.4	37.2 ± 3.2	0.63

Results are expressed as mean ± SEM. $n = 11$ subjects per group except for ghrelin, acyl-ghrelin, and desacyl-ghrelin, where $n = 9$ for placebo group. Hormone measurements are from the fasted state unless otherwise indicated. [a]$P < 0.05$; week 16 is statistically different from week 0 within group. [b]$P < 0.05$; difference in values is statistically significant between groups at the same week. $^*P < 0.05$; statistically significant values for group by time interaction.

Table 3: Visual analog scale.

	Placebo		Leptin	
	Week 0	Week 16	Week 0	Week 16
How hungry are you?	2460 ± 479	3761 ± 569	2747 ± 524	3200 ± 592
How satisfied are you?	7606 ± 819	6537 ± 924	5283 ± 885	4719 ± 969
How full do you feel?	7428 ± 785	6241 ± 890	5286 ± 848	5197 ± 933
How much do you crave something sweet?	2120 ± 517	2898 ± 563*	2391 ± 543	1461 ± 585*
How much can you eat now?	3221 ± 454	4264 ± 529	3453 ± 492	3246 ± 557
How much nausea or discomfort do you feel?	2958 ± 508	2330 ± 577	3056 ± 549	2888 ± 604

Values represent mean ± SEM AUC from fasting to 120 min after consumption of the test meal. $^*P < 0.05$ for within-group change.

PYY levels following a short term fast [40], which is similar to our findings in surgically induced weight loss, suggesting that PYY is not dependent on leptin to exert satiating effects.

Postprandial GLP-1 levels have been found to increase following RYGB [41] and may contribute to a reduction in appetite, weight loss, and improved glucose homeostasis. GLP-1 and leptin interact to cause a reduction in food intake in *ob/ob* mice and rat models, with GLP-1 secretion being enhanced following leptin administration [15, 16]. We did not observe an increase in GLP-1 levels after leptin treatment. Although AUC measurement of GLP-1 decreased after leptin treatment, which is contrary to animal models and counter to what we expected based on the role of GLP-1, the group by time interaction was not significant. A possible limitation of this study is that total, and not active, GLP-1 was measured.

Leptin, glucagon, and insulin work collaboratively to maintain normoglycemia through inhibitory and stimulatory effects on each other. The relationship between insulin and leptin has been heavily studied, with findings most recently suggesting that leptin has an inhibitory effect on insulin secretion and reduces glucagon secretion in rodents with type 1 diabetes, resulting in improved glycemic control in the absence of insulin [24]. Restoration of leptin receptors on proopiomelanocortin neurons in obese mice normalized blood glucose and ameliorated hepatic insulin resistance and hyperglucagonemia independent of changes in body weight [24]. In humans, leptin administration had a minimal impact on glycemic control (HbA1c decreased from 8.01% to 7.96%) in patients with type 2 diabetes who maintained a stable weight over the 4 month study period [42]. Similarly, leptin treatment did not have a clinically important effect on insulin action in obese people with newly diagnosed type 2 diabetes [43]. Our findings are in disagreement with mouse studies showing a reduction in glucagon secretion in the presence of leptin. A limitation of our study is that although subjects were obese, they were not hyperglycemic or insulin resistant

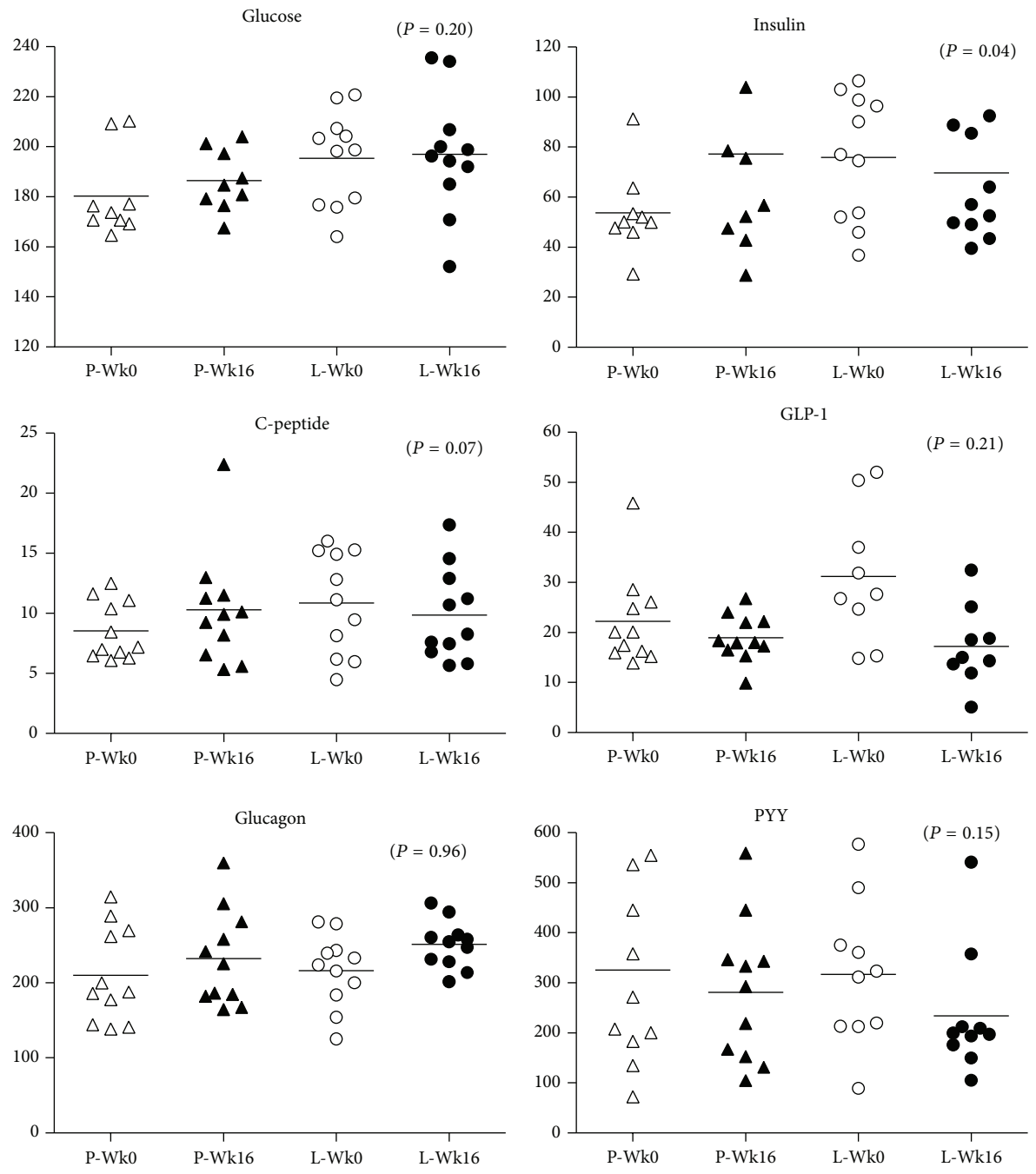


Figure 1: Levels of glucose, insulin, C-peptide, GLP-1, glucagon, and PYY. GLP-1 in placebo and leptin treated groups at weeks 0 and 16 (triangles, placebo group; circles, leptin group). Individual values and group mean (solid line) are represented.

as evaluated by HOMA-IR; thus, results may not be indicative of results that might be obtained using leptin therapy in a population with glucose intolerance or before diabetes. Also, the small study size would not be able to detect very small changes.

Feelings of hunger and satiety data were not different between groups with the exception of a decreased craving for something sweet in the leptin treated group at week 16 compared to placebo treated subjects at that time point. Leptin has been shown to modulate sweet sensitivity at the level of the taste receptors of the tongue. *Ob*/*ob* mice were found to have increased cravings for sweet items that were decreased following administration of leptin [44]. This did not occur in the leptin receptor deficient *db*/*db* mice. Similarly, obese humans were found to have obliterated diurnal variation in sweet cravings that are present in nonobese humans and to have a higher threshold for sensitivity to the effects of leptin on sweet cravings, thought to be due to a higher basal leptin level [45]. Our findings suggest that this threshold can be overcome with leptin administration;

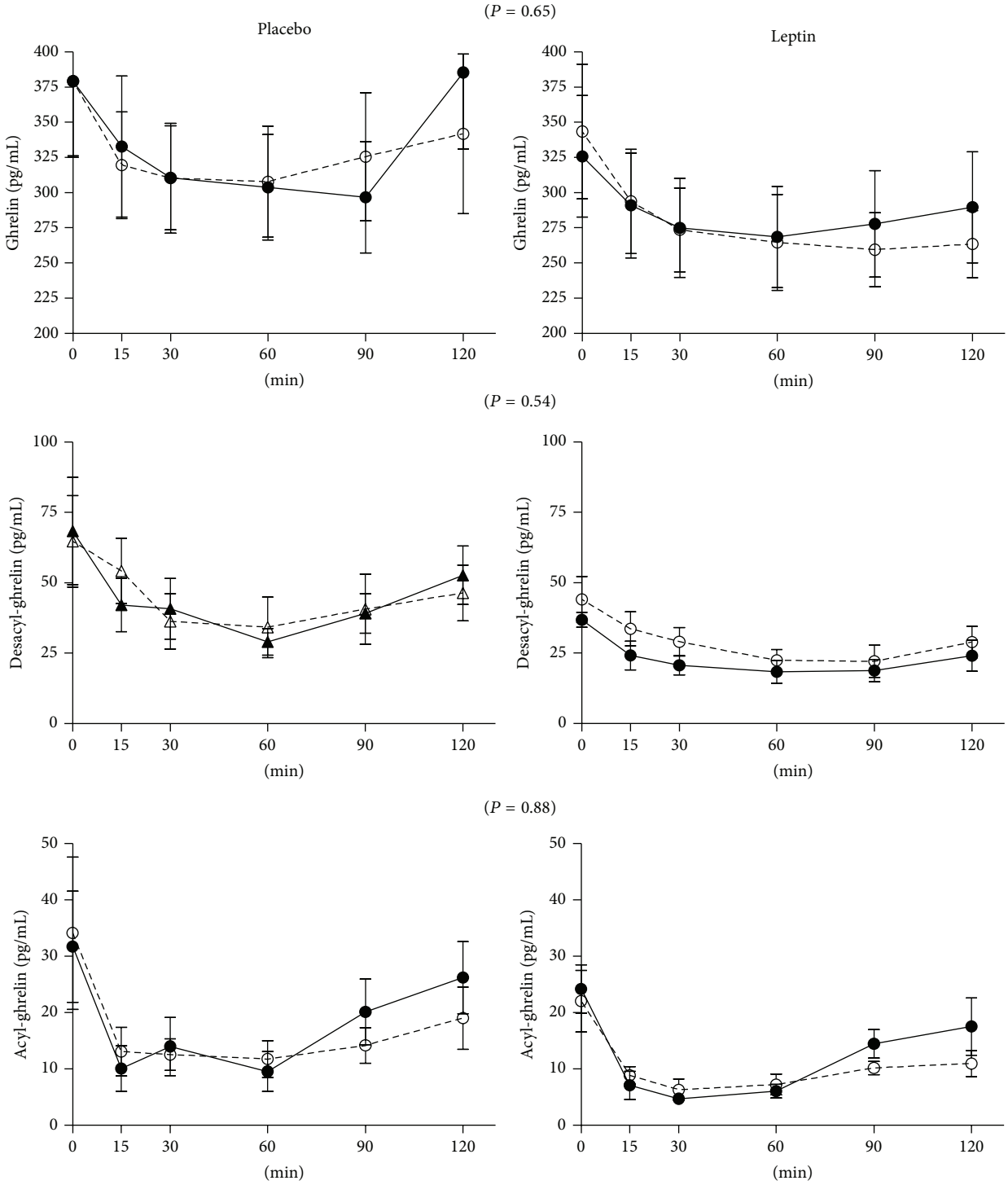


Figure 2: Fasting and postprandial plasma levels of total ghrelin, acyl-ghrelin, and desacyl-ghrelin (open circles and dashed line, week 0; closed circles and solid line, week 16).

however, such decreases in cravings were unable to elicit significant alterations in weight or glucose homeostasis but might conceivably play a role in the maintenance of weight loss over time.

Our study subjects were still overweight or obese at the time of study entry, and while they had lost a considerable amount of weight and had leptin levels below those predicted for their current BMI, there is a possibility that they continued to be leptin resistant, manifesting no significant changes to gut related hormones or feelings of satiety and satiation. Our results are specific to a stable weight-reduced state and, thus, may not indicate whether leptin administration

can modulate hormone levels during active weight loss. The question then becomes, at what level of leptin or at what weight or at what amount of weight loss would the resistance begin to subside, thus allowing those undergoing weight loss to experience the weight regulating benefits of leptin and possibly of appetitive hormones regulated by leptin. There may also be subpopulations, such as individuals with sweet cravings, who may benefit from longer exposure to exogenous leptin administration in order to help maintain a reduced body weight.

5. Conclusion

Leptin administration to women after RYGB who are in a state of relative leptin insufficiency does not lead to alterations in gut hormones or hormones that control glucose homeostasis in the absence of weight reduction, suggesting that the effects of these hormones to promote weight loss are not necessarily leptin-dependent. Sweet cravings were found to be decreased in those subjects receiving leptin, suggesting a possible therapeutic benefit of leptin administration in a subset of individuals.

Conflict of Interests

Dr. Korner receives research support from Covidien, has served as consultant for the Federal Trade Commission, Office of Professional Misconduct, Expert Network Group, and Unigene Laboratories, and was on the scientific advisory board for Nutrisystem. Dr. L. Aronne does contracted research with Amylin Pharmaceuticals Inc., Aspire Bariatrics Inc., GI Dynamics, High Point Pharmaceuticals LLC, Medical University of South Carolina (MUSC), and Novo Nordisk. He is on the advisory board of Amylin Pharmaceuticals Inc., Ethicon Endo-Surgery Inc., GlaxoSmithKline Consumer Healthcare LP, Novo Nordisk, Orexigen Therapeutics Inc., VIVUS Inc., Takeda Pharmaceuticals, and Zafgen Inc. Dr. L. Aronne is on the speakers bureau for VIVUS Inc. and has ownership interest in Cardiometabolic Support Network, LLC, Myos Corporation. The rest of the authors have nothing to disclose. Amylin Pharmaceuticals Inc. generously supplied placebo and leptin for this trial.

Acknowledgments

The authors would like to gratefully acknowledge the participants of this study. This work was supported by NIH/NIDDK Grant R21DK081050 (Judith Korner), Grant no. UL1 RR024156 from NCATS-NCRR/NIH, National Center for Advancing Translations Sciences Grant UL1 TR000040UL1 (Columbia University), and Institutional Research Training Grant (T32) in Pediatric Endocrinology T32 DK065522-05. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Metreleptin was generously provided by Amylin Pharmaceuticals, Inc. (San Diego, CA).

References

[1] M. G. Myers Jr., S. B. Heymsfield, C. Haft et al., "Challenges and opportunities of defining clinical leptin resistance," *Cell Metabolism*, vol. 15, no. 2, pp. 150–156, 2012.

[2] J. Korner, W. Inabnet, G. Febres et al., "Prospective study of gut hormone and metabolic changes after adjustable gastric banding and Roux-en-Y gastric bypass," *International Journal of Obesity*, vol. 33, no. 7, pp. 786–795, 2009.

[3] S. H. Jacobsen, S. C. Olesen, C. Dirksen et al., "Changes in gastrointestinal hormone responses, insulin sensitivity, and beta-cell function within 2 weeks after gastric bypass in non-diabetic subjects," *Obesity Surgery*, vol. 22, no. 7, pp. 1084–1096, 2012.

[4] C. W. le Roux, S. J. B. Aylwin, R. L. Batterham et al., "Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters," *Annals of Surgery*, vol. 243, no. 1, pp. 108–114, 2006.

[5] A. P. Coll, I. S. Farooqi, and S. O. O'Rahilly, "The hormonal control of food intake," *Cell*, vol. 129, no. 2, pp. 251–262, 2007.

[6] N. V. Christou, D. Look, and L. D. MacLean, "Weight gain after short- and long-limb gastric bypass in patients followed for longer than 10 years," *Annals of Surgery*, vol. 244, no. 5, pp. 734–740, 2006.

[7] M. Shah, V. Simha, and A. Garg, "REVIEW: long-term impact of bariatric surgery on body weight, comorbidities, and nutritional status," *The Journal of Clinical Endocrinology & Metabolism*, vol. 91, no. 11, pp. 4223–4231, 2006.

[8] N. Haddad, R. Howland, G. Baroody, and C. Daher, "The modulatory effect of leptin on the overall insulin production in ex-vivo normal rat pancreas," *Canadian Journal of Physiology and Pharmacology*, vol. 84, no. 2, pp. 157–162, 2006.

[9] M. Nakano, A. Asakawa, and A. Inui, "Long-term correction of type 1 and 2 diabetes by central leptin gene therapy independent of effects on appetite and energy expenditure," *Indian Journal of Endocrinology and Metabolism*, vol. 16, no. 9, pp. 556–561, 2012.

[10] M. C. Klempel and K. A. Varady, "Reliability of leptin, but not adiponectin, as a biomarker for diet-induced weight loss in humans," *Nutrition Reviews*, vol. 69, no. 3, pp. 145–154, 2011.

[11] J. Korner, M. Bessler, L. J. Cirilo et al., "Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 1, pp. 359–365, 2005.

[12] T. A. Dardeno, S. H. Chou, H.-S. Moon, J. P. Chamberland, C. G. Fiorenza, and C. S. Mantzoros, "Leptin in human physiology and therapeutics," *Frontiers in Neuroendocrinology*, vol. 31, no. 3, pp. 377–393, 2010.

[13] R. Janeckova, "The role of leptin in human physiology and pathophysiology," *Physiological Research*, vol. 50, no. 5, pp. 443–459, 2001.

[14] S. Unniappan and T. J. Kieffer, "Leptin extends the anorectic effects of chronic PYY(3-36) administration in ad libitum-fed rats," *American Journal of Physiology: Regulatory Integrative and Comparative Physiology*, vol. 295, no. 1, pp. R51–R58, 2008.

[15] S. Zhao, S. E. Kanoski, J. Yan, H. J. Grill, and M. R. Hayes, "Hindbrain leptin and glucagon-like-peptide-1 receptor signaling interact to suppress food intake in an additive manner," *International Journal of Obesity*, vol. 36, pp. 1522–1528, 2012.

[16] Y. Anini and P. L. Brubaker, "Role of leptin in the regulation of glucagon-like peptide-1 secretion," *Diabetes*, vol. 52, no. 2, pp. 252–259, 2003.

[17] D. Kohno, M. Nakata, F. Maekawa et al., "Leptin suppresses ghrelin-induced activation of neuropeptide Y neurons in the arcuate nucleus via phosphatidylinositol 3-kinase- and phosphodiesterase 3-mediated pathway," *Endocrinology*, vol. 148, no. 5, pp. 2251–2263, 2007.

[18] S. P. Kalra, N. Ueno, and P. S. Kalra, "Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action," *The Journal of Nutrition*, vol. 135, no. 5, pp. 1331–1335, 2005.

[19] H. Dhillon, S. P. Kalra, and P. S. Kalra, "Dose-dependent effects of central leptin gene therapy on genes that regulate body weight and appetite in the hypothalamus," *Molecular Therapy*, vol. 4, no. 2, pp. 139–145, 2001.

[20] H. R. Kissileff, J. C. Thornton, M. I. Torres et al., "Leptin reverses declines in satiation in weight-reduced obese humans," *The American Journal of Clinical Nutrition*, vol. 95, no. 2, pp. 309–317, 2012.

[21] J. R. McDuffie, P. A. Riggs, K. A. Calis et al., "Effects of exogenous leptin on satiety and satiation in patients with lipodystrophy and leptin insufficiency," *The Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 9, pp. 4258–4263, 2004.

[22] E. D. Berglund, C. R. Vianna, J. Donato Jr. et al., "Direct leptin action on POMC neurons regulates glucose homeostasis and hepatic insulin sensitivity in mice," *The Journal of Clinical Investigation*, vol. 122, no. 3, pp. 1000–1009, 2012.

[23] B. P. Cummings, A. Bettaieb, J. L. Graham et al., "Subcutaneous administration of leptin normalizes fasting plasma glucose in obese type 2 diabetic UCD-T2DM rats," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 35, pp. 14670–14675, 2011.

[24] X. Yu, B.-H. Park, M.-Y. Wang, Z. V. Wang, and R. H. Unger, "Making insulin-deficient type 1 diabetic rodents thrive without insulin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 37, pp. 14070–14075, 2008.

[25] S. B. Heymsfield, A. S. Greenberg, K. Fujioka et al., "Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial," *The Journal of the American Medical Association*, vol. 282, no. 16, pp. 1568–1575, 1999.

[26] M. Rosenbaum, E. M. Murphy, S. B. Heymsfield, D. E. Matthews, and R. L. Leibel, "Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones," *The Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 5, pp. 2391–2394, 2002.

[27] M. Rosenbaum, R. Goldsmith, D. Bloomfield et al., "Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight," *The Journal of Clinical Investigation*, vol. 115, no. 12, pp. 3579–3586, 2005.

[28] G. Paz-Filho, C. Mastronardi, T. Delibasi, M.-L. Wong, and J. Licinio, "Congenital leptin deficiency: diagnosis and effects of leptin replacement therapy," *Arquivos Brasileiros de Endocrinologia & Metabologia*, vol. 54, no. 8, pp. 690–697, 2010.

[29] I. S. Farooqi, S. A. Jebb, G. Langmack et al., "Effects of recombinant leptin therapy in a child with congenital leptin deficiency," *The New England Journal of Medicine*, vol. 341, no. 12, pp. 879–884, 1999.

[30] E. D. Javor, E. K. Cochran, C. Musso, J. R. Young, A. M. DePaoli, and P. Gorden, "Long-term efficacy of leptin replacement in patients with generalized lipodystrophy," *Diabetes*, vol. 54, no. 7, pp. 1994–2002, 2005.

[31] S. H. Chou, J. P. Chamberland, X. Liu et al., "Leptin is an effective treatment for hypothalamic amenorrhea," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 16, pp. 6585–6590, 2011.

[32] J. Korner, R. Conroy, G. Febres et al., "Randomized double-blind placebo-controlled study of leptin administration after gastric bypass," *Obesity*, vol. 21, no. 5, pp. 951–956, 2013.

[33] A. Flint, A. Raben, J. E. Blundell, and A. Astrup, "Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies," *International Journal of Obesity*, vol. 24, no. 1, pp. 38–48, 2000.

[34] L. Plum, L. Ahmed, G. Febres et al., "Comparison of glucostatic parameters after hypocaloric diet or bariatric surgery and equivalent weight loss," *Obesity*, vol. 19, no. 11, pp. 2149–2157, 2011.

[35] C. Prudom, J. Liu, J. Patrie et al., "Comparison of competitive radioimmunoassays and two-site sandwich assays for the measurement and interpretation of plasma ghrelin levels," *The Journal of Clinical Endocrinology & Metabolism*, vol. 95, no. 5, pp. 2351–2358, 2010.

[36] T. Tsubone, T. Masaki, I. Katsuragi, K. Tanaka, T. Kakuma, and H. Yoshimatsu, "Leptin downregulates ghrelin levels in streptozotocin-induced diabetic mice," *American Journal of Physiology: Regulatory Integrative and Comparative Physiology*, vol. 289, no. 6, pp. R1703–R1706, 2005.

[37] R. H. Unger and A. D. Cherrington, "Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover," *The Journal of Clinical Investigation*, vol. 122, no. 1, pp. 4–12, 2012.

[38] H. Kirchner, K. M. Heppner, J. Holland, D. Kabra, M. H. Tschop, and P. T. Pfluger, "Ablation of ghrelin O-acyltransferase does not improve glucose intolerance or body adiposity in mice on a leptin-deficient ob/ob background," *PLoS ONE*, vol. 8, no. 4, article e61822, 2013.

[39] K. M. Heppner, C. L. Piechowski, A. Müller et al., "Both acyl and des-acyl ghrelin regulate adiposity and glucose metabolism via central nervous system ghrelin receptors," *Diabetes*, 2013.

[40] J. L. Chan, V. Stoyneva, T. Kelesidis, P. Raciti, and C. S. Mantzoros, "Peptide YY levels are decreased by fasting and elevated following caloric intake but are not regulated by leptin," *Diabetologia*, vol. 49, no. 1, pp. 169–173, 2006.

[41] N. A. Rhee, T. Vilsboll, and F. K. Knop, "Current evidence for a role of GLP-1 in Roux-en-Y gastric bypass-induced remission of type 2 diabetes," *Diabetes, Obesity and Metabolism*, vol. 14, no. 4, pp. 291–298, 2012.

[42] H.-S. Moon, G. Matarese, A. M. Brennan et al., "Efficacy of metreleptin in obese patients with type 2 diabetes: cellular and molecular pathways underlying leptin tolerance," *Diabetes*, vol. 60, no. 6, pp. 1647–1656, 2011.

[43] B. Mittendorfer, J. F. Horowitz, A. M. DePaoli, M. A. McCamish, B. W. Patterson, and S. Klein, "Recombinant human leptin treatment does not improve insulin action in obese subjects with type 2 diabetes," *Diabetes*, vol. 60, no. 5, pp. 1474–1477, 2011.

[44] R. Yoshida, M. Niki, M. Jyotaki, K. Sanematsu, N. Shigemura, and Y. Ninomiya, "Modulation of sweet responses of taste receptor cells," *Seminars in Cell & Developmental Biology*, vol. 24, no. 3, pp. 226–231, 2013.

[45] N. Horio, M. Jyotaki, R. Yoshida, K. Sanematsu, N. Shigemura, and Y. Ninomiya, "New frontiers in gut nutrient sensor research: nutrient sensors in the gastrointestinal tract: modulation of sweet taste sensitivity by leptin," *Journal of Pharmacological Sciences*, vol. 112, no. 1, pp. 8–12, 2010.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 512013, 9 pages
http://dx.doi.org/10.1155/2014/512013

Research Article

Appetite Response among Those Susceptible or Resistant to Obesity

Rachel C. Brown,[1] Rebecca T. McLay-Cooke,[1] Sara L. Richardson,[1] Sheila M. Williams,[2] David R. Grattan,[3] and Alexandra W.-A. H. Chisholm[1]

[1] *Department of Human Nutrition, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand*
[2] *Department of Preventive and Social Medicine, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand*
[3] *Department of Anatomy, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand*

Correspondence should be addressed to Rebecca T. McLay-Cooke; rebecca.cooke@otago.ac.nz

Received 21 October 2013; Revised 31 January 2014; Accepted 9 February 2014; Published 16 March 2014

Academic Editor: Joseph R. Vasselli

An alternative approach in determining cause, treatment, and prevention of obesity is to study those who appear resistant to the obesogenic environment. We examined appetite responses in 33 obesity resistant individuals (ORI) versus 28 obesity susceptible individuals (OSI). Fingerprick blood samples to measure ghrelin, total peptide YY (PYY), leptin, glucose, and insulin along with appetite ratings were collected at baseline and 15, 30, 60, 120, and 180 min following consumption of a standardized meal. Fasting, area under the curve (AUC), peak/nadir, and time to peak/nadir were compared. Participants completed the three factor eating questionnaire (TFEQ). No significant differences were observed for ghrelin or PYY. Higher leptin concentrations in the OSI disappeared after controlling for percent body fat (%BF). Significant differences in appetite ratings included a lower hunger nadir among OSI compared with ORI ($P = 0.017$). Dietary restraint ($P < 0.001$) and disinhibition ($P < 0.001$) were lower in ORI compared with OSI, with and without adjustment for %BF. Given the differential body weight of the study groups, similar observed ghrelin concentrations were unexpected, perhaps indicating OSI and ORI respond differently to the same ghrelin concentration. Also ORI response to hunger appears different as they exhibit lower levels of dietary restraint and disinhibition compared with OSI.

1. Introduction

Obesity is well-recognised as a disease process leading to multiple pathological consequences. The WHO declared obesity to be an epidemic in 1998 [1]. In countries where data are available, prevalence rates of obesity today are about four times those of thirty years ago [1, 2]. Unfortunately, most strategies to reduce or even curb increases in rates of obesity have been largely unsuccessful [3–6]. Our so-called obesogenic environment has been blamed for the dramatic increase in obesity rates in recent years, as genetics alone cannot account for such dramatic changes in a relatively short time-frame.

Some obesity experts have made dire predictions for future obesity prevalence rates. For instance, Wang et al. [7] have predicted that by the year 2048, all American adults would become overweight or obese. However, evidence suggests that these predictions may be flawed. Information from cross-sectional and prospective studies on temporal changes in body mass index (BMI) indicate that the population distribution of BMI is positively skewed and that over time the degree of skew has increased [8–13]. This means that there is proportionally more shifting at the upper end of the distribution curve with the lower end of the distribution remaining relatively static. That is, within a population, more people are becoming overweight and obese; however, there still remains a substantial sector of the population who have remained lean, seemingly resistant to this obesogenic environment.

Most obesity researchers have investigated the characteristics of overweight and obese individuals and populations in an attempt to determine the cause, treatment, and prevention of obesity. An alternative approach is to study those who have

remained lean despite living in an obesogenic environment. Information from this group may allow us to develop novel strategies to benefit those who continually struggle to maintain a healthy body weight.

One potential difference between those who struggle with their weight and those who remain lean with seeming ease may involve appetite regulation. Several hormones have important roles in regulating appetite. For example, ghrelin, an orexigenic peptide, appears important in meal initiation [14], whereas PYY may reduce food intake [15]. A previous study compared concentrations of ghrelin, PYY, glucagon-like peptide 1 (GLP-1), and leptin measured every 4 h over a 24 h period among those considered constitutionally thin (CT) and a group of healthy weight individuals [16]. Individuals were considered CT if they met the following criteria: a BMI between 14.5 and 16.5 $kg \cdot m^{-2}$, stable weight throughout the postpubertal period, presence of menstrual periods without estroprogestative treatment, and the desire to gain weight. Ghrelin, GLP-1, and leptin concentrations were significantly lower, while PYY was significantly higher in the CT group compared to normal weight controls. One could propose that the hormone profile of CT individuals may protect them from overeating and therefore in part explain how thin individuals remain lean despite living in an obesogenic environment. However, it is equally likely that potential differences in eating patterns between the CT and normal weight controls result in changes in the hormone profile.

Some [17–19], but not all [20] research has reported lower baseline and postprandial ghrelin concentrations in obese individuals when compared to their lean counterparts. The suppression of ghrelin in response to a meal may indicate some form of dysregulation of appetite hormones in obese individuals. In addition, ghrelin levels increase with subsequent weight loss, implying a role in long-term weight regulation. It has also been suggested that lower fasting ghrelin may be a consequence of downregulation caused by overeating [21]. Two anorexigenic hormones, Peptide YY and GLP-1, are reported to be lower in obese individuals, suggesting differences in these hormones may be contributing to higher energy intakes in the obese [19, 22, 23].

It would therefore be of interest to compare the hormone profiles of those who report they remain lean with relative ease (ORI) with those who report they struggle to maintain a healthy body weight (OSI). As some studies have shown that ghrelin secretion mirrors reported hunger [14, 20, 24], it would also be worthwhile to investigate any differences in appetite indices between these two diverse groups of individuals.

We investigated hormone concentrations and appetite indices in response to a standard meal among those who remain lean with ease and those who constantly struggle with their weight.

2. Materials and Methods

2.1. Subjects. Sixty-one participants were recruited from the general public in Dunedin, New Zealand, via the distribution of flyers, designed with specific questions to target obesity susceptible and obesity resistant individuals, around local supermarkets, advertising in the local newspaper, and emails sent to University staff.

To be eligible, participants were required to be healthy males or females aged between 20 and 45 years. Participants completed a screening questionnaire to see if they met our study criteria as either an obesity susceptible individual (OSI) (struggles to maintain their weight, despite perceived low energy intakes) or an obesity resistant individual (ORI) (remains lean with relative ease and can eat whatever they like). Participants were classified as OSI if they answered positively to either or both of the following two statements:

(i) *I am a person who needs to eat small amounts of food to manage my weight,*

(ii) *I am a person who gains weight easily.*

Conversely, participants were classified as ORI if they answered positively to any of the following statements:

(i) *I am a person who can eat whatever I like without gaining weight,*

(ii) *I am a person who maintains my weight easily,*

(iii) *I am a person who loses weight easily,*

(iv) *I am a person who finds it difficult to put on weight.*

Participants were excluded if they did not answer positively to any of the screening tool questions, had a thyroid disorder, or were menopausal. In total 28 OSI and 33 ORI were recruited. Obesity resistant individuals had a BMI of 17.5–27.7 kg/m^2, had always been lean (as indicated by self-reported weight history), and found it difficult to gain but not lose weight. In contrast, OSI had a BMI of 21.6–44.0 kg/m^2, were likely to experience fluctuations in weight (as indicated by self-reported weight history), and found it difficult to lose but not gain weight. The study protocol was approved by the Human Ethics Committee of the University of Otago, New Zealand. All participants provided written informed consent.

2.2. Experimental Design and Procedures. Each participant attended a 4 h clinic visit at the Department of Human Nutrition, University of Otago. Participants arrived at the clinic after an overnight fast of at least 10 h. A fasting fingerprick blood sample using a disposable lancet was taken for measurement of ghrelin (active), PYY (total), leptin, glucose, and insulin. Capillary blood samples were collected in microcentrifuge tubes containing potassium EDTA. This was followed by the consumption of a standardised meal that participants were asked to consume within 15 min. Further capillary blood samples were collected at 15, 30, 60, 120, and 180 min following the start of ingestion of the meal. Participants also completed an appetite questionnaire at each blood sampling time-point.

2.3. Standardized Meal. The standardized meal was designed to provide 2440 kJ (584 kcal) for females and 2928 kJ (700 kcal) for males made up of 55, 29, and 15 percent of

the total energy intake from carbohydrate, fat, and protein, respectively.The meal was comprised of a muesli cereal (containing oats, wheat germ, Special K (Kelloggs), brown sugar, desiccated coconut, skimmed milk powder, full fat milk, canola oil, almonds, sultanas, dried apricots, sunflower seeds), milk, yoghurt, and orange juice. Participants were required to consume the entire standardized meal. Because the response of important study variables (namely, ghrelin, PYY, and appetite scores) has been shown to be proportional to caloric intake and because this study was cross-sectional, we decided to fix caloric intake for each sex to reduce interindividual variability.

2.4. Sampling and Biochemical Analysis. Capillary blood samples (1 mL) were collected in 1.5 mL microcentrifuge tubes containing 10 μL of sodium EDTA. Immediately prior to blood collection, 10 μL of serine protease inhibitor was added for the ghrelin (active) measurement. Upon blood collection the tubes were gently inverted and stored on ice. Samples were then centrifuged for 15 min to obtain plasma, which was stored in microcentrifuge tubes at −80°C until assay. Whole capillary blood was also collected into a HemoCue cuvette and blood glucose concentration measured using a HemoCue Glucose 201+ Analyzer (Helsingborg, Sweden).

Ghrelin (active), PYY (total), leptin, and insulin were analysed using immunoassay (Human Gut Hormone Panel LINCOplex Kit, LINCO Research, St. Charles, MO, USA). The minimum detectable concentrations for the hormones were 1.8 pg·mL^{-1} for ghrelin (active), 8.4 pg·mL^{-1} for PYY (total), 157.2 pg·mL^{-1} for leptin, and 44.5 pg·mL^{-1} for insulin. The coefficient of variation for measurements of ghrelin, PYY (total), leptin, and insulin was 13.0%, 8.1%, 11.8%, and 8.4% respectively.

Area under the curve (AUC) for ghrelin, PYY (total), leptin, glucose, and insulin was calculated by the trapezoid method.

2.5. Appetite Ratings. At each blood sampling time-point participants completed a series of appetite related questions using a 10 cm visual analogue scale (VAS) [25, 26]. In relation to each question, there were extreme states anchored at either end of the line. The questions asked were "how hungry are you right now?" (not at all hungry/as hungry as I've ever felt); "preoccupation with thoughts of food" (no thoughts of food/very preoccupied, difficult to concentrate); "how strong is your desire to eat right now" (very weak/very strong); and "how full are you right now?" (not at all full/as full as I have ever felt). The VAS was measured by an investigator blinded to the study group. Area under the curve (AUC) for each rating was calculated by the trapezoid method. The observed peak/nadir and time to peak/nadir were recorded. The three-factor eating questionnaire (TFEQ) [27] was administered on a separate occasion before the 4 h clinic visit.

2.6. Body Composition. Body weight was measured on calibrated electronic scales (Wedderburn) that measured to the nearest 0.01 kg. Height was measured to the nearest millimeter using a stadiometer. Body composition including lean mass, fat mass, and body fat percentage (%BF) was measured using dual-energy X-ray absorptiometry (DXA) (manufacturer info DPX-L Scanner, Lunar Corp., Cincinnati, OH, USA) using software version 1.35 (Lunar, Cincinnati, OH, USA) at the Dunedin Public Hospital Dual X-Ray Absorptiometry Scanning Unit. Following screening, participants also completed a questionnaire regarding past-weight history. Weight history information was not used to further categorise the participants, but it did confirm their status as obesity resistant or obesity susceptible. When entering the study participants self-reported being weight stable.

2.7. Statistical Analysis. The primary outcome measure to be assessed was the postprandial change in ghrelin. Thirty participants per group (OSI and ORI) were required to detect a difference of 5% in the serial measurements of ghrelin with a power of 90% and alpha 0.05. Participant characteristics are presented as arithmetic means and standard deviations (SD). The results show the differences for sex adjusted for obesity resistance/susceptibility category (ORS) and differences for ORS adjusted for sex from regression analysis. A further adjustment %BF was conducted by including a term for %BF in the regression model. An interaction between sex and ORS group was considered but as it was not statistically significant it was not included in the final model. The fasting and AUC hormone variables were log transformed before analysis. The results are presented as medians (interquartile range). No adjustment was made for multiple testing. Statistical analysis was performed using STATA Version 12 (STATA Inc., College Station, TX).

3. Results

3.1. Participant Characteristics. The characteristics of study participants are shown in Table 1.

3.2. Hormone Analyses. Fasting, AUC, peak or nadir, and time to peak or nadir results for ghrelin, PYY (total), leptin, glucose, and insulin adjusted for ORS and sex with and without adjustment for %BF are presented in Table 2.

3.3. Ghrelin and PYY. No differences related to ORS or sex were observed in the analysis of ghrelin or PYY.

3.4. Leptin. Fasting leptin concentration and leptin AUC were significantly greater in females compared to males. The nadir for leptin was also higher in females compared with males. However, these differences disappeared when controlling for %BF. Fasting leptin concentration, leptin AUC, and nadir for leptin were all lower in ORI compared with OSI. Again, these differences disappeared when controlling for %BF. No differences were observed in the leptin time to nadir.

3.5. Glucose and Insulin. There were no significant differences for fasting, AUC, or time to peak for blood glucose for OSI versus ORI or males versus females. Peak blood glucose was significantly higher in males compared to females. Fasting insulin and insulin AUC were significantly lower in ORI

Table 1: Characteristics of obesity resistant individuals (ORI) and obesity susceptible individuals (OSI).

	ORI		OSI		P value*
	Females	Males	Females	Males	
n	16	17	15	13	
Age (years)	32.6 (7.6)	30.6 (7.7)	35.0 (7.7)	35.5 (9.1)	0.081
Weight (kg)	56.3 (5.3)	73.3 (10.7)	86.6 (15.2)	94.1 (11.0)	<0.001
Height (m)	1.65 (0.06)	1.81 (0.08)	1.66 (0.05)	1.79 (0.04)	0.400
WC (cm)	71.6 (6.0)	80.4 (4.7)	95.6 (10.8)	99.4 (11.7)	<0.001
BMI (kg/m^2)	20.6 (1.8)	22.3 (2.9)	31.6 (6.2)	29.5 (3.3)	<0.001
LBM (kg)	40.1 (4.2)	58.5 (9.1)	45.8 (3.7)	63.7 (7.6)	0.002
Fat Mass (kg)	13.1 (2.9)	11.3 (4.6)	37.2 (14.2)	26.1 (8.1)	<0.001
% Body Fat	23.4 (4.8)	15.4 (5.6)	41.9 (9.9)	27.6 (7.1)	<0.001
TSH (μIU·mL^{-1})	1.45 (0.96)	1.78 (0.97)	1.67 (0.77)	1.58 (0.82)	0.958

All values are means (standard deviation).
BMI: Body Mass Index, LBM: Lean Body Mass, TSH: Thyroid Stimulating Hormone (reference range = 0.3–5 μIU·mL^{-1} for adults with no known thyroid dysfunction), WC: waist circumference.
* P value from regression analysis for ORS adjusted for sex.

compared with OSI. These differences disappeared when controlling for %BF.

3.6. Subjective Ratings of Hunger and Satiety. Fasting, AUC, peak or nadir, and time to peak or nadir results for "hunger," "desire to eat," "fullness," and "preoccupation with thoughts of food" adjusted for ORS and sex with and without adjustment for %BF are presented in Table 3.

The nadir for "hunger" was significantly lower for OSI compared with ORI. The fasting rating of "preoccupation with thoughts of food" was significantly higher in females compared with males. Differences were also observed in the time to nadir for the "preoccupation with thoughts of food" rating, with the nadir occurring significantly later in ORI versus OSI. These differences disappeared after controlling for %BF. There were no significant differences observed in fasting, AUC, peak, or time to peak for ratings of "fullness" or "desire to eat."

3.7. Three-Factor Eating Questionnaire (TFEQ). The TFEQ results are presented in Table 3 and Figure 1. Dietary restraint scores were significantly lower in ORI versus OSI ($P < 0.001$). Disinhibition scores were significantly lower in ORI versus OSI ($P < 0.001$), while no significant differences were observed in scores for hunger. These differences remained statistically significant after adjustment for %BF.

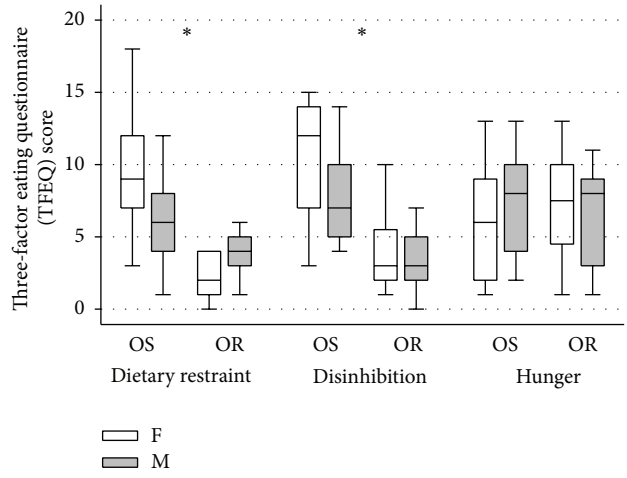


Figure 1: Three-factor eating questionnaire (TFEQ) scores for obesity resistant (OR) females (F) and males (M) versus obesity susceptible (OS) females (F) and males (M). Values are medians in 25th and 75th interquartile range. * = obesity resistant < obesity susceptible $P < 0.001$.

4. Discussion

Why some individuals remain lean with relative ease while others constantly struggle with their body weight, despite living in a similar environment, is an intriguing question. Although the majority of research in the obesity field has focused on the characteristics of obese individuals, an alternative approach is to compare the characteristics of those who are seemingly resistant to obesity (ORI) with those who appear highly susceptible (OSI). One reason for the difference in weight gain susceptibility may be due to physiological differences in appetite control. We studied the hormonal response to a standardized meal amongst ORI and OSI. Despite some differences in absolute values of these hormones, the patterns of change in response to a standard meal were remarkably similar between ORI and OSI.

As expected, ghrelin concentrations decreased in all groups upon feeding reaching a nadir between 30 and 60 min. Ghrelin, an orexigenic hormone, is acutely negatively regulated by the ingestion of meals and positively regulated by fluxes in overall energy balance [14]. Most previous studies have shown that total ghrelin is negatively correlated with %BF (obese individuals tend to have lower fasting ghrelin levels compared to lean controls) [17–19]. Based on these previous observations, one might have anticipated ORI (%BF: 23.4 in females, 15.4 in males) to have a higher fasting

Table 2: Hormone profiles in obesity resistant individuals (ORI) and obesity susceptible individuals (OSI) in response to a standardised meal.

	ORI						OSI								
	Females			Males			Females			Males			P value*	P value†	P value‡
	n	Median	Interquartile Range	n	Median	Interquartile Range	n	Median	Interquartile Range	n	Median	Interquartile Range			
Ghrelin															
Fasting (pg·mL^{-1})	14	70.7	39.2–129.5	17	55.2	38.6–137.3	12	69.3	59.5–105.5	12	55.0	35.6–65.1	0.319	0.600	0.662
AUC (mmol·min^{-1})	11	12596	7470–16987	16	11341	8747–21848	9	11232	9252–14968	9	9889	6136–10693	0.857	0.460	0.751
Nadir (pg·mL^{-1})	16	44.9	31.5–55.3	17	33.7	27.1–56.7	14	39.4	31.2–46.7	13	35.3	18.0–38.7	0.419	0.287	0.822
Time to nadir (min)	16	60	30–60	17	60	60-60	14	30	30–60	13	60	60-60	0.058	0.518	0.580
PYY															
Fasting (pg·mL^{-1})	14	47.9	40.1–53.7	17	55	47.8–65.3	14	47.1	37.5–67.3	12	56.4	49.4–62.0	0.161	0.889	0.761
AUC (mmol·min^{-1})	13	11700	10954–13230	16	12511	10524–13597	12	12779	10777–18752	9	12877	12436–13739	0.825	0.601	0.736
Peak (pg·mL^{-1})	16	72.6	68.2–82.0	17	77.8	66.7–87.5	15	75.4	55.0–112.4	13	75.7	68.7–87.5	0.981	0.877	0.916
Time to peak (min)	16	60	45–120	17	60	30–120	15	120	60–180	13	30	30-30	0.275	0.584	0.526
Leptin															
Fasting (pg·mL^{-1})	16	2426	1367–3626	17	693.4	440.9–1495.0	15	14367	4135–24941	12	2480	1424–8725	**<0.001**	**<0.001**	0.324
AUC (mmol·min^{-1})	16	425506	231672–582562	16	101878	74897–237294	14	1753714	502753–4671533	9	410572	268776–1746261	**<0.001**	**<0.001**	0.378
Nadir (pg·mL^{-1})	16	2003	1184–2986	17	531.7	339.7–1313.4	15	9015	2661–24450	13	2108	1198–6836	**0.003**	**0.001**	0.946
Time to nadir (min)	16	60	15–120	17	60	30–120	15	60	15–120	13	60	30–120	0.781	0.558	0.949
Glucose															
Fasting (mmol·L^{-1})	16	5.30	5.00–5.53	17	5.25	4.95–5.40	15	5.25	5.05–5.30	13	5.25	5.05–5.50	0.851	0.900	0.887
AUC (mmol·min^{-1})	16	1030	995–1063	17	1054	1013–1081	15	1062	970–1128	13	1048.0	992–1113	0.298	0.252	0.954
Peak (mmol·L^{-1})	16	6.9	6.70–7.55	17	7.3	7.00–8.00	15	7.2	6.7–7.8	13	7.5	7.10–7.60	**0.038**	0.540	0.759
Time to peak (min)	16	15	15–30	17	30	30-30	15	30	15–30	13	30	30-30	0.103	0.072	0.352
Insulin															
Fasting (pg·mL^{-1})	13	157	131.1–177.1	15	236.7	110.3–299.6	14	252.2	187.0–355.5	11	398	281.3–466.5	0.085	**0.004**	0.213
AUC (mmol·min^{-1})	12	105038	92640–143522	14	139943	108035–171234	12	175434	104685–217197	8	213250	131498–321104	0.057	**0.016**	0.802
Peak (pg·mL^{-1})	16	1345.1	965.9–1867.8	17	1677.5	1250.4–2298.3	15	1467.9	1098.9–2294.6	13	1849	1453–2475	0.145	0.196	0.632
Time to peak (min)	16	30	30-30	17	30	30-30	15	30	30-30	13	30	30-30	0.466	0.379	0.716

%BF: percent body fat, AUC: area under the curve, ORS: obesity resistance/susceptibility category.

Fasting and AUC data have been log transformed, *P value for sex adjusted for ORS, †P value for ORS adjusted for sex, ‡P value for ORS adjusted for sex and %BF.

Table 3: Appetite ratings for obesity resistant individuals (ORI) and obesity susceptible individuals (OSI) in response to a standardised meal.

	ORI						OSI								
	Females			Males			Females			Males			P value*	P value†	P value‡
	n	Median	Interquartile Range	n	Median	Interquartile Range	n	Median	Interquartile Range	n	Median	Interquartile Range			
Hunger															
Fasting (mm)	16	73	55–86	17	67	49–84	15	65	51–77	13	60	40–70	0.515	0.074	0.341
AUC (mm·min^{-1})	16	9049	4819–10084	17	6413	4200–10358	15	4275	2798–6780	13	6158	5528–9443	0.506	0.056	0.785
Nadir (mm)	16	22	2–30	17	19	10–36	15	1	0–4	13	9	1–27	0.170	**0.017**	0.414
Time to Nadir (min)	16	15	15–30	17	30	15–60	15	15	15–30	13	15	15–30	0.656	0.305	0.252
Fullness															
Fasting (mm)	15	13	5–20	17	16	6–28	15	14	3-41	13	17	1–28	0.871	0.918	0.826
AUC (mm·min^{-1})	15	7095	6158–8670	17	6720	4800–10125	15	10185	6600–11295	13	7088	5595–7598	0.053	0.451	0.470
Peak (mm)	16	76	64–95	17	71	49–77	15	90	74–96	13	63	51–77	0.817	0.482	0.754
Time to peak (min)	16	15	15–30	17	15	15–30	15	15	15–30	13	30	15–30	0.380	0.119	0.860
Preoccupation with Food															
Fasting (mm)	16	63	49–85	17	34	28–63	15	57	30–70	13	41	15–68	**0.015**	0.448	0.987
AUC (mm·min^{-1})	16	6008	3173–8948	17	4628	2805–10830	15	2813	1875–7155	13	6660	2378–8663	0.496	0.294	0.455
Nadir (mm)	16	15	4–31	17	10	4–27	15	1	0–8	13	15	1–25	0.309	0.193	0.871
Time to Nadir (min)	15	15	15–30	17	30	15–30	15	15	15–30	13	15	15–60	0.092	**0.040**	0.888
Desire to Eat															
Fasting (mm)	16	76	58–85	17	64	45–85	15	69	49–79	13	61	35–72	0.336	0.199	0.243
AUC (mm·min^{-1})	16	8456	4943–10609	17	6473	4118–10913	15	3900	2753–6938	13	6705	5723–9788	0.389	0.148	0.427
Nadir (mm)	16	25	2–31	17	18	10–31	15	2	0–5	13	15	1–31	0.134	0.059	0.745
Time to Nadir (min)	16	15	15–30	17	15	15–30	15	15	15–30	13	30	15–60	0.770	0.923	0.940
TFEQ															
Dietary Restraint	16	4	0–12	17	4	3–5	15	9	3–18	13	6	4–8	0.337	**<0.001**	**<0.001**
Disinhibition	16	3	1–10	17	3	2–5	15	12	3–15	13	7	5–10	0.255	**<0.001**	**0.005**
Hunger	16	8	1–13	17	8	3–9	15	6	1–13	13	8	4–10	0.791	0.749	0.665

%BF: percent body fat, AUC: area under the curve, ORS: obesity resistance/susceptibility category, TFEQ: three factor eating questionnaire.

*P value for sex adjusted for ORS, †P value for ORS adjusted for sex, ‡P value for ORS adjusted for sex and %BF.

ghrelin compared to OSI (%BF: 41.9 in females, 27.6 in males). However, our study showed no differences in ghrelin concentration between ORI and OSI. Perhaps this is evidence that ORI and OSI respond differently, in terms of eating behaviour, to the same ghrelin concentration. That is, the OSI's higher level of dietary restraint and disinhibition may indicate that they are either more responsive to orexigenic signals and/or less responsive to anorectic factors.

Similar gherlin concentrations, despite different body composition and therefore energy stores, have also been observed in the study cohorts of two previous studies. Khoury et al. [28] compared gut hormone response to three standardized meals in a group with the metabolic syndrome and lean controls. Despite a 15% difference in mean body fat (33.5% versus 17.9%) there was no difference in active ghrelin. Mittelman et al. [19] showed that although fasting active ghrelin concentration was lower in obese, postprandial increases were similar in both lean and obese children.

The similarities in ghrelin concentration among the two groups may reflect two different mechanisms. Whereas the OSI ghrelin levels are due to increased energy stores, the ORI may have lower than predicted ghrelin levels due to a possible underlying protective mechanism which theoretically could protect against overeating and subsequent weight gain. Thus, no obvious difference between the two groups is apparent. Obesity resistance individuals may differ from other populations previously studied in that they largely struggle to gain weight rather than simply being lean. One previous study that investigated constitutionally thin (i.e., those who find it difficult to gain weight) also found lower ghrelin levels than expected given their low %BF [16]. As ghrelin is orexigenic this finding may indicate a possible mechanism that prevents these particularly lean individuals from overeating.

PYY is an anorexigenic hormone that has been associated with meal satiety and thus theoretically meal termination [15]. One may expect that those who remain lean with relative ease have a higher concentration of these hormones in response to feeding when compared to those who struggle to maintain a healthy body weight. In the majority of previous studies investigating overweight and/or obese compared to lean subjects, PYY levels were higher amongst lean individuals postprandially, suggesting a blunted response in overweight/obese individuals [16, 19, 20, 22, 24]. In contrast, we found no difference in PYY concentrations in response to a meal. This finding compliments the ghrelin results and may also indicate a differential response to the same PYY level in ORI versus OSI. Khoury et al. [28] also observed no differences in PYY responses and AUC between individuals with the metabolic syndrome and controls in response to a variety of meals.

Consistent with results from previous studies where higher leptin concentrations have been associated with increases in BMI [17, 29], the fasting and postprandial leptin concentrations in the present study were also higher in the group with greater BMI (OSI). This is in line with previous literature that has highlighted the concept of leptin resistance in overweight and obese individuals as a result of increased adipose tissue stores [30]. In our study OSI have markedly higher leptin concentrations than ORI but equivalent satiety scores. This suggests that OSI are not responding fully to the high concentrations of leptin.

In the face of similar hormone patterns it would have been of interest to observe how much our two groups would have eaten when presented with an *ad libitum* meal. Would they choose a similar meal size or would OSI have actually wanted to eat more? Future research should seek to investigate this as it represents a more realistic eating situation.

In addition to the potential differential response to the hormones in these two distinct groups of individuals there were some differences in perceived appetite ratings. Most notably, the ORI experienced smaller fluctuations in hunger ratings. In addition, the TFEQ indicates that ORI may respond differently to hunger in that they are less likely to engage in dietary restraint and disinhibition behaviours. This style of eating could be an artifact of the differential response to the hormones or may be in response to some psychological factors or learned behaviour. Overeating and lack of response to satiety cues may be a learned response that affects some to a greater extent than others. Physiological signals and behavioural cues both regulate appetite and energy intake. Which one predominates may depend on a number of factors including genetics, the environment, past experiences, parental influence, sensory stimulation, social situation, and psychological well-being.

There are some limitations that should be considered when interpreting the results of this study. Firstly, the cross-sectional design of the study does not allow us to draw casual inferences and the sample size was relatively small. Further, given that ghrelin plasma concentrations differ throughout the day in cyclic fashion in relation to meal taking and diurnal rhythms [14], it may be that the analyses simply did not capture ghrelin differences as values were only assessed over a 4 h period in the morning. In addition, any differences observed between males and females may be attributable to differences in the energy content of the standardized meal as this was based on sex rather than estimated energy requirements. This method for assigning energy content to the standardized meal could also potentially have resulted in the OSI eating less and the ORI eating more than they are used to. Furthermore, current dieting patterns may have influenced the results as individualswho were in a pattern of energy restriction may have responded differently compared to someone who was not actively trying to lose weight.

In conclusion, comparing people who remain lean despite living in an obesegenic environment (ORI) to those who struggle to maintain a healthy weight (OSI) has provided us with a novel approach to investigate the aetiology of obesity. Given the differential body weights observed in the two study groups in the present study, a similar ghrelin concentration was unexpected. This could indicate that OSI respond differently to the same ghrelin concentration. Conversely, the lower than expected fasting ghrelin levels observed in the ORI may provide a protective mechanism that enables these individuals to remain lean. This warrants further investigation. The higher levels of dietary restriction and disinhibition amongst OSI indicate that psychosocial factors are likely also important regulators of energy balance in this group.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank those who participated in the study. This project was funded by a University of Otago Research Grant.

References

[1] World Health Organisation, "Obesity: preventing and managing the global epidemic," A report of a WHO consultation Report Series 894, WHO, Geneva, Switzerland, 2000.

[2] M. Bajekal, R. Borehan, B. Erens et al., *Health Survey for England 1998*, The Stationery Office, London, UK, 1999.

[3] J. W. Anderson, E. C. Konz, R. C. Frederich, and C. L. Wood, "Long-term weight-loss maintenance: a meta-analysis of US studies," *American Journal of Clinical Nutrition*, vol. 74, no. 5, pp. 579–584, 2001.

[4] T. Brown, S. Kelly, and C. Summerbell, "Prevention of obesity: a review of interventions," *Obesity Reviews*, vol. 8, no. 1, supplement, pp. 127–130, 2007.

[5] F. Grodstein, R. Levine, L. Troy, T. Spencer, G. A. Colditz, and M. J. Stampfer, "Three-year follow-up of participants in a commercial weight loss program: can you keep it off?" *Archives of Internal Medicine*, vol. 156, no. 12, pp. 1302–1306, 1996.

[6] A. G. Tsai and T. A. Wadden, "Systematic review: an evaluation of major commercial weight loss programs in the United States," *Annals of Internal Medicine*, vol. 142, no. 1, pp. 56–66, 2005.

[7] Y. Wang, M. A. Beydoun, L. Liang, B. Caballero, and S. K. Kumanyika, "Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic," *Obesity*, vol. 16, no. 10, pp. 2323–2330, 2008.

[8] K. M. Flegal, "Trends in body weight and overweight in the U.S. population," *Nutrition Reviews*, vol. 54, no. 4, pp. S97–S100, 1996.

[9] K. M. Flegal and R. P. Troiano, "Changes in the distribution of body mass index of adults and children in the US population," *International Journal of Obesity*, vol. 24, no. 7, pp. 807–818, 2000.

[10] C. E. Lewis, D. E. Smith, D. D. Wallace, O. Dale Williams, D. E. Bild, and D. R. Jacobs Jr., "Seven-year trends in body weight and associations with lifestyle and behavioral characteristics in Black and White young adults: the CARDIA study," *American Journal of Public Health*, vol. 87, no. 4, pp. 635–642, 1997.

[11] C. E. Lewis, D. R. Jacobs Jr., H. McCreath et al., "Weight gain continues in the 1990s 10-year trends in weight and overweight from the CARDIA study," *American Journal of Epidemiology*, vol. 151, no. 12, pp. 1172–1181, 2000.

[12] K. Midthjell, Ø. Krüger, J. Holmen et al., "Rapid changes in the prevalence obesity and known diabetes in an adult Norwegian population: the Nord-Trondelag Health Surveys: 1984–1986 and 1995–1997," *Diabetes Care*, vol. 22, no. 11, pp. 1813–1820, 1999.

[13] M. Shah, P. J. Hannan, and R. W. Jeffery, "Secular trend in body mass index in the adult population of three communities from the upper mid-western part of the USA: the Minnesota Heart Health Program," *International Journal of Obesity*, vol. 15, no. 8, pp. 499–503, 1991.

[14] D. E. Cummings, J. Q. Purnell, R. S. Frayo, K. Schmidova, B. E. Wisse, and D. S. Weigle, "A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans," *Diabetes*, vol. 50, no. 8, pp. 1714–1719, 2001.

[15] E. Karra, K. Chandarana, and R. L. Batterham, "The role of peptide YY in appetite regulation and obesity," *Journal of Physiology*, vol. 587, no. 1, pp. 19–25, 2009.

[16] N. Germain, B. Galusca, C. W. Le Roux et al., "Constitutional thinness and lean anorexia nervosa display opposite concentrations of peptide YY, glucagon-like peptide 1, ghrelin, and leptin," *American Journal of Clinical Nutrition*, vol. 85, no. 4, pp. 967–971, 2007.

[17] J. M. Beasley, B. A. Ange, C. A. M. Anderson, E. R. Miller III, J. T. Holbrook, and L. J. Appel, "Characteristics associated with fasting appetite hormones (obestatin, Ghrelin, and Leptin)," *Obesity*, vol. 17, no. 2, pp. 349–354, 2009.

[18] C. W. Le Roux, M. Patterson, R. P. Vincent, C. Hunt, M. A. Ghatei, and S. R. Bloom, "Postprandial plasma ghrelin is suppressed proportional to meal calorie content in normal-weight but not obese subjects," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 2, pp. 1068–1071, 2005.

[19] S. D. Mittelman, K. Klier, S. Braun, C. Azen, M. E. Geffner, and T. A. Buchanan, "Obese adolescents show impaired meal responses of the appetite-regulating hormones ghrelin and PYY," *Obesity*, vol. 18, no. 5, pp. 918–925, 2010.

[20] J. P. Lomenick, J. L. Clasey, and J. W. Anderson, "Meal-related changes in ghrelin, peptide YY, and appetite in normal weight and overweight children," *Obesity*, vol. 16, no. 3, pp. 547–552, 2008.

[21] A. Geliebter, S. A. Hashim, and M. E. Gluck, "Appetite-related gut peptides, ghrelin, PYY, and GLP-1 in obese women with and without binge eating disorder (BED)," *Physiology and Behavior*, vol. 94, no. 5, pp. 696–699, 2008.

[22] P. T. Pfluger, J. Kampe, T. R. Castaneda et al., "Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 2, pp. 583–588, 2007.

[23] C. Verdich, S. Toubro, B. Buemann, J. Lysgård Madsen, J. Juul Holst, and A. Astrup, "The role of postprandial releases of insulin and incretin hormones in meal-induced satiety—effect of obesity and weight reduction," *International Journal of Obesity*, vol. 25, no. 8, pp. 1206–1214, 2001.

[24] S. Stock, P. Leichner, A. C. K. Wong et al., "Ghrelin, peptide YY, glucose-dependent insulinotropic polypeptide, and hunger responses to a mixed meal in anorexic, obese, and control female adolescents," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 4, pp. 2161–2168, 2005.

[25] J. Blundell, C. de Graaf, T. Hulshof et al., "Appetite control: methodological aspects of the evaluation of foods," *Obesity Reviews*, vol. 11, no. 3, pp. 251–270, 2010.

[26] R. J. Stubbs, D. A. Hughes, A. M. Johnstone et al., "The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings," *British Journal of Nutrition*, vol. 84, no. 4, pp. 405–415, 2000.

[27] A. J. Stunkard and S. Messick, "The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger," *Journal of Psychosomatic Research*, vol. 29, no. 1, pp. 71–83, 1985.

[28] D. E. Khoury, N. Hwalla, V. Frochot, J.-M. Lacorte, M. Chabert, and A. D. Kalopissis, "Postprandial metabolic and hormonal

responses of obese dyslipidemic subjects with metabolic syndrome to test meals, rich in carbohydrate, fat or protein," *Atherosclerosis*, vol. 210, no. 1, pp. 307–313, 2010.

[29] R. V. Considine, M. K. Sinha, M. L. Heiman et al., "Serum immunoreactive-leptin concentrations in normal-weight and obese humans," *New England Journal of Medicine*, vol. 334, no. 5, pp. 292–295, 1996.

[30] J. F. Caro, M. K. Sinha, J. W. Kolaczynski, P. L. Zhang, and R. V. Considine, "Leptin: the tale of an obesity gene," *Diabetes*, vol. 45, no. 11, pp. 1455–1462, 1996.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 356289, 9 pages
http://dx.doi.org/10.1155/2014/356289

Research Article

Identification of Psychological Dysfunctions and Eating Disorders in Obese Women Seeking Weight Loss: Cross-Sectional Study

Maude Panchaud Cornut,[1] Jennifer Szymanski,[1] Pedro Marques-Vidal,[2] and Vittorio Giusti[1,3]

[1] *Service of Endocrinology, Diabetes and Metabolism, University Hospital CHUV, rue du Bugnon, 1011 Lausanne, Switzerland*
[2] *Institute of Social and Preventive Medicine, University Hospital CHUV, rue du Bugnon, 1011 Lausanne, Switzerland*
[3] *Département de Médecine, Hôpital Intercantonal de la Broye, 1470 Estavayer-le-Lac, Switzerland*

Correspondence should be addressed to Vittorio Giusti; vittorio.giusti@hibroye.ch

Received 21 October 2013; Revised 16 January 2014; Accepted 6 February 2014; Published 11 March 2014

Academic Editor: Yi-Hao Yu

Objective. The aim of this study is to analyse associations between eating behaviour and psychological dysfunctions in treatment-seeking obese patients and identify parameters for the development of diagnostic tools with regard to eating and psychological disorders. *Design and Methods*. Cross-sectional data were analysed from 138 obese women. Bulimic Investigatory Test of Edinburgh and Eating Disorder Inventory-2 assessed eating behaviours. Beck Depression Inventory II, Spielberger State-Trait Anxiety Inventory, form Y, Rathus Assertiveness Schedule, and Marks and Mathews Fear Questionnaire assessed psychological profile. *Results*. 61% of patients showed moderate or major depressive symptoms and 77% showed symptoms of anxiety. Half of the participants presented with a low degree of assertiveness. No correlation was found between psychological profile and age or anthropometric measurements. The prevalence and severity of depression, anxiety, and assertiveness increased with the degree of eating disorders. The feeling of ineffectiveness explained a large degree of score variance. It explained 30 to 50% of the variability of assertiveness, phobias, anxiety, and depression. *Conclusion*. Psychological dysfunctions had a high prevalence and their severity is correlated with degree of eating disorders. The feeling of ineffectiveness constitutes the major predictor of the psychological profile and could open new ways to develop screening tools.

1. Introduction

The prevalence of obesity has increased markedly worldwide during the past 20 years [1]. Among US adults, approximately 127 million are overweight, 60 million are obese, and 9 million have morbid obesity (body mass index (BMI) > 40 kg/m^2) [2–4].

This increase has a major impact on public health and on health care costs because of the raise of obesity-related diseases [4]. Further, and despite more than $30 billion spent per year on weight-reduction programs [4–6], their efficacy has not increased accordingly [7]. A recent review suggested that the standard conservative treatments (diet, physical activity, cognitive-behavioural therapy, and drugs) are ineffective in the long term in 95% of the patients [8]. After diet alone, 75% of patients regain most of their weight [9] and the addition of behavioural treatments only modestly improves the results [10]. Bariatric surgery is currently the only treatment achieving a sufficient and durable weight loss [11, 12]; still, follow-up studies show that a number of patients present a weight regain as early as 1 to 2 years after surgery [13, 14].

One of the major reasons for the treatments' ineffectiveness is the large prevalence of eating disorders in obese patients trying to lose weight [15], namely, binge eating disorder (BED) [16]. The impact of weight loss programs on the reduction of BED is low and actually tends to exacerbate the severity of BED and obesity [17, 18]. Specific

treatments including psychological support are essential in those patients to improve long term results and to escape from weight cycling [19–22]. Interestingly, most patients with BED start binge eating prior to the onset of dieting. The eating disorder therefore seems to be the *primum movens* leading to weight gain [23, 24]. Other psychological dysfunctions such as depression are also frequent among obese subjects [25]. Moreover, obese patients with BED present a higher prevalence and/or severity of most psychological dysfunctions than obese patients without BED [26–32]. The detection of psychological dysfunctions in obese patients is essential as these are associated with lower weight self-efficacy and limited weight loss [20, 33]. This evidence suggests that the identification of potential psychological dysfunctions is a very important step in the assessment of an obese patient, as are the detecting potential cardiovascular and metabolic comorbidities. Unfortunately, the implementation of psychological assessment is complex and time consuming and requires the use of specific questionnaires by psychologists.

Despite the fact that a number of studies already showed that binge eating in obese women is a marker for greater psychiatric morbidity, no data is available concerning potential predictive factors for the identification of psychological distress in patients who suffer from eating disorders.

Thus, the aim of this study was to analyse the associations between eating behaviour and psychological dysfunctions in obese patients searching weight loss and to identify possible predictive parameters for future development of diagnostic tools in the field of eating and psychological disorders.

2. Materials and Methods

2.1. Patient's Sampling. This work was approved by the Ethical Committee of Lausanne University Medicine School and was conducted at the Outpatient Obesity Clinic of the University Hospital of Lausanne, Switzerland. Inclusion criteria were female gender, willingness to lose weight, and agreeing to participate; as men represent less than 5% of our Obesity Clinic, it was decided to exclude them as it would be very difficult to obtain an adequate sample size. The current use of psychotropic medication was an exclusion criterion. Overall, one hundred and fifty women trying to lose weight and to control food compulsion accepted to participate.

2.2. Anthropometric Measurements and Weight History. Body weight was measured in kg with a Detecto scale with a precision of 0.2 kg; height was measured in cm with a stadiometer with a precision of 0.5 cm. For the weight the clothes and shoes were left off and for the height the shoes and socks were left off. For each parameter only one measurement was taken. BMI was calculated as weight/height squared (kg/m^2). Waist circumference was taken at the smallest standing horizontal circumference between the ribs and the iliac crest using a TEC anthropometric tape (Rollfix, Hoechst Mass, Germany). Three measurements were taken with the criterion that difference between the measurements had to be less than 2 cm apart and an average of these three values was calculated. Additional measurements were taken when needed until this criterion was fulfilled.

A specific case history was taken in order to estimate weight history and fluctuation during the patient's life. The participation in organised weight loss programs defined as a diet following a defined program through a nutritionist or an organisation and the number of intentional weight loss attempts were collected. The previous use of weight loss drugs was also registered. The presence of a weight cycling syndrome (WCS), defined as at least 3 weight reductions of ≥5 kg with a subsequent regain of ≥50% of the weight loss, was also assessed [34, 35].

2.3. Eating Behaviours and Eating Disorders. The eating behaviours over the last six months before evaluation were assessed by the use of a clinical specific interview and two specific questionnaires: the Bulimic Investigatory Test of Edimburgh (BITE) [36] and the Eating Disorder Inventory-2 (EDI-2) [37, 38].

Regarding the BITE scales the score used was proposed previously by Henderson and Freeman [36]: symptom score was divided in three groups: high (≥20, indicating presence of binge eating), medium (10–19, suggesting unusual eating pattern), and low (<10, being within normal limits) score; for severity scale, a score ≥5 was considered as clinically significant. For the ineffectiveness item of the EDI-2, since the absence of standardized scores, three groups were arbitrarily established for statistical analysis: low (0–5), medium (6–10), and high (≥11) score.

2.4. Psychological Profile. All patients were evaluated by the same trained psychologist with a large experience of years of practice and a specific formation in eating disorders, through three one-hour semistructured interviews. During these interviews, depression, anxiety, phobias, and assertiveness were assessed by the following questionnaires: the Beck Depression Inventory II (BDI-II) [39]; the Spielberger State-Trait Anxiety Inventory (STAI) [40], form Y (French-Canadian adaptation IASTA-Y) [41]; the Rathus Assertiveness Schedule (RAS) [42], and the Marks and Mathews Fear Questionnaire (Fear M and M) [43]. These questionnaires were used to assess characteristic attitudes and symptoms of depression, anxiety, phobia, and assertiveness and not to perform a clinical diagnosis.

The following cutoff values were used for grouping total BDI-II scores: 0–9 not depressed; 10–15 mildly depressed; 16–24 moderately depressed; ≥25 severely depressed [44]. For the STAI-Y, considering the sex and the mean age of our cohort, a value ≥40 was applied to define clinically significant symptoms of transient and enduring levels of anxiety [45–47]. For the RAS, a score ≥105 was considered representative of a low degree of assertiveness, as proposed by Bouvard and Cottraux in the French version of the questionnaire [48]. The Fear M and M questionnaire has no formal cutoff point, so the following cutoff values were defined according to Cottraux [43] in the validated French version of the questionnaire: agoraphobia ≥27 and for social phobia ≥23.

2.5. Statistical Analysis. All analysis was performed using JMP 8 statistical package (SAS Institute, Cary, NC, USA).

Results were expressed as number of patients and percentages or as mean ± standard deviation (SD). Between-group comparisons were performed using Chi-square for qualitative variables and Student's t-test or analysis of variance (ANOVA) for quantitative variables. The associations between anthropometric measurements, eating behaviours, and psychological profile were evaluated by univariate non-parametric Spearman's correlation. The associations between eating behaviours and psychological profile were further refined by Cochran-Armitage trend test and by multivariate forward stepwise regression (P value for entry is 0.05) using the scores of the psychological questionnaires (BDI-II, STAI-Y, and RAS), as dependent variables, and age, BMI, BITE score, and items score of EDI-2 as independent variables. The R^2 values for each final (i.e. including all significant variables) model were computed. Statistical significance was considered for $P < 0.05$.

3. Results

3.1. Patients' Characteristics. Of the 150 women, 12 (8%) were excluded from the analysis because of missing data for height ($n = 3$), waist circumference ($n = 12$), and/or STAI-Y ($n = 9$). The remaining 138 women had a mean age (±SD) of 41.4 ± 11.6 years, a mean BMI of 39.3 ± 6.4 kg/m^2, and a mean waist circumference of 108.6 ± 14.3 cm.

One hundred and ten (80%) of all patients had made >5 intentional weight loss attempts and 109 (79%) presented with WCS. The BMI of patient with WCS was significantly higher than in patients without WCS: 40.3 ± 7 versus 35.8 ± 4 ($P < 0.01$).

3.2. Eating Profile. The mean BITE symptom and severity scores were 18.3±6.4 and 4.0±3.3, respectively. Almost half of the patients (48.6%) had a high (≥20) BITE symptom score, and 41% of them had a clinically significant BITE severity score. No correlations were found between the questionnaire scores and age or BMI or waist circumference of the patients. The mean scores of EDI-2's items were drive for thinness 8.9 ± 5.3; bulimia 5.2 ± 4.3; body dissatisfaction 20.8 ± 6.6; ineffectiveness 8.1±6.9; perfectionism 5.7±4.3; interpersonal distrust 4.0 ± 3.8; interoceptive awareness 7.6 ± 5.8; maturity fears 3.8±4.1; asceticism 5.9±3.2; impulse regulation 4.0±4.4; social insecurity 5.6 ± 4.4.

3.3. Psychological Profile. The psychological profile of the patients is summarized in Table 1. Over half of the patients showed moderate (26%) or major (35%) depressive symptoms. Clinically significant signs of enduring levels of anxiety were found in 77% of patients, and a low degree of assertiveness was found in approximately half of the patients. Agoraphobia was identified in about 4% of patients and social phobia was identified in 20%. Conversely, no differences in BMI and waist circumference were found within all subclasses of the different psychological groups evaluated by the four questionnaires.

3.4. Association between Eating Behaviours and Psychological Profile. No correlations were found between psychological markers and age or BMI, while strong positive correlations were found between psychological markers and BITE components. Similarly, strong correlations were found between psychological markers and most EDI-2 components, namely, ineffectiveness, social insecurity, interoceptive awareness, and impulse regulation (Table 2).

The results of the stepwise regression analyses using psychological (BDI-II, STAI-Y State-Trait, and RAS) scores as dependent variables and the scores of BITE and EDI-2 items and age as independent variables are summarized in Table 3. Overall, depression and RAS were associated with BITE symptom score and EDI-2 ineffectiveness score, while anxiety was associated with BITE severity score and EDI-2 ineffectiveness score. In all models, EDI-2 ineffectiveness score was the variable most related with psychological scores, and in all models the percentage of variance explained was over 30%, with a value >50% for the Beck Depression score (Table 3).

The results of the different psychological questionnaires (BDI-II, STAI-Y State-Trait, RAS, and Fear M and M anxiety-depression and social phobia items) according to the BITE symptoms categories and EDI-2 ineffectiveness groups are summarized in Figures 1 and 2. Depression scores as well as the number of patients presenting with major depressive symptoms increased with high severity at BITE symptoms; similarly, STAI-Y Trait score and prevalence of patients presenting clinically significant symptoms of anxiety also increased with high severity of assertiveness. Finally, RAS scores increased with high severity of BITE symptoms while a borderline significant (P value <8%) trend was found for the prevalence of a high degree of the assertiveness (Figures 1 and 2).

4. Discussion

This study confirms that obese women with an obesity level 2 and seeking weight loss present high prevalence of typical psychological characteristics especially depressive and anxious symptoms and a low degree of assertiveness. These results are compatible with those shown in the literature [25, 49–56].

The prevalence and the severity of eating disorders and of psychological dysfunctions were not correlated with the degree and type of obesity. In a previous study, Onyike et al. [57] reported a positive correlation between major depression and obesity. However, they analyzed data from the Third National Health and Nutrition Examination Survey, which included underweight and obese subjects. Ahlberg et al. [58] also documented a significant correlation between depressive and anxious symptoms and abdominal distribution of body fat, but not with degree of obesity, and Hill and Williams [59] showed that a higher severity of the obesity was not bound with a higher frequency of psychological disorders.

On the other hand the prevalence and severity of depression, anxiety, and assertiveness increased according to the degree of eating disorders. Several studies showed that obese patients with BED presented with a higher prevalence and/or severity of most of psychological dysfunctions in comparison to obese patients without BED [26–32]. Didie and Fitzgibbon

Table 1: Distribution and characteristics of patients according to psychological profile.

	Score	N° patients (%)	Age (years)	BMI (kg/m^2)
BDI-II	0–9	37 (26.8)	40.9 ± 11.4	39.5 ± 6.7
	10–15	17 (12.3)	45.2 ± 11.6	39.0 ± 8.6
	16–24	36 (26.1)	40.7 ± 10.2	39.8 ± 6.5
	≥25	48 (34.8)	40.9 ± 12.7	38.9 ± 5.3
Test (*P* value)			0.98 (0.40)	0.10 (0.96)
STAI-Y State	≤39	48 (34.8)	40.8 ± 9.8	39.2 ± 7.3
	≥40	90 (65.2)	41.7 ± 12.4	39.3 ± 5.9
Test (*P* value)			0.19 (0.67)	0.01 (0.91)
STAI-Y Trait	≤39	31 (22.5)	42.0 ± 10.8	39.2 ± 6.6
	≥40	107 (77.5)	41.2 ± 11.8	39.3 ± 6.3
Test (*P* value)			0.12 (0.73)	0.01 (0.96)
RAS	<105	66 (47.8)	41.8 ± 11.4	40.2 ± 7.2
	≥105	72 (52.2)	41.1 ± 11.8	38.4 ± 5.3
Test (*P* value)			0.31 (0.76)	1.49 (0.14)
Fear M and M				
Agoraphobia	≤26	133 (96.4)	41.6 ± 11.5	39.3 ± 6.4
	≥27	5 (3.6)	36.8 ± 13.7	40.3 ± 4.2
Test (*P* value)			0.82 (0.37)	0.13 (0.71)
Social phobia	≤22	111 (80.4)	42.5 ± 11.5	39.5 ± 5.0
	≥23	27 (19.6)	37.0 ± 11.0	38.3 ± 5.0
Test (*P* value)			4.93 (0.03)	0.79 (0.38)

Results are expressed as number of subjects and percentages or as mean ± standard deviation. BMI: body mass index; BDI-II: Beck Depression Inventory II; STAI-Y: Spielberger State-Trait Anxiety Inventory, form Y; RAS: Rathus Assertiveness Schedule; Fear M and M: Marks and Mathews Fear Questionnaire. Statistical analyses performed using Student's *t*-test or analysis of variance for quantitative variables.

Table 2: Spearman's correlations between anthropometric parameters, eating behaviour, and psychological profile.

	BDI-II	STAI-Y State	STAI-Y Trait	RAS
Age	-0.102^{NS}	-0.084^{NS}	-0.150^{NS}	0.026^{NS}
BMI	-0.003^{NS}	0.041^{NS}	-0.005^{NS}	-0.104^{NS}
Waist circumference	0.027^{NS}	-0.166^{NS}	-0.008^{NS}	0.008^{NS}
BITE				
Symptom	0.374^{***}	0.398^{***}	0.338^{***}	0.275^{***}
Severity	0.261^{**}	0.314^{***}	0.283^{***}	0.184^{*}
EDI-2				
Ineffectiveness	0.677^{***}	0.616^{***}	0.709^{***}	0.548^{***}
Interpersonal distrust	0.221^{**}	0.088^{NS}	0.333^{***}	0.234^{**}
Desire for thinness	0.291^{***}	0.375^{***}	0.294^{***}	0.133^{NS}
Bulimia	0.335^{***}	0.292^{***}	0.285^{***}	0.322^{***}
Body dissatisfaction	0.105^{NS}	0.250^{**}	0.251^{**}	0.200^{*}
Perfectionism	0.190^{*}	0.218^{*}	0.162^{NS}	0.082^{NS}
Interoceptive awareness	0.564^{***}	0.486^{***}	0.488^{***}	0.438^{***}
Maturity fears	0.304^{***}	0.265^{**}	0.360^{***}	0.115^{NS}
Asceticism	0.260^{**}	0.366^{***}	0.262^{**}	0.232^{**}
Impulse regulation	0.461^{***}	0.387^{***}	0.472^{***}	0.315^{***}
Social insecurity	0.576^{***}	0.462^{***}	0.565^{***}	0.437^{***}

BMI: body mass index; BITE: Bulimic Investigatory Test of Edinburgh; EDI-2: Eating Disorder Inventory-2; BDI-II: Beck Depression Inventory II; STAI-Y: Spielberger State-Trait Anxiety Inventory, form Y; RAS: Rathus Assertiveness Schedule. Statistical analysis by Spearman's rank correlations: ^{NS}not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

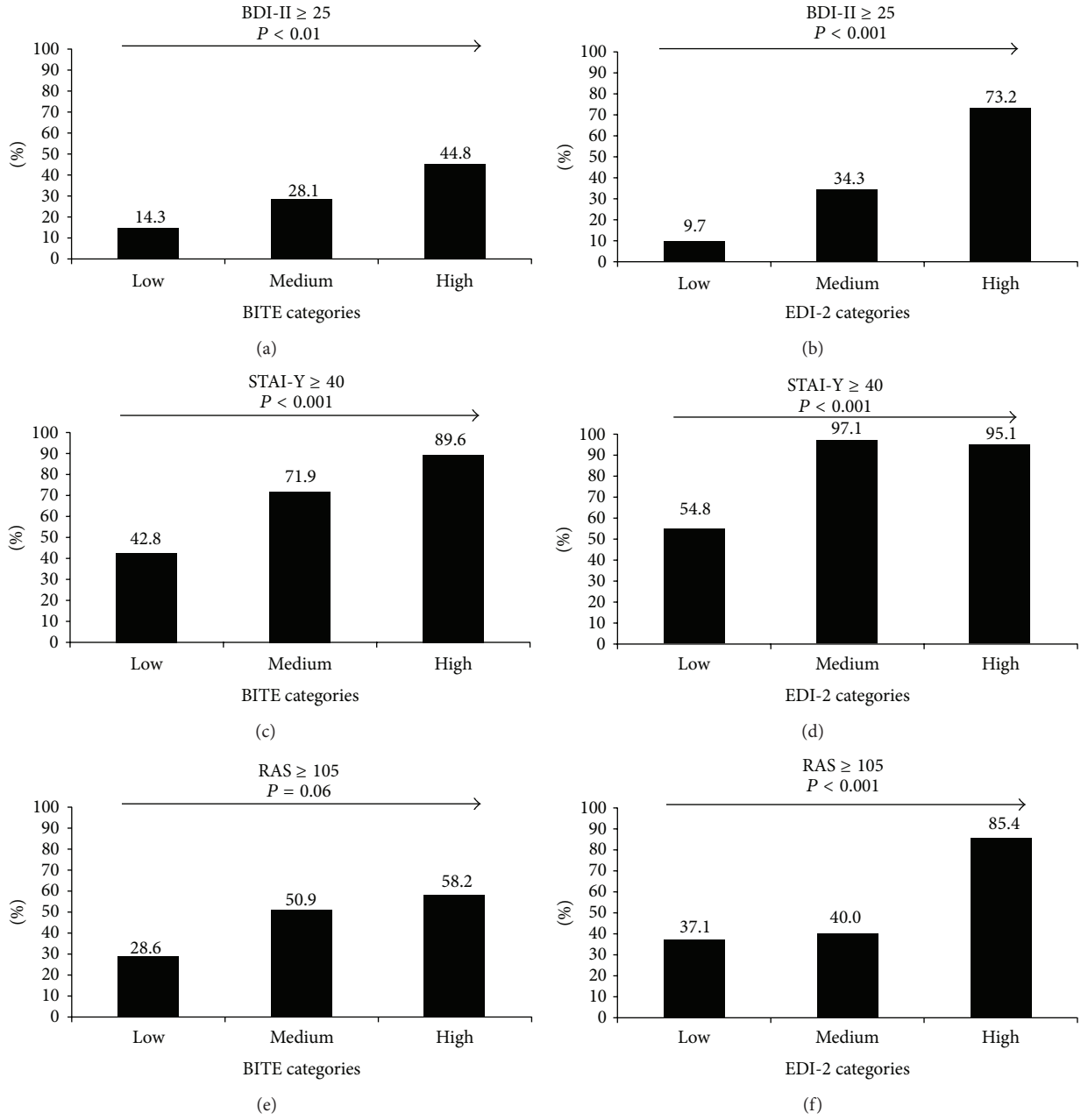


Figure 1: Psychological profile according to eating behaviour categories, as assessed by the Bulimic Investigatory Test of Edinburgh (BITE) symptoms score, and to ineffectiveness groups, as assessed by the Eating Disorder Inventory-2 (EDI-2). Results are expressed as percentage. BDI-II: Beck Depression Inventory II; STAI-Y: Spielberger State-Trait Anxiety Inventory, form Y; RAS: Rathus Assertiveness Schedule. Statistical analyses were performed using Cochran-Armitage trend test for qualitative variables and analysis of variance for quantitative variables.

[60] showed that eating disorders account for psychological dysfunction independently of weight status and Fassino et al. [27, 28] showed that obese patients with eating disorders were at higher risk of being diagnosed with personality disorders and concluded that the presence of binge eating in obese women is a marker for greater medical and psychiatric morbidity. Particularly Behar et al. [61] showed that a lack of assertiveness was a significant trait in patients with eating disorders and may be considered as a predictive factor in the development of an eating disorder and Elfhag [62] showed that a lack of assertiveness characterized obese patients with more problematic eating behaviours and that a greater self-assertiveness was found in patients with a relatively more efficient eating strategy.

Furthermore Iliceto et al. [63] showed that overweight or obese women have higher scores on the EDI-2 subscales and Villarejo et al. [64] showed that obese women with BED had still higher scores on some EDI-2 subscales compare with obese women without BED or control group with normal weight. In another study Barry et al. [65] found also that

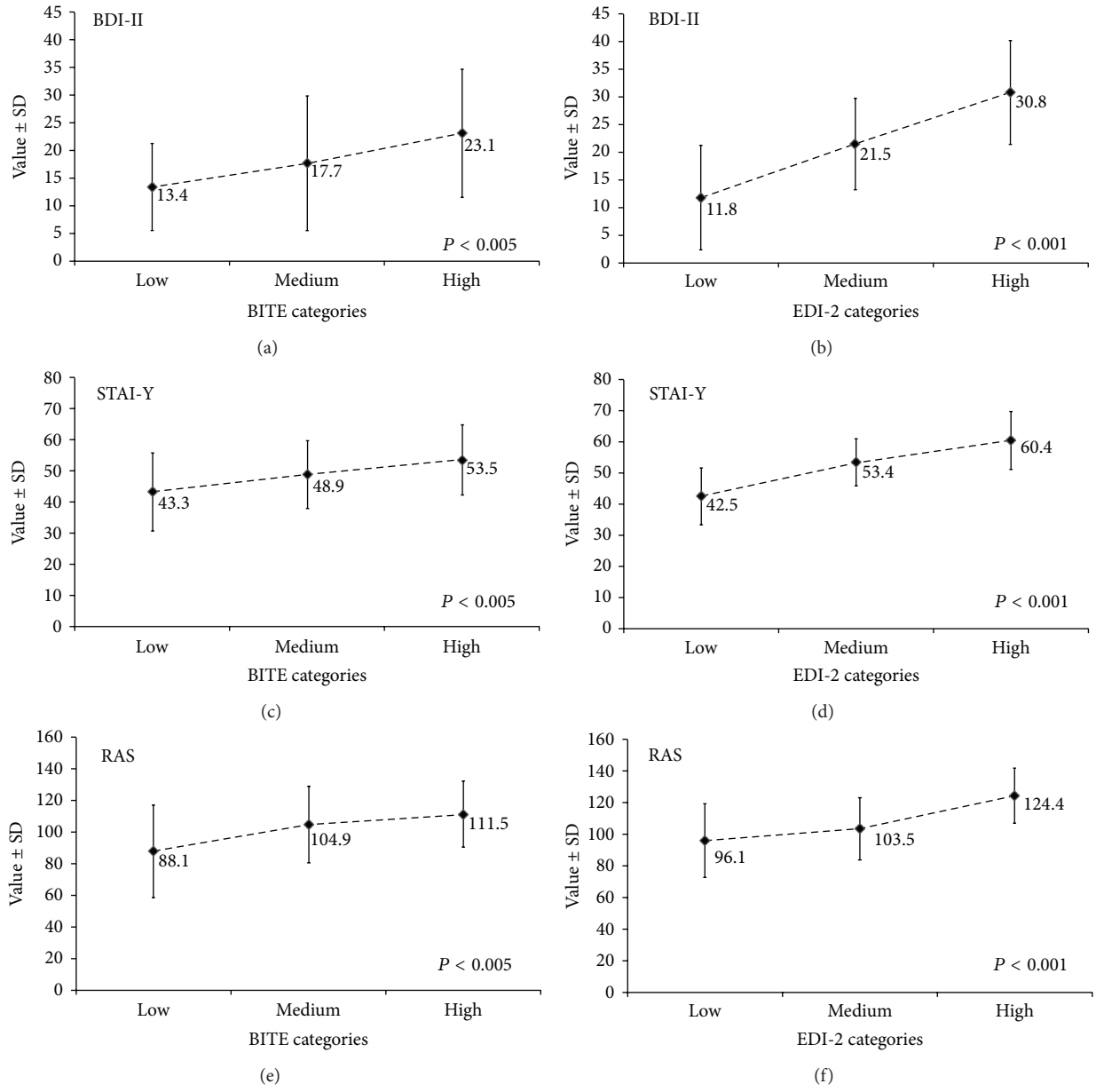


Figure 2: Psychological profile according to eating behaviour categories, as assessed by the Bulimic Investigatory Test of Edinburgh (BITE) symptoms score, and to ineffectiveness groups, as assessed by the Eating Disorder Inventory-2 (EDI-2). Results are expressed as mean ± standard deviation. BDI-II: Beck Depression Inventory II; STAI-Y, Spielberger State-Trait Anxiety Inventory, form Y; RAS: Rathus Assertiveness Schedule. Statistical analyses were performed using Cochran-Armitage trend test for qualitative variables and analysis of variance for quantitative variables.

patients with BED differ also in subscale of EDI-2. This variation was bound to the presence of BED but not with the obesity.

Stepwise regression analyses of eating status and psychological profile identified the ineffectiveness score of the EDI-2 as the major determinant of each of the questionnaires used (BDI-II, STAI-Y, and RAS). This is the most relevant and new finding of this work. In fact, for the first time, a potential predictive parameter for the identification of psychological distress has been recognized.

This finding has clinical implications in the health care of obese patients. Indeed, in order to recognize patients at risk of psychological dysfunctions, the first step in patient's evaluation should include a specific interview focusing on emotional behavioral aspects, such as body dissatisfaction, ineffectiveness, or perfectionism, for example. The development of such a questionnaire based on these emotional behavioral aspects could complement the interview and generate a promising data basis to conduct longitudinal cohort studies comparing results of long-term weight loss depending on different screening methods.

From a psychopathological point of view, these results (high ineffectiveness score, low interoceptive awareness, and impulsivity among obese) are in line with Hilde Bruch

Table 3: Multivariate analysis of the associations between eating behaviours and psychological profile.

	BDI-II	STAI-Y State	STAI-Y Trait	RAS
Age	—	—	—	0.150
BITE				
Symptom	0.222	—	—	0.190
Severity	—	0.188	0.199	—
EDI-2				
Ineffectiveness	0.653	0.636	0.651	0.495
Interpersonal distrust	—	−0.149	—	—
R^2 of model	**0.546**	**0.400**	**0.478**	**0.323**

Statistical analysis by forward stepwise linear regression. BITE: Bulimic Investigatory Test of Edinburgh; EDI-2: Eating Disorder Inventory-2; BDI-II: Beck Depression Inventory II; STAI-Y: Spielberger State-Trait Anxiety Inventory, form Y; RAS: Rathus Assertiveness Schedule.
Results are expressed as standardized slope. —: not retained. The other items of the EDI-2 (desire for thinness, bulimia, body dissatisfaction; perfectionism, interoceptive awareness, maturity fears, asceticism, impulse regulation, and social insecurity) were not retained.

and Bergeret's psychoanalytical theories, as well as with Fairburn and Apfeldorfer's cognitive-behavioral models of the obese patient's psychological functioning. Fairburn's concept regarding the factors contributing to the persistence of eating disorders is of particular interest. He suggested that a biopsychological vulnerability, together with ambivalent relationships with the environment, results in low self-esteem. The eating disorder resulting from this low self-esteem attempts to compensate it, followed by a self-controlling attitude with restrictive eating behaviors, which provides an immediate feeling of control, thus positively reinforcing self-esteem. However this behavior cannot be maintained in the long term and inevitably results in loss of control (binging behaviors). The fragile self-esteem which had momentarily been established is then shattered and feelings of frustration, guilt, and inefficacy take over. This experience of failure produces feelings of anxiety, which remind us of the similarities between eating disorders and the phobic functioning described by Apfeldorfer.

The results of this study also highlight that psychiatric diagnosis per se might not be useful for outcome prediction and that identification of underlying psychological dynamics might be more promising. Recent critics with regard to DSM criteria for research purposes are in line with these assumptions. The fact that the underlying construct of "feeling of ineffectiveness" has been identified as clinically relevant by both, psychoanalytic and cognitive-behavioral theories, also adds to its potential value.

This study has some limitations. First of all it is a cross-sectional data. Secondly only women have been included and further research is needed to confirm the results in men. We have chosen to analyze depression, anxiety, phobias, and assertiveness by means of specific questionnaires, to facilitate an objective and standardized statistical evaluation, but without relying on DSM-IV diagnostic criteria for psychiatric disorders; the lack of psychiatric diagnoses therefore hampers an evaluation of their predictive value. The arbitrarily established groups for the ineffectiveness item of the EDI-2 lead also to a limitation and a study for standardized scores should be done. Finally, this outpatient obesity clinic is a tertiary centre for eating disorders and obesity and we may therefore assume that the studied population consists of the most severe cases. In future study with a greater number of patients or patients of different types should be done to confirm these results

5. Conclusions

In conclusion this study confirms the high prevalence of psychological dysfunction such as depression, anxiety, self-affirmation, and phobia in obese women trying to lose weight and that their severity is correlated to the degree of eating disorders. In addition, the fact that the feeling of ineffectiveness constitutes the major determinant of these patients' psychological profiles was demonstrated. This evidence opens new ways for the development of screening tools for outcome prediction in the field of eating and psychological disorders and for early and targeted intervention for patients at risk for unfavorable developments after bariatric surgery. For example, the future development of easy questionnaires based on these parameters done by patients alone at home will be a great development and a great benefit in time and in money for a better care of these patients.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] M. M. Finucane, G. A. Stevens, M. J. Cowan et al., "National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants," *The Lancet*, vol. 377, no. 9765, pp. 557–567, 2011.

[2] K. M. Flegal, M. D. Carroll, C. L. Ogden, and L. R. Curtin, "Prevalence and trends in obesity among US adults, 1999–2008," *Journal of the American Medical Association*, vol. 303, no. 3, pp. 235–241, 2010.

[3] K. M. Flegal, R. Wei, C. L. Ogden, D. S. Freedman, C. L. Johnson, and L. R. Curtin, "Characterizing extreme values of body mass index-for-age by using the 2000 Centers for Disease Control and Prevention growth charts," *American Journal of Clinical Nutrition*, vol. 90, no. 5, pp. 1314–1320, 2009.

[4] Y. Wang, M. A. Beydoun, L. Liang, B. Caballero, and S. K. Kumanyika, "Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic," *Obesity*, vol. 16, no. 10, pp. 2323–2330, 2008.

[5] M. Forster, J. L. Veerman, J. J. Barendregt, and T. Vos, "Cost-effectiveness of diet and exercise interventions to reduce overweight and obesity," *International Journal of Obesity*, vol. 35, no. 8, pp. 1071–1078, 2011.

[6] L. Roux, K. M. Kuntz, C. Donaldson, and S. J. Goldie, "Economic evaluation of weight loss interventions in overweight and obese women," *Obesity*, vol. 14, no. 6, pp. 1093–1106, 2006.

[7] J. D. Douketis, C. Macie, L. Thabane, and D. F. Williamson, "Systematic review of long-term weight loss studies in obese

adults: clinical significance and applicability to clinical practice," *International Journal of Obesity*, vol. 29, no. 10, pp. 1153–1167, 2005.

[8] C. C. Curioni and P. M. Lourenço, "Long-term weight loss after diet and exercise: a systematic review," *International Journal of Obesity*, vol. 29, no. 10, pp. 1168–1174, 2005.

[9] F. M. Sacks, G. A. Bray, V. J. Carey et al., "The new England journal of medicine: comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates," *New England Journal of Medicine*, vol. 360, no. 9, pp. 859–873, 2009.

[10] J. W. Anderson, E. C. Konz, R. C. Frederich, and C. L. Wood, "Long-term weight-loss maintenance: a meta-analysis of US studies," *American Journal of Clinical Nutrition*, vol. 74, no. 5, pp. 579–584, 2001.

[11] R. Padwal, S. Klarenbach, N. Wiebe et al., "Bariatric surgery: a systematic review of the clinical and economic evidence," *Journal of General Internal Medicine*, vol. 26, no. 10, pp. 1183–1194, 2011.

[12] J. Picot, J. Jones, J. L. Colquitt et al., "The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation," *Health Technology Assessment*, vol. 13, no. 41, pp. 1–190, 215–357, 2009.

[13] L. Sjöström, K. Narbro, C. D. Sjöström et al., "Effects of bariatric surgery on mortality in Swedish obese subjects," *New England Journal of Medicine*, vol. 357, no. 8, pp. 741–752, 2007.

[14] J. A. Tice, L. Karliner, J. Walsh, A. J. Petersen, and M. D. Feldman, "Gastric banding or bypass? A systematic review comparing the two most popular bariatric procedures," *American Journal of Medicine*, vol. 121, no. 10, pp. 885–893, 2008.

[15] A. E. Dingemans, M. J. Bruna, and E. F. Van Furth, "Binge eating disorder: a review," *International Journal of Obesity*, vol. 26, no. 3, pp. 299–307, 2002.

[16] R. H. Striegel-Moorel and D. L. Franko, "Epidemiology of binge eating disorder," *International Journal of Eating Disorders*, vol. 34, supplement 1, pp. S19–S29, 2003.

[17] D. E. Pankevich, S. L. Teegarden, A. D. Hedin, C. L. Jensen, and T. L. Bale, "Caloric restriction experience reprograms stress and orexigenic pathways and promotes binge eating," *Journal of Neuroscience*, vol. 30, no. 48, pp. 16399–16407, 2010.

[18] J. G. Thomas, M. L. Butryn, E. Stice, and M. R. Lowe, "A prospective test of the relation between weight change and risk for bulimia nervosa," *International Journal of Eating Disorders*, vol. 44, no. 4, pp. 295–303, 2011.

[19] R. Dalle Grave, S. Calugi, M. L. Petroni, S. Di Domizio, and G. Marchesini, "Weight management, psychological distress and binge eating in obesity. A reappraisal of the problem," *Appetite*, vol. 54, no. 2, pp. 269–273, 2010.

[20] T. M. Legenbauer, M. de Zwaan, B. Mühlhans, F. Petrak, and S. Herpertz, "Do mental disorders and eating patterns affect long-term weight loss maintenance?" *General Hospital Psychiatry*, vol. 32, no. 2, pp. 132–140, 2010.

[21] S. M. Somerset, L. Graham, and K. Markwell, "Depression scores predict adherence in a dietary weight loss intervention trial," *Clinical Nutrition*, vol. 30, no. 5, pp. 593–598, 2011.

[22] P. J. Teixeira, S. B. Going, L. B. Sardinha, and T. G. Lohman, "A review of psychosocial pre-treatment predictors of weight control," *Obesity Reviews*, vol. 6, no. 1, pp. 43–65, 2005.

[23] J. Fandiño, R. O. Moreira, C. Preissler et al., "Impact of binge eating disorder in the psychopathological profile of obese women," *Comprehensive Psychiatry*, vol. 51, no. 2, pp. 110–114, 2010.

[24] G. Gariepy, D. Nitka, and N. Schmitz, "The association between obesity and anxiety disorders in the population: a systematic review and meta-analysis," *International Journal of Obesity*, vol. 34, no. 3, pp. 407–419, 2010.

[25] J. B. Dixon, M. E. Dixon, and P. E. O'Brien, "Depression in association with severe obesity: changes with weight loss," *Archives of Internal Medicine*, vol. 163, no. 17, pp. 2058–2065, 2003.

[26] C. M. Bulik, P. F. Sullivan, and K. S. Kendler, "Medical and psychiatric morbidity in obese women with and without binge eating," *International Journal of Eating Disorders*, vol. 32, no. 1, pp. 72–78, 2002.

[27] S. Fassino, P. Leombruni, A. Pierò, G. Abbate-Daga, and G. G. Rovera, "Mood, eating attitudes, and anger in obese women with and without Binge Eating Disorder," *Journal of Psychosomatic Research*, vol. 54, no. 6, pp. 559–566, 2003.

[28] S. Fassino, P. Leombruni, A. Pierò et al., "Temperament and character in obese women with and without binge eating disorder," *Comprehensive Psychiatry*, vol. 43, no. 6, pp. 431–437, 2002.

[29] G. Gariepy, J. Wang, A. D. Lesage, and N. Schmitz, "The longitudinal association from obesity to depression: results from the 12-year national population health survey," *Obesity*, vol. 18, no. 5, pp. 1033–1038, 2010.

[30] F. S. Luppino, L. M. De Wit, P. F. Bouvy et al., "Overweight, obesity, and depression: a systematic review and meta-analysis of longitudinal studies," *Archives of General Psychiatry*, vol. 67, no. 3, pp. 220–229, 2010.

[31] C. E. Ramacciotti, E. Coli, E. Bondi, A. Burgalassi, G. Massimetti, and L. Dell'Osso, "Shared psychopathology in obese subjects with and without binge-eating disorder," *International Journal of Eating Disorders*, vol. 41, no. 7, pp. 643–649, 2008.

[32] K. N. Javaras, H. G. Pope Jr., J. K. Lalonde et al., "Co-occurrence of binge eating disorder with psychiatric and medical disorders," *Journal of Clinical Psychiatry*, vol. 69, no. 2, pp. 266–273, 2008.

[33] M. Livhits, C. Mercado, I. Yermilov et al., "Behavioral factors associated with successful weight loss after gastric bypass," *American Surgeon*, vol. 76, no. 10, pp. 1139–1142, 2010.

[34] V. Giusti, E. Héraïef, R. C. Gaillard, and P. Burckhardt, "Predictive factors of binge eating disorder in women searching to lose weight," *Eating and Weight Disorders*, vol. 9, no. 1, pp. 44–49, 2004.

[35] "National Task Force on the Prevention and Treatment of obesity: weight cycling," *Journal of the American Medical Association*, vol. 272, no. 15, pp. 1196–1202, 1994.

[36] M. Henderson and C. P. L. Freeman, "A self-rating scale for bulimia: the 'BITE'," *The British Journal of Psychiatry*, vol. 150, pp. 18–24, 1987.

[37] D. M. Garner, M. P. Olmstead, and J. Polivy, "Development and validation of a multidimensional eating disorder inventory for anorexia nervosa and bulimia," *International Journal of Eating Disorders*, vol. 2, no. 2, pp. 15–34, 1982.

[38] J. B. Hurley, R. L. Palmer, and D. Stretch, "The specificity of the eating disorders inventory: a reappraisal," *International Journal of Eating Disorders*, vol. 9, no. 4, pp. 419–424, 1990.

[39] A. T. Beck and R. A. Steer, *Manual for the Beck Depression Inventory: II*, Psychological Corporation, San Antonio, Tex, USA, 2nd edition, 1996.

[40] K. Kvaal, I. Ulstein, I. H. Nordhus, and K. Engedal, "The Spielberger State-Trait Anxiety Inventory (STAI): the state scale in detecting mental disorders in geriatric patients," *International Journal of Geriatric Psychiatry*, vol. 20, no. 7, pp. 629–634, 2005.

[41] J. G. Gauthier and S. Bouchard, "Adaptation canadienne-française de la forme révisée du State—trait anxiety Inventory de Spielberger," *Revue Canadienne des Sciences du Comportement*, vol. 2, no. 4, pp. 559–578, 1993.

[42] S. A. Rathus, "A 30 item schedule for assessing assertive behavior," *Behavior Therapy*, vol. 4, no. 3, pp. 398–406, 1973.

[43] J. Cottraux, M. Bouvard, and P. Messy, "Validation study on the French version of the fear questionnaire," *Encephale*, vol. 13, no. 1, pp. 23–29, 1987.

[44] A. McPherson and C. R. Martin, "A narrative review of the Beck Depression Inventory (BDI) and implications for its use in an alcohol-dependent population," *Journal of Psychiatric and Mental Health Nursing*, vol. 17, no. 1, pp. 19–30, 2010.

[45] G. Addolorato, C. Ancona, E. Capristo et al., "State and trait anxiety in women affected by allergic and vasomotor rhinitis," *Journal of Psychosomatic Research*, vol. 46, no. 3, pp. 283–289, 1999.

[46] C. H. Kindler, C. Harms, F. Amsler, T. Ihde-Scholl, and D. Scheidegger, "The visual analog scale allows effective measurement of preoperative anxiety and detection of patients' anesthetic concerns," *Anesthesia and Analgesia*, vol. 90, no. 3, pp. 706–712, 2000.

[47] D. Stark, M. Kiely, A. Smith, G. Velikova, A. House, and P. Selby, "Anxiety disorders in cancer patients: their nature, associations, and relation to quality of life," *Journal of Clinical Oncology*, vol. 20, no. 14, pp. 3137–3148, 2002.

[48] M. C. J. Bouvard, E. Mollard, Ph. Messy, and M. Defayolle, "Validation et analyse factorielle de l'échelle d'affirmation de soi de Rhatus," *Psychologie Médicale*, vol. 18, no. 5, pp. 759–463, 1986.

[49] G. Hasler, D. S. Pine, A. Gamma et al., "The associations between psychopathology and being overweight: a 20-year prospective study," *Psychological Medicine*, vol. 34, no. 6, pp. 1047–1057, 2004.

[50] S. Herpertz, R. Burgmer, A. Stang et al., "Prevalence of mental disorders in normal-weight and obese individuals with and without weight loss treatment in a German urban population," *Journal of Psychosomatic Research*, vol. 61, no. 1, pp. 95–103, 2006.

[51] M. S. Faith, P. E. Matz, and M. A. Jorge, "Obesity—depression associations in the population," *Journal of Psychosomatic Research*, vol. 53, no. 4, pp. 935–942, 2002.

[52] J. A. Linde, R. W. Jeffery, R. L. Levy et al., "Binge eating disorder, weight control self-efficacy, and depression in overweight men and women," *International Journal of Obesity*, vol. 28, no. 3, pp. 418–425, 2004.

[53] R. E. Roberts, G. A. Kaplan, S. J. Shema, and W. J. Strawbridge, "Are the obese at greater risk for depression?" *American Journal of Epidemiology*, vol. 152, no. 2, pp. 163–170, 2000.

[54] M. Q. Werrij, S. Mulkens, H. J. Hospers, and A. Jansen, "Overweight and obesity: the significance of a depressed mood," *Patient Education and Counseling*, vol. 62, no. 1, pp. 126–131, 2006.

[55] L. Lee and C. M. Shapiro, "Psychological manifestations of obesity," *Journal of Psychosomatic Research*, vol. 55, no. 6, pp. 477–479, 2003.

[56] E. S. Becker, J. Margraf, V. Türke, U. Soeder, and S. Neumer, "Obesity and mental illness in a representative sample of young women," *International Journal of Obesity*, vol. 25, no. 1, pp. S5–S9, 2001.

[57] C. U. Onyike, R. M. Crum, H. B. Lee, C. G. Lyketsos, and W. W. Eaton, "Is obesity associated with major depression? Results from the third national health and nutrition examination survey," *American Journal of Epidemiology*, vol. 158, no. 12, pp. 1139–1147, 2003.

[58] A. C. Ahlberg, T. Ljung, R. Rosmond et al., "Depression and anxiety symptoms in relation to anthropometry and metabolism in men," *Psychiatry Research*, vol. 112, no. 2, pp. 101–110, 2002.

[59] A. J. Hill and J. Williams, "Psychological health in a non-clinical sample of obese women," *International Journal of Obesity*, vol. 22, no. 6, pp. 578–583, 1998.

[60] E. R. Didie and M. Fitzgibbon, "Binge eating and psychological distress: is the degree of obesity a factor?" *Eating Behaviors*, vol. 6, no. 1, pp. 35–41, 2005.

[61] A. R. Behar, G. R. Manzo, and Z. D. Casanova, "Lack of assertiveness in patients with eating disorders," *Revista Medica de Chile*, vol. 134, no. 3, pp. 312–319, 2006.

[62] K. Elfhag, "Personality correlates of obese eating behaviour: Swedish universities scales of personality and the three factor eating questionnaire," *Eating and Weight Disorders*, vol. 10, no. 4, pp. 210–215, 2005.

[63] P. Iliceto, M. Pompili, G. Candilera et al., "Gender-related differences concerning anger expression and interpersonal relationships in a sample of overweight/obese subjects," *La Clinica Terapeutica*, vol. 163, no. 5, pp. 279–285, 2012.

[64] C. Villarejo, S. Jiménez-Murcia, E. Álvarez-Moya et al., "Loss of control over eating: a description of the eating disorder/obesity spectrum in women," *European Eating Disorders Review*, vol. 22, no. 1, pp. 25–31, 2014.

[65] D. T. Barry, C. M. Grilo, and R. M. Masheb, "Comparison of patients with bulimia nervosa, obese patients with binge eating disorder, and nonobese patients with binge eating disorder," *Journal of Nervous and Mental Disease*, vol. 191, no. 9, pp. 589–594, 2003.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 323728, 8 pages
http://dx.doi.org/10.1155/2014/323728

Clinical Study

Fasting Leptin Is a Metabolic Determinant of Food Reward in Overweight and Obese Individuals during Chronic Aerobic Exercise Training

Mark Hopkins,[1,2] Catherine Gibbons,[2] Phillipa Caudwell,[2] Dominic-Luc Webb,[3] Per M. Hellström,[4] Erik Näslund,[5] John E. Blundell,[2] and Graham Finlayson[2]

[1] *Academy of Sport and Physical Activity, Faculty of Health and Wellbeing, Sheffield Hallam University, Sheffield S10 2BP, UK*
[2] *Institute of Psychological Sciences, Faculty of Medicine and Health, University of Leeds, Leeds LS2 9JT, UK*
[3] *Department of Medical Sciences, Gastroenterology and Hepatology, Uppsala University, Uppsala, Sweden*
[4] *Department of Medical Sciences, Uppsala University, Uppsala, Sweden*
[5] *Division of Surgery, Department of Clinical Sciences, Danderyd Hospital, Karolinska Institute, Stockholm, Sweden*

Correspondence should be addressed to Mark Hopkins; m.hopkins@shu.ac.uk

Received 15 November 2013; Revised 15 January 2014; Accepted 18 January 2014; Published 11 March 2014

Academic Editor: Yi-Hao Yu

Changes in food reward have been implicated in exercise-induced compensatory eating behaviour. However, the underlying mechanisms of food reward, and the physiological correlates of exercise-induced changes in food reward, are unknown. *Methods.* Forty-six overweight and obese individuals completed 12 weeks of aerobic exercise. Body composition, food intake, and fasting metabolic-related hormones were measured at baseline, week six, and postintervention. On separate days, the reward value of high-and-low-fat food (explicit liking and implicit wanting) was also assessed at baseline, week six, and postintervention. *Results.* Following the intervention, FM, FFM, and VO_{2peak} improved significantly, while fasting leptin decreased. However, food intake or reward did not change significantly. Cross-sectional analyses indicated that FM ($P = 0.022$) and FFM ($P = 0.046$) were associated with explicit liking for high-fat food, but implicit wanting was associated with FM only ($P = 0.005$). Fasting leptin was associated with liking ($P = 0.023$) and wanting ($P = 0.021$) for high-fat food. Furthermore, a greater exercise-induced decline in fasting leptin was associated with increased liking ($P = 0.018$). *Conclusion.* These data indicate that food reward has a number of physiological correlates. In particular, fasting leptin appears to play an active role in mediating food reward during exercise-induced weight loss.

1. Introduction

Day-to-day food intake involves the coordination of both homeostatic and nonhomeostatic signals in the overall expression of eating behaviour [1]. Homeostatic feeding is often described through a series of physiological processes that initiate and terminate feeding (i.e., satiation) and suppress intermeal hunger (i.e., satiety) [2]. This pattern of eating is thought to be driven by tonic and episodic inhibitory signals (arising from adipose tissue and the gastrointestinal tract) that modulate an intrinsic excitatory drive to eat [3]. However, extrinsic determinants of eating behaviour such as food palatability and hedonic reward, linked closely to perceived fat and energy content of food, interact with these homeostatic mechanisms with the potential to enhance or undermine appetite control [4]. Recent attention has started to focus on the hedonic determinants of eating behaviour and has highlighted the importance of distinguishing liking (i.e., the perceived pleasurable sensory properties of food) from wanting (i.e., the attraction towards a specific food over available alternatives) [5, 6]. Both components of food reward are thought to act in parallel to facilitate eating behaviour [7]. Indeed, heightened liking and wanting for food have been noted in overweight and obese individuals [8] and those who demonstrate binge eating [9].

Changes in food reward may also play a role in compensatory eating behaviour following exercise [10–12]. For example, following 50 minutes of cycling, lean women who

overconsumed relative to the energy cost of exercise exhibited increased wanting for food compared to those who did not exhibit postexercise compensatory eating [10]. Furthermore, overweight and obese individuals who exhibited an immediate postexercise increase in explicit liking and wanting for food (particularly, high fat sweet foods) demonstrated smaller fat mass losses following a program of aerobic exercise training [11]. However, while these findings suggest that exercise-induced changes in food reward influence compensatory eating behaviour, the physiological processes that influence food reward are not well understood. Given that appetite control is a psychobiological process [13], it is plausible that a prolonged and potent metabolic stimulus such as aerobic exercise training would be reflected in an increased motivational drive for high energy yielding foods. However, whether adaptive changes in metabolism influence food reward during chronic exercise training has yet to be examined. Therefore, this study aimed to examine whether exercise-induced changes in body composition or metabolic-related hormones influenced food reward in overweight and obese individuals during 12 weeks of aerobic exercise.

2. Materials and Methods

2.1. Participants. Forty-six overweight and obese individuals participated in the present study (30 females, BMI = 30.8 ± 3.5 kg/m^2; 16 males, BMI = 30.5 ± 4.7 kg/m^2). Participants were recruited from the University of Leeds, UK, and surrounding areas using poster advertisements and recruitment emails. Participants were initially physically inactive (≤2 hrs.wk^{-1} of exercise over the previous six months), weight stable (±2 kg for the previous three months), nonsmokers, and not taking medication known to effect metabolism or appetite. At baseline mean dietary restraint and disinhibition scores, as measured using the Three-Factor Eating Questionnaire [14], were 7.21 ± 0.55 and 8.10 ± 0.50, respectively, which are within the normal ranges for healthy adults [15]. The study was conducted in accordance with the Declaration of Helsinki (1964), and all participants provided written informed consent before taking part. In addition, ethical approval was granted by the Institute of Psychological Science's Ethics Board, University of Leeds, and the Leeds West NHS Research Ethics Committee (09/H1307/7).

2.2. Study Design. Participants completed a 12-week supervised aerobic exercise program designed to expend 2500 kcal.wk^{-1}. Body composition, food intake, and fasting metabolic-related hormones (glucose, insulin, and leptin) were measured at baseline, week six, and postintervention. In addition, explicit liking and implicit wanting for a standardised array of high fat and low fat foods were assessed before a fixed-energy meal, at baseline, week six, and postintervention, using a validated computer based task, for example, the Leeds Food Preference Questionnaire [7].

2.3. Exercise Protocol. Participants completed a 12-week aerobic exercise program in which they exercised five days per week, expending 500 kcal per session at 70% of age-predicted maximum heart rate. All exercise sessions were supervised in the research laboratory, and participants could choose from a range of exercise modes (running, cycling, rowing, or stepping). Individual exercise prescriptions were calculated using standard stoichiometric equations [16], based on the relationship between heart rate and VO_2/VCO_2 during a maximal incremental treadmill test. To account for changes in cardiovascular fitness during the intervention, the incremental test was performed at baseline, week six, and postintervention, with the exercise prescription adjusted accordingly. To verify and record the duration and intensity of exercise, participants wore a heart rate monitor during each session (Polar RS400, Polar, Kempele, Finland). Total exercise-induced energy expenditure during the intervention was 27960 ± 3479 kcal, which represented >98% of the prescribed exercise-induced energy expenditure.

2.4. Physiological Measures. At baseline, week six, and postintervention, venous blood, body composition, and maximal aerobic capacity were measured in the morning (7–9am) following an overnight fast (10–12 hrs). Baseline measures were taken prior to the start of the intervention, while postintervention measures were taken upon completion of the exercise intervention (a minimum of 48 hrs after the final exercise bout and within one week of finishing the intervention). Body composition was measured using air-displacement plethysmography (BOD POD Body Composition System, Life Measurement, Inc., Concord, USA). After voiding, participants were weighed (to the nearest 0.01 kg) and instructed to sit in the BOD POD. Measurements were taken according to manufacturers' instructions, with thoracic gas volumes estimated using the manufacturer's software. In addition, the fat mass index (FMI; fat mass/height^2) and the fat-free mass index (FFMI; fat-free mass/height^2) were calculated from these body composition data. Maximal aerobic capacity (VO_{2peak}) was determined using a validated maximal incremental treadmill test [17], with expired air (Sensormedics Vmax29, Yorba Linda, USA) and heart rate (Polar RS400, Polar, Kempele, Finland) measured continuously. The respiratory and heart rate data from this test were also used to calculate the exercise prescriptions used in the exercise intervention.

2.5. Metabolic- and Appetite-Related Hormones. Fasting glucose, insulin, and leptin were measured at baseline, week six, and postintervention in a subsample of 32 participants who completed the exercise intervention. Fasting venous blood samples were collected into EDTA-containing Monovette tubes. After collection, blood samples were centrifuged for 10 min at 4°C at 3500 rpm and were immediately pipetted into Eppendorf tubes and stored at −80°C until analysis. Insulin and leptin were analysed using a magnetic bead based multiples kit (Millipore, Billerica, MA, USA). Furthermore, insulin resistance was calculated using the homeostatic model of assessment (HOMA) [18].

2.6. Assessment of Food Reward and Food Intake. A laboratory-based test meal protocol was used to measure food intake at baseline, week six, and postintervention. At each time point, participants consumed test meals at 4-hour intervals. No exercise was performed on these days. A detailed description of the foods provided can be found elsewhere [19]. Meals consisted of an individualised energy breakfast (*ad libitum* at baseline and then fixed at baseline levels for the remainder of intervention), a fixed-energy lunch (800 kcal), and an *ad libitum* dinner meal. After the dinner meal, participants were free to leave the research unit but were given an *ad libitum* snack box of foods to consume if desired during the evening. All meals consumed in the research unit were eaten in isolation, with participants instructed to eat as much or as little as they wanted until comfortably full (during *ad libitum* meal consumption).

Prior to the lunch test meal, food reward was assessed using the Leeds Food Preference Questionnaire (LFPQ; [7]). The LFPQ measured liking and wanting for foods according to differences in fat content (i.e., >50% or <20% energy from fat). Each food category was represented by 8 photographs of ready-to-eat foods that were matched for familiarity, taste, protein, and acceptability. Firstly, to measure "implicit wanting" a forced-choice paradigm was used in which participants were presented with two foods from different categories and were required to press a key as quickly as possible to indicate which food "they most want to eat at that moment." This was repeated until all food photograph pairs had been presented. Following Dalton et al. [20], the parameters were set as 96 randomised food pair trials presented in three blocks, with each stimulus appearing 8 times. Stimuli were presented until a valid response was detected up to a maximum of 4000 ms with a 500 ms washout between presentations in which a central fixation cross was displayed. Mean response times for choices outside of each food category, adjusted for choice frequency, were subtracted from response times for choices towards each category, adjusted for frequency. Therefore positive scores for a specific category indicated a more rapid preference (i.e., "implicit wanting"). Secondly, to measure explicit liking, the food images were presented individually in randomised order, and the participant rated the extent to which they liked each food image presented to them using a 100 mm visual analogue scale; for example, how pleasant would it be to taste this food now? Mean scores for high fat and low fat food categories were calculated. The LFPQ has been shown to demonstrate reliable immediate postexercise and postmeal changes [21] and is a good predictor of food choice and intake in laboratory and community settings [22, 23].

2.7. Statistical Analysis. Data are reported as mean ± SEM throughout. Statistical analyses were performed using IBM SPSS for Windows (Chicago, Illinois, Version 21). For food reward measures, mean scores for high fat and low fat categories were computed for implicit wanting and explicit liking outcomes. Mean low fat scores were then subtracted from the mean for high fat scores to provide a composite score representing reward value for high fat relative to low fat food for both liking and wanting. Using this approach, a positive score indicated greater liking or wanting for high fat foods over low fat foods; a negative score indicated greater liking or wanting for low fat foods over high fat foods; and a score of zero indicated an equal liking or wanting for high and low fat foods. Scores on each food reward outcome were calculated at baseline, week six, and postintervention and analysed using one-way repeated measures ANOVAs.

Changes in body composition, metabolic-related hormones, and total daily energy intake were examined using one-way repeated measures ANOVAs. Where appropriate, Greenhouse-Geisser probability levels were used to adjust for sphericity, and Bonferroni adjustments were applied to control for multiple post hoc comparisons. To test for associations between physiological variables and food reward, Pearson partial correlation coefficients were used, controlling for sex. Firstly, cross-sectional models were examined using mean scores on each variable collapsed across the three time points of the exercise intervention (i.e., baseline, week six, and postintervention). Secondly, associations between changes in physiological variables and changes in explicit liking and implicit wanting following the exercise intervention were performed. Change variables were calculated by subtracting baseline scores from postintervention scores. To control for confounding effects of body composition, metabolic hormones were tested both with and without adjustment for adiposity by dividing by percentage body fat.

3. Results

3.1. Changes in Body Composition and Metabolism following the Exercise Intervention. As can be seen in Table 1, there was a significant reduction in body mass (−1.72 ± 0.41 kg; $P < 0.001$), fat mass (−2.23 ± 0.38 kg; $P < 0.001$), and percentage body fat (−1.90 ± 0.22%; $P < 0.001$) following the exercise intervention, while fat-free mass was preserved at baseline levels (+0.52 ± 0.17 kg; $P = 0.081$). Furthermore, FMI decreased (−0.76 ± 0.14 kg/m^2; $P < 0.001$) and FFMI increased significantly (0.17 ± 0.62 kg/m^2; $P < 0.01$). VO_{2peak} increased from 33.33 ± 1.17 mL.kg.min^{-1} at baseline to 39.16 ± 0.09 mL.kg.min^{-1} after intervention ($P < 0.001$). There were no significant changes in fasting glucose (−0.20 ± 0.25 mmol.L^{-1}; $P = 0.415$) or fasting insulin (−42.98 ± 82.94 ng.L^{-1}; $P = 0.230$) following the exercise intervention. However, fasting leptin decreased significantly following the exercise intervention (−6215.93 ± 3076.37 ng.L^{-1}; $P = 0.023$).

3.2. Changes in Food Intake and Food Reward following the Exercise Intervention. Table 2 shows that total daily energy intake, explicit liking, and implicit wanting for high fat food did not differ significantly between baseline and week 6 or baseline and postintervention. There was a nonsignificant trend for implicit wanting to shift from a small bias for high fat food at baseline, towards a bias for low fat food following the intervention ($P = 0.114$).

Table 1: Body composition and metabolic values during the 12-week exercise intervention ($n = 46$).

	Baseline	Week six	Postintervention	Delta Δ	P value
Body mass (kg)	88.21 (2.04)	87.39 (2.00)	86.49 (2.04)	−1.72 (0.41)	0.000*
Fat mass (kg)	35.71 (1.34)	34.48 (1.35)	33.49 (1.43)	−2.23 (0.38)	0.000*
Fat mass index (kg/m^2)	12.61 (0.52)	12.17 (0.53)	11.85 (0.56)	−0.76 (0.14)	0.000*
Body fat (%)	40.33 (1.13)	39.24 (1.16)	38.43 (1.22)	−1.90 (0.32)	0.000*
Fat-free mass (kg)	52.48 (1.43)	52.91 (1.41)	53.00 (1.39)	0.52 (0.17)	0.081
Fat-free mass index (kg/m^2)	18.25 (0.31)	18.40 (0.30)	18.41 (0.30)	0.17 (0.62)	0.009*
VO_{2peak} (mL·kg^{-1}·min^{-1})	33.33 (1.17)	37.45 (1.08)	39.16 (0.09)	5.83 (0.95)	0.000*
Fasting glucose (mmol·L^{-1})	4.93 (0.15)	4.88 (0.17)	4.73 (0.19)	−0.20 (0.25)	0.415
Fasting insulin (ng·L^{-1})	1034.32 (106.24)	918.77 (105.33)	991.34 (113.24)	−42.98 (82.94)	0.230
HOMA index	3.18 (0.31)	2.92 (0.31)	3.02 (0.33)	−0.16 (0.25)	0.554
Fasting leptin (ng·L^{-1})	38318.80 (4832.26)	369923.92 (4612.41)	32102.87 (5333.58)	−6215.93 (3076.37)	0.023*

Delta Δ: baseline to postintervention change. VO_{2peak}: maximal aerobic capacity. HOMA: homeostatic model of assessment. *Significant difference between baseline and postintervention ($P < 0.05$).

3.3. Physiological Correlates of Food Reward: Cross-Sectional Associations. As can be seen in Table 3, liking for high fat food was positively associated with body mass ($P = 0.008$) and fat mass ($P = 0.022$) and marginally associated with fat-free mass ($P = 0.046$). However, there were no significant associations between these components of body composition when adjusted for height (i.e., FMI or FFMI). Wanting for high fat foods was also positively associated with body mass ($P = 0.004$), fat mass ($P = 0.005$), and FMI ($P = 0.018$), but not fat-free mass ($P = 0.129$) or FFMI ($P = 0.161$). Of the metabolic hormones, fasting leptin was positively associated with both greater liking ($P = 0.023$) and wanting ($P = 0.021$) responses. Moreover, these relationships remained after adjusted fasting leptin values for percentage body fat (liking, $P = 0.043$; wanting, $P = 0.041$), suggesting they were independent of adiposity.

3.4. Physiological Correlates of Food Reward: Exercise-Induced Changes. No associations existed between changes in food reward and changes in body composition following the intervention (Table 3). Furthermore, no associations existed between the changes in food reward and the changes in fasting glucose, insulin, HOMA index, or VO_{2peak}. However, the change in fasting leptin (absolute or adjusted leptin) was found to be negatively associated with the change in liking for high fat foods following the exercise intervention ($P = 0.018$ and $P = 0.031$, resp.). As can been seen in Figure 1, a decline in fasting leptin following the exercise intervention was associated with increased liking for high fat food. No associations were found between the change in implicit wanting for high fat foods and the change in fasting leptin (absolute or adjusted leptin).

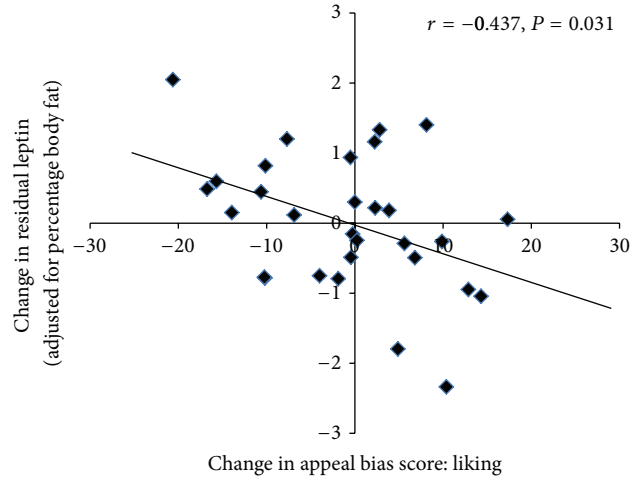


Figure 1: Scatter plot illustrating the relationship between the change in fasting leptin (adjusted for percentage body fat) following the exercise intervention and the change in appeal bias scores for liking for high fat foods ($n = 32$). Positive appeal bias = preference for high fat foods > low fat foods. Negative appeal bias score = preference for low fat foods > high fat foods.

4. Discussion

This study examined whether components of body composition and metabolic-related hormones were associated with food reward in overweight and obese individuals during 12 weeks of aerobic exercise. Cross-sectional analyses disclosed associations between body composition (fat mass and fat-free mass), fasting leptin, and food reward. Furthermore, a novel relationship was also disclosed between the change in fasting leptin and the change in explicit liking for high fat foods following the exercise intervention. Specifically, a decline in fasting leptin was associated with an increased liking for high fat foods relative to low fat foods following the intervention. This relationship was independent of changes in fat mass and suggests that leptin may have a key role in mediating changes in food reward during exercise-induced weight loss.

4.1. The Effect of Exercise on Body Composition, Metabolism, and Food Reward. The 12-week exercise intervention resulted in significant (but modest) reductions in body mass, fat mass, and percentage body fat, while fat-free mass was preserved at baseline levels. In addition, significant improvements in VO_{2peak} were seen following the exercise intervention. When

Table 2: Changes in food intake, explicit liking, and implicit wanting for high fat versus low fat foods during the 12-week exercise intervention ($n = 46$).

	Baseline	Week six	Postintervention	Delta Δ	P value
Total daily EI (kcal·day^{-1})	2949.29 (79.15)	2877.24 (92.77)	2892.81 (88.11)	−56.48 (60.15)	0.438
Explicit liking (appeal bias score)	−0.20 (2.25)	−1.08 (2.16)	−0.85 (2.02)	−0.65 (1.72)	0.919
Implicit wanting (appeal bias score)	1.10 (4.18)	−2.56 (4.47)	−3.17 (3.98)	−4.27 (2.58)	0.114

EI: energy intake; Delta Δ: baseline to postintervention change. Positive appeal bias score = preference for high fat foods > low fat foods. Negative appeal bias score = preference for low foods > high fat foods.

Table 3: Pearson partial correlation coefficients (controlling for sex) between food reward and the cross-sectional and exercise-induced changes in body composition and fasting metabolic-related hormones.

Body composition and VO_{2peak}			Metabolic hormones		
	Liking	Wanting		Liking	Wanting
BM	0.393**	0.417**	Glucose	0.019	0.060
ΔBM	−0.251	0.116	ΔGlucose	−0.014	0.077
FM	0.341*	0.414**	Adjusted Glucose	−0.267	−0.336
ΔFM	−0.196	0.004	ΔAdjusted Glucose	−0.039	0.061
FMI	0.265	0.351*	Insulin	−0.236	0.311
ΔFMI	−0.223	−0.016	ΔInsulin	−0.206	−0.216
BF%	0.212	0.324*	Adjusted Insulin	0.155	0.194
ΔBF%	−0.210	−0.101	ΔAdjusted Insulin	−0.213	−0.178
FFM	0.295*	0.230	Leptin	0.358*	0.401*
ΔFFM	−0.138	0.265	ΔLeptin	−0.437*	−0.110
FFMI	0.213	0.184	Adjusted Leptin	0.373*	0.370*
ΔFFMI	−0.121	−0.094	ΔAdjusted Leptin	−0.378*	−0.159
VO_{2peak}	−0.224	−0.231	HOMA	0.213	0.008
ΔVO_{2peak}	−0.178	−0.179	ΔHOMA	−0.123	−0.151
			Adjusted HOMA	0.090	0.139
			ΔAdjusted HOMA	−0.124	−0.152

VO_{2peak}: maximal aerobic capacity; FM: fat mass; FMI: fat mass index; FFM: fat-free mass; FFMI: fat-free mass index; %BF: percentage body fat; HOMA: homeostatic model of assessment. Delta Δ: baseline to postintervention change.* $P < 0.05$; ** $P < 0.01$. Of note: the metabolic-related hormones have been adjusted for percentage fat mass. Cross-sectional models represent the mean scores on each variable collapsed across baseline, week six, and postintervention.

the changes in food intake and reward were examined, no mean changes in total daily energy intake, explicit liking, or implicit wanting were found. However, it has become clear that examination of the mean (group) response to exercise masked marked heterogeneity in eating behaviour and exercise-induced weight loss following acute [24] and chronic exercise [25–28]. Previous studies have suggested that exercise-induced changes in food reward may mediate compensatory eating behaviour and in turn body weight regulation following exercise [10–12]. However, the physiological correlates of food reward during exercise-induced weight loss have not previously been examined.

4.2. Body Composition and Food Reward. Recent evidence has demonstrated the importance of distinguishing explicit perceptions of liking from behavioural operations of wanting, with these components of food reward considered to be separable risk factors in overconsumption and weight gain [5, 6]. During the present study, fat mass and fat-free mass were associated with explicit liking for high fat foods. However, implicit wanting was only associated with fat mass cross-sectionally (i.e., when the baseline, week six, and postintervention measures were combined). These data therefore suggest that fat mass may predict food reward (particularly food wanting) independently of fat-free mass. These findings are consistent with recent observations that fat mass and fat-free mass operated differentially in the control of appetite, with separate roles for fat-free mass in satiation [29] and hunger [19, 30] and fat mass in hedonic eating behaviour traits [31] and neural activation to high energy foods[32]. However, these findings are so far limited to obese individuals and need to be confirmed in a range of different populations, that is, lean versus obese and active versus inactive.

4.3. Leptin and Food Reward. It has been suggested that obese individuals display a loss of hedonic control over eating when exposed to highly palatable foods compared to lean individuals [33]. This increase in the susceptibility to overconsumption in obese individuals may be related to increased leptin and insulin resistance (resulting from the

excessive accumulation of adipose tissue), which may reduce the sensitivity of short-term appetite control [34, 35]. In the present study, cross-sectional associations were found between explicit liking and implicit wanting for high fat foods relative to low fat foods and fasting leptin, but not fasting glucose, insulin, HOMA, or VO_{2peak}. These findings are supported by Raynaud and colleagues [36], who examined the relationships between body composition, serum leptin, insulin, and self-reported palatability of a high CHO breakfast in a sample of predominantly obese adults. A positive relationship was noted between serum leptin and palatability, but not insulin and palatability, and this association remained after controlling for BMI or fat mass. In the present study, the cross-sectional associations indicated that a greater implicit wanting for high fat foods was associated with greater *ad libitum* food intake, suggesting that differences in food reward are expressed behaviourally through differences in food intake (data not reported). However, it should be noted that as the test meal design employed in the present study incorporated both fixed-energy and *ad libitum* meals, the measures of daily energy intake in the present study do not reflect "true" *ad libitum* daily intake.

Interestingly, the present study also disclosed a novel relationship between the changes in fasting leptin and explicit liking for high fat foods following the exercise intervention, with a decline in fasting leptin associated with an increase in liking for high fat foods relative to low fat foods. This relationship is consistent with the proposed role of leptin in food reward, in which leptin is thought to tonically inhibit brain reward pathways [34]. It is hypothesised that a reduction in leptin would act to increase the sensitivity of reward brain circuitry, potentially increasing the motivation to consume highly palatable energy dense foods via its action on dopamine reuptake transporters [37]. Furthermore, leptin's role as an adiposity signal is well established [34], with a decline in leptin thought to stimulate increased hunger and, in turn, food intake, via a downregulation in the hypothalamic expression of anorexigenic neuropeptides, such as proopiomelanocortin and alpha-melanocyte stimulating hormone, and an upregulation in the expression of orexigenic neuropeptides, such as neuropeptide Y and agouti gene-related peptide [38, 39]. Importantly, the present data suggest that a decline in leptin may also promote a greater perceived liking for high fat foods, thereby helping to further promote increased food intake and the restoration of energy homeostasis.

The present findings are in keeping with the idea that leptin is primarily a "starvation" signal rather than a "satiety" signal [40]. While a decline in leptin is thought to promote increases in hunger and food intake [39], an increase in fat mass and leptin does not appear to exert a proportional downregulation in eating [40]. As such, it could be argued that the inhibitory action of fat mass (and associated adiposity signals) on food intake is actually weaker at higher levels of fat mass, and this asymmetry may reflect increased "leptin resistance" with obesity. Indeed, leptin resistance may account for the positive (cross-sectional) correlations between fasting leptin and food reward seen in the present study. However, a decline in leptin (independent of fat mass) during the exercise intervention was also found to be associated with increased explicit liking for high fat foods. While these findings may initially appear contradictory, it has been argued that it is the fall in circulating leptin below a critical (and individualised) threshold level that triggers corrective hypothalamic responses to restore energy homeostasis [41–43]. Theoretically, increased leptin sensitivity resulting from the exercise intervention could have made individuals more sensitive to perturbations in peripheral leptin concentrations, with a decline in leptin perceived by the brain in some individuals as a state of relative leptin deficiency despite an actual surplus of stored energy, that is, fat mass [41–43]. However, clearly this can only be speculated, upon and the precise role of leptin and leptin resistance in food reward remains an important area for future research.

It has previously been reported that the change in leptin (independent of fat mass) during weight loss was negatively associated with the changes in subjective appetite [44]. These observations were made in the context of a 12-week weight loss program in which subjects lost an average of 7 kg fat mass (through diet and exercise). The present intervention on the other hand resulted in a relatively modest 2.2 kg loss of fat mass. Therefore, the subtle effects of the exercise intervention on food reward are perhaps unsurprising. It should be noted that a role for leptin in the hedonic control of food intake during exercise-induced weight loss is a novel hypothesis, and, as such, further work is needed to examine the physiological correlates of food reward in more targeted research. Nevertheless, these findings are consistent with other recent observations that some individuals experience a greater than expected decline in resting metabolic rate following exercise-induced weight loss, and this compensatory downregulation in resting metabolic rate was again associated with a decline in fasting leptin (independent of fat mass). Importantly, those individuals who experienced a compensatory downregulation in resting metabolic rate also experienced a concomitant upregulation in food intake during exercise-induced weight loss [45].

The present study has some limitations that deserve comment. When interpreting the findings of the present study, it is important to note that a nonexercise control condition was not included. However, the observed improvements in body composition, VO_{2peak} are unlikely to have occurred independent of the exercise intervention. Furthermore, due to the need to measure body composition and metabolism at standardised time points during the exercise intervention, no control was made for menstrual cycle phase in female participants. This may have contributed to the variability seen in food reward, as studies have shown that eating behaviour and food reward are influenced by the phases of the menstrual cycle [46, 47].

5. Conclusion

Through the concurrent measurement of physiological and behavioural components of energy balance, this study has disclosed novel relationships between food reward, body composition, and metabolic-related hormones in overweight

and obese individuals. Cross-sectional relationships were found between measures of explicit liking and both fat mass and fat-free mass. However, only fat mass was found to be associated with implicit wanting, suggesting that aspects of body composition may differentially affect the separate components of food reward. Independent of adiposity, a positive relationship between fasting leptin and liking and wanting for high fat food was demonstrated. Furthermore, a decline in fasting leptin following the exercise intervention was found to be associated with an increase in liking for high fat relative to low fat foods. Taken together, these findings suggest a dynamic role for fasting leptin as a regulatory signal of food reward during exercise-induced weight loss.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

This research was supported by BBSRC Grant nos. BBS/B/05079 and BB/G005524/1 (DRINC), EU FP7 Full4Health (#266408), Uppsala University and the Stockholm County Council (ALF).

References

[1] M. W. Schwartz, S. C. Woods, D. Porte Jr., R. J. Seeley, and D. G. Baskin, "Central nervous system control of food intake," *Nature*, vol. 404, no. 6778, pp. 661–671, 2000.

[2] J. C. G. Halford and J. E. Blundell, "Separate systems for serotonin and leptin in appetite control," *Annals of Medicine*, vol. 32, no. 3, pp. 222–232, 2000.

[3] J. E. Blundell and A. Gillett, "Control of food intake in the obese," *Obesity Research*, vol. 9, pp. 263S–270S, 2001.

[4] H.-R. Berthoud, "Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance," *Obesity*, vol. 14, pp. 197S–200S, 2006.

[5] K. C. Berridge, "Food reward: brain substrates of wanting and liking," *Neuroscience and Biobehavioral Reviews*, vol. 20, no. 1, pp. 1–25, 1996.

[6] G. Finlayson, N. King, and J. E. Blundell, "Liking vs. wanting food: importance for human appetite control and weight regulation," *Neuroscience and Biobehavioral Reviews*, vol. 31, no. 7, pp. 987–1002, 2007.

[7] G. Finlayson, N. King, and J. Blundell, "The role of implicit wanting in relation to explicit liking and wanting for food: implications for appetite control," *Appetite*, vol. 50, no. 1, pp. 120–127, 2008.

[8] I. M. T. Nijs, P. Muris, A. S. Euser, and I. H. A. Franken, "Differences in attention to food and food intake between overweight/obese and normal-weight females under conditions of hunger and satiety," *Appetite*, vol. 54, no. 2, pp. 243–254, 2010.

[9] C. A. Davis, R. D. Levitan, C. Reid et al., "Dopamine for wanting and opioids for liking: a comparison of obese adults with and without binge eating," *Obesity*, vol. 17, no. 6, pp. 1220–1225, 2009.

[10] G. Finlayson, E. Bryant, J. E. Blundell, and N. A. King, "Acute compensatory eating following exercise is associated with implicit hedonic wanting for food," *Physiology and Behavior*, vol. 97, no. 1, pp. 62–67, 2009.

[11] G. Finlayson, P. Caudwell, C. Gibbons, M. Hopkins, N. King, and J. Blundell, "Low fat loss response after medium-term supervised exercise in obese is associated with exercise-induced increase in food reward," *Journal of Obesity*, vol. 2011, Article ID 615624, 8 pages, 2011.

[12] M.-A. Cornier, E. L. Melanson, A. K. Salzberg, J. L. Bechtell, and J. R. Tregellas, "The effects of exercise on the neuronal response to food cues," *Physiology and Behavior*, vol. 105, no. 4, pp. 1028–1034, 2012.

[13] J. E. Blundell, "The control of appetite: basic concepts and practical implications," *Schweizerische Medizinische Wochenschrift*, vol. 129, no. 5, pp. 182–188, 1999.

[14] A. J. Stunkard and S. Messick, "The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger," *Journal of Psychosomatic Research*, vol. 29, no. 1, pp. 71–83, 1985.

[15] M. Lowe and J. Thomas, "Measures of restrained eating: conceptual evolution and psychometric update," in *Handbook of Assessment Methods for Obesity and Eating Behaviours*, D. Allison and M. Baskin, Eds., pp. 137–185, Sage, New York, NY, USA, 2009.

[16] F. Péronnet and D. Massicotte, "Table of nonprotein respiratory quotient: an update," *Canadian Journal of Sport Sciences*, vol. 16, no. 1, pp. 23–29, 1991.

[17] J. Achten and A. E. Jeukendrup, "Maximal Fat Oxidation during Exercise in Trained Men," *International Journal of Sports Medicine*, vol. 24, no. 8, pp. 603–608, 2003.

[18] D. R. Matthews, J. P. Hosker, and A. S. Rudenski, "Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man," *Diabetologia*, vol. 28, no. 7, pp. 412–419, 1985.

[19] P. Caudwell, G. Finlayson, C. Gibbons et al., "Resting metabolic rate is associated with hunger, self-determined meal size, and daily energy intake and may represent a marker for appetite American," *Journal of Clinical Nutrition*, vol. 97, pp. 7–14, 2013.

[20] M. Dalton, J. Blundell, and G. S. Finlayson, "Examination of food reward and energy intake under laboratory and free-living conditions in a trait binge eating subtype of obesity," *Frontiers in Psychology*, vol. 4, article 757, 2013.

[21] G. Finlayson, N. King, and J. E. Blundell, "Is it possible to dissociate "liking" and "wanting" for foods in humans? A novel experimental procedure," *Physiology and Behavior*, vol. 90, no. 1, pp. 36–42, 2007.

[22] S. Griffioen-Roose, M. Mars, E. Siebelink, G. Finlayson, D. Tomé, and C. de Graaf, "Protein status elicits compensatory changes in food intake and food preferences," *The American Journal of Clinical Nutrition*, vol. 95, no. 1, pp. 32–38, 2012.

[23] S. A. French, N. R. Mitchell, G. Finlayson, J. E. Blundell, and R. W. Jeffery, "Questionnaire and laboratory measures of eating behavior. Associations with energy intake and BMI in a community sample of working adults," *Appetite*, vol. 72, pp. 50–58, 2013.

[24] M. Hopkins, J. E. Blundell, and N. A. King, "Individual variability in compensatory eating following acute exercise in overweight and obese women," *British Journal of Sports Medicine*, 2013.

[25] N. A. King, M. Hopkins, P. Caudwell, R. J. Stubbs, and J. E. Blundell, "Individual variability following 12 weeks of supervised exercise: identification and characterization of compensation for exercise-induced weight loss," *International Journal of Obesity*, vol. 32, no. 1, pp. 177–184, 2008.

[26] N. D. Barwell, D. Malkova, M. Leggate, and J. M. R. Gill, "Individual responsiveness to exercise-induced fat loss is associated

with change in resting substrate utilization," *Metabolism*, vol. 58, no. 9, pp. 1320–1328, 2009.

[27] T. S. Church, C. K. Martin, A. M. Thompson, C. P. Earnest, C. R. Mikus, and S. N. Blair, "Changes in weight, waist circumference and compensatory responses with different doses of exercise among sedentary, overweight postmenopausal women," *PLoS ONE*, vol. 4, no. 2, Article ID e4515, 2009.

[28] M. Rosenkilde, P. Auerbach, M. H. Reichkendler, T. Ploug, B. M. Stallknecht, and A. Sjödin, "Body fat loss and compensatory mechanisms in response to different doses of aerobic exercise—a randomized controlled trial in overweight sedentary males," *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, vol. 303, pp. R571–R579, 2012.

[29] J. E. Blundell, P. Caudwell, C. Gibbons et al., "Body composition and appetite: fat-free mass (but not fat mass or BMI) is positively associated with self-determined meal size and daily energy intake in humans," *British Journal of Nutrition*, vol. 107, no. 3, pp. 445–449, 2012.

[30] C. M. Weise, M. G. Hohenadel, J. Krakoff, and S. B. Votruba, "Body composition and energy expenditure predict ad-libitum food and macronutrient intake in humans," *International Journal of Obesity*, 2013.

[31] B. V. O'Neill, E. T. Bullmore, S. Miller et al., "The relationship between fat mass, eating behaviour and obesity-related psychological traits in overweight and obese individuals," *Appetite*, vol. 59, no. 3, pp. 656–661, 2012.

[32] S. Luo, A. Romero, T. C. Adam, H. H. Hu, J. Monterosso, and K. A. Page, "Abdominal fat is associated with a greater brain reward response to high calorie food cues in hispanic women," *Obesity*, vol. 21, pp. 2029–22036, 2013.

[33] L. H. Epstein, J. L. Temple, B. J. Neaderhiser, R. J. Salis, R. W. Erbe, and J. J. Leddy, "Food reinforcement, the dopamine D2 receptor genotype, and energy intake in obese and nonobese humans," *Behavioral Neuroscience*, vol. 121, no. 5, pp. 877–886, 2007.

[34] G. J. Morton, D. E. Cummings, D. G. Baskin, G. S. Barsh, and M. W. Schwartz, "Central nervous system control of food intake and body weight," *Nature*, vol. 443, no. 7109, pp. 289–295, 2006.

[35] A. Flint, N. T. Gregersen, L. L. Gluud et al., "Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies," *British Journal of Nutrition*, vol. 98, no. 1, pp. 17–25, 2007.

[36] E. Raynaud, J.-F. Brun, A. Perez-Martin et al., "Serum leptin is associated with the perception of palatability during a standardized high-carbohydrate breakfast test," *Clinical Science*, vol. 96, no. 4, pp. 343–348, 1999.

[37] S. Fulton, B. Woodside, and P. Shizgal, "Modulation of brain reward circuitry by leptin," *Science*, vol. 287, no. 5450, pp. 125–128, 2000.

[38] N. R. Lenard and H.-R. Berthoud, "Central and peripheral regulation of food intake and physical activity: pathways and genes," *Obesity*, vol. 16, no. 3, pp. S11–S22, 2008.

[39] A. Sainsbury and L. Zhang, "Role of the arcuate nucleus of the hypothalamus in regulation of body weight during energy deficit," *Molecular and Cellular Endocrinology*, vol. 316, no. 2, pp. 109–119, 2010.

[40] J. L. Chan, K. Heist, A. M. DePaoli, J. D. Veldhuis, and C. S. Mantzoros, "The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men," *Journal of Clinical Investigation*, vol. 111, no. 9, pp. 1409–1421, 2003.

[41] R. L. Leibel, "The role of leptin in the control of body weight," *Nutrition Reviews*, vol. 60, no. 10, pp. S15–S19, 2002.

[42] M. Rosenbaum, H. R. Kissileff, L. E. S. Mayer, J. Hirsch, and R. L. Leibel, "Energy intake in weight-reduced humans," *Brain Research*, vol. 1350, pp. 95–102, 2010.

[43] H. R. Kissileff, J. C. Thornton, M. I. Torres et al., "Leptin reverses declines in satiation in weight-reduced obese humans," *The American Journal of Clinical Nutrition*, vol. 95, no. 2, pp. 309–317, 2012.

[44] N. L. Keim, J. S. Stern, and P. J. Havel, "Relation between circulating leptin concentrations and appetite during a prolonged, moderate energy deficit in women," *The American Journal of Clinical Nutrition*, vol. 68, no. 4, pp. 794–801, 1998.

[45] M. Hopkins, C. Gibbons, P. Caudwell et al., "The adaptive metabolic response to exercise-induced weight loss influences both energy expenditure and energy intake," *European Journal of Clinical Nutrition*, 2013.

[46] J. McNeil and É. Doucet, "Possible factors for altered energy balance across the menstrual cycle: a closer look at the severity of PMS, reward driven behaviors and leptin variations," *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol. 163, no. 1, pp. 5–10, 2012.

[47] J. McNeil, J. D. Cameron, G. Finlayson, and J. E. Blundell, "Greater overall olfactory performance, explicit wanting for high fat foods and lipid intake during the mid-luteal phase of the menstrual cycle," *Physiology and Behavior*, vol. 112-113, pp. 84–89, 2013.

Hindawi Publishing Corporation
International Journal of Endocrinology
Volume 2014, Article ID 674069, 9 pages
http://dx.doi.org/10.1155/2014/674069

Research Article

Effect of *GAS6* and *AXL* Gene Polymorphisms on Adiposity, Systemic Inflammation, and Insulin Resistance in Adolescents

Fone-Ching Hsiao,[1,2] Yuh-Feng Lin,[1] Po-Shiuan Hsieh,[3] Nain-Feng Chu,[4] Yii-Der Ida Chen,[5] Yi-Shing Shieh,[6] Chang-Hsun Hsieh,[2] Chien-Hsing Lee,[2] Ting-I Lee,[1,7] and Yi-Jen Hung[2]

[1] *Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei City 110, Taiwan*
[2] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei City 114, Taiwan*
[3] *Department of Physiology and Biophysics, National Defense Medical Center, Taipei City 114, Taiwan*
[4] *School of Public Health Department, National Defense Medical Center, Taipei City 114, Taiwan*
[5] *Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA 90509, USA*
[6] *Department of Oral Diagnosis and Pathology, School of Dentistry, Tri-Service General Hospital, National Defense Medical Center, Taipei City 114, Taiwan*
[7] *Division of Endocrinology and Metabolism, Wan Fang Hospital, Taipei Medical University, Taipei City 116, Taiwan*

Correspondence should be addressed to Yi-Jen Hung; metahung@yahoo.com

Received 18 October 2013; Revised 21 December 2013; Accepted 7 January 2014; Published 18 February 2014

Academic Editor: Yi-Hao Yu

The present study was designed to explore the effects of *GAS6* and *AXL* gene polymorphisms on adiposity, systemic inflammation, and insulin resistance in adolescents. After multistage sampling from the data of the Taipei Children Heart Study-III, we collected 358 boys and 369 girls with an average age of 13.3 years. We genotyped the adolescents' *GAS6* rs8191973, *GAS6* rs8191974, *AXL* rs4802113, and *AXL* rs2304232 polymorphisms. Significantly higher body mass index (BMI), waist circumference (WC), and hsCRP levels were found in boys with the GG genotype of *GAS6* rs8191974 than A allele carriers; higher IL-6 and insulin levels and increased HOMA-IR were found in boys with the GG genotype of *AXL* rs2304232 than the A allele carriers. There was a significant difference in hsCRP levels of boys with the TT, TC, and CC genotypes of *AXL* rs4802113. Boys with both the GG genotype of *GAS6* rs8191973 and the GG genotype of *GAS6* rs8191974 exhibited higher BMI, WC, IL-6, and hsCRP levels than the boys carrying both the C allele of the *GAS6* rs8191973 and the A allele of the *GAS6* rs8191974. In conclusion, *GAS6* and *AXL* polymorphisms are associated with adiposity, systemic inflammation, and insulin resistance in adolescents, especially in boys.

1. Introduction

Childhood obesity is a serious and growing public health problem that has arisen over the past three decades [1]. The increasing occurrence of disorders such as type 2 diabetes during childhood is believed to be a consequence of this obesity epidemic [1]. In addition to several behavioral and dietary risk factors, genetic predisposition is an important factor in the pathogenesis of childhood obesity [2]. It is estimated that 40–70% of adiposity variance can be explained by direct or indirect genetic factors [3].

Growth arrest-specific 6 (Gas6), cloned in 1988 and characterized in 1993, is a secreted vitamin K-dependent protein present in the human circulatory system [4, 5]. Initially, Gas6 was shown to be upregulated in growth-arrested fibroblasts, suggesting that it plays a protective role in certain cellular stresses such as during apoptosis [6]. Gas6 expression is widespread in many tissues, including immune cells, endothelial cells, vascular smooth muscle cells, and adipocytes [7–9]. The protein is also a ligand for the TAM (Tyro-3/Axl/Mer) family tyrosine kinase receptor [4]. The Gas6/TAM system has been implicated in cell survival and

proliferation, cell adhesion and migration, hemostasis, and inflammatory cytokine release [4, 10].

Recently, the Gas6/TAM pathway was found to be involved in mediating adipocyte survival and proliferation *in vitro* [11, 12]. Experiments with mice fed a high-fat diet indicated that overexpression of Gas6 might enhance body-fat accumulation [9], but blocking Gas6 signaling using an Axl antagonist could reduce body-fat mass and body weight [13]. Interestingly, transgenic animals that ectopically express the Axl tyrosine kinase receptor also develop progressive obesity with elevated circulating proinflammatory cytokines and severe systemic insulin resistance [14]. This protein-array study also revealed higher levels of Axl mRNA in subcutaneous adipose tissue of obese humans than their lean control counterparts had. This indicates that the Axl receptor may be involved in the development of human obesity [15]. In addition, some studies in transgenic mice indicate that Gas6/Axl signaling might recruit macrophages and other immune cells into the adipose tissue resulting in the production and secretion of proinflammatory mediators. This suggests that the Gas6/Axl signaling might play a role in the pathogenesis of obesity-associated systemic inflammation [8, 16, 17]. Recent studies have indicated that systemic inflammation, a hallmark of childhood and adult obesity, is a pivotal mechanism linking obesity to insulin resistance and type 2 diabetes [18–21].

Although *GAS6* gene polymorphisms are reported to be associated with stroke, acute coronary syndrome, and type 2 diabetes [22–24], to our knowledge, both *GAS6* and *AXL* gene polymorphisms associated with childhood obesity have not yet been identified. In order to address this issue, we conducted a community-based study to determine whether common variations in the *GAS6* and *AXL* genes correlate with adiposity, systemic inflammation, insulin resistance among adolescents.

2. Materials and Methods

2.1. Study Design and Sampling. The Taipei Children Heart Study-III was an epidemiological survey that investigated obesity and cardiovascular disease risk factors among adolescents in Taipei City during 2006. The sampling method and results have been previously described [25]. Briefly, the survey included junior high school students in Taipei City to collect a representative distribution of demographic, lifestyle, and biochemical characteristics to measure their risk for cardiovascular disease. After multistage sampling, researchers randomly selected 1283 Taipei adolescents. Those with autoimmune diseases, cancers, or active infection and those taking medications known to interfere with insulin or glucose metabolism were excluded. Excluding any missing data, 727 adolescents (358 boys and 369 girls) were included in the final analyses.

2.2. Data Collection. The institutional review board of the Tri-Service General Hospital approved these studies and obtained informed consent from both parents and adolescents. All the participants completed a structured questionnaire detailing their gender, age, puberty development, and lifestyle characteristics (including cigarette smoking, alcohol consumption, and physical activity). Based on their responses, the subjects were divided into young adolescents with history of smoking, those without, and those who currently smoke. The study divided alcohol consumption into 2 categories: present or no consumption. Physical activity was divided into 5 levels based on amount of exercise per week: less than 1 h, 1–3 h, 3–5 h, 5–7 h, and over 7 h. Survey questions concerning puberty onset included the development of the penis/testis and pubic hair for boys and development of breasts and pubic hair for girls. Pubertal status was evaluated according to the Tanner criteria [26].

2.3. Anthropometric Measurements. Body weight was measured of barefoot students wearing light indoor clothing and was rounded to the nearest 0.1 kg. Body height was recorded to the nearest 0.1 cm. Waist circumference (WC) was measured at the midway point between the inferior margin of the last rib and the crest of the ilium in a horizontal plane and was recorded to the nearest 0.1 cm. Hip circumference was measured at its widest point to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

2.4. Analytical Methods. To reduce extraneous variation between subjects, we collected blood samples from the students after 12 h fasting and who had consumed their usual diet for the previous 3 days. Children who had recently attended a holiday or family celebration were contacted for a blood sample several weeks after the event. Biochemical assays were performed within 2 weeks of blood collection and storage at −4°C. Plasma was stored at −70°C until used for biochemical analysis.

Plasma glucose concentrations were determined by the glucose oxidase method by using the Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). The intra- and interassay coefficients of variation (CVs) for glucose were 0.6% and 1.5%, respectively. Plasma insulin was measured using a commercial immunoradiometric kit (BioSource Europe, Nivelles, Belgium). The intra- and interassay CVs for insulin were 2.2% and 6.5%, respectively. Serum levels for high-sensitivity C-reactive protein (hsCRP) were measured using the Tina-Quant (Latex) high-sensitivity assay (Roche, Mannheim, Germany). The intra- and interassay CVs for hsCRP were 3.7% and 4.9%, respectively. Serum IL-6 concentrations were determined using a human high-sensitivity enzyme-linked immunosorbent assay (ELISA) (Innotest, Besancon, France). The intra- and interassay CVs for IL-6 were 1.5% and 5.3%, respectively. Serum TNF-α was measured with the Biotrak high-sensitivity human ELISA kit from Amersham Biosciences (Buckinghamshire, UK). The intra- and interassay CVs for TNF-α were 3.5% and 5.3%, respectively. All concentrations of the above biochemical variables are the means of 2 samples. Insulin resistance was assessed using the homeostasis model assessment (HOMA),

Table 1: Anthropometric and biochemical data with different *GAS6* rs8191973 genotypes among boys and girls.

	Boys			Girls		
	GG ($n = 277$)	GC ($n = 72$)	CC ($n = 9$)	GG ($n = 277$)	GC ($n = 85$)	CC ($n = 7$)
BMI (kg/m^2)	22.4 ± 4.0	21.7 ± 4.0	24.2 ± 2.8	21.1 ± 3.2	21.6 ± 3.7	21.1 ± 2.0
WC (cm)	80.1 ± 10.5	79.2 ± 10.4	84.7 ± 8.6	75.2 ± 7.8	76.5 ± 9.2	78.6 ± 6.8
hsCRP (mg/L)	0.8 ± 1.3	1.0 ± 1.3	1.0 ± 1.7	0.6 ± 0.9	0.6 ± 0.7	0.3 ± 0.2
TNF-α (pg/mL)	26.8 ± 2.9	23.5 ± 4.1	25.3 ± 6.2	22.7 ± 4.7	27.3 ± 5.4	25.1 ± 7.5
IL-6 (pg/mL)	3.2 ± 2.1	3.5 ± 3.1	3.9 ± 3.0	3.3 ± 3.1	3.0 ± 1.4	2.8 ± 1.1
Glucose (mg/dL)	94.1 ± 6.4	92.7 ± 5.8	93.7 ± 4.1	91.7 ± 6.4	91.1 ± 7.3	88.3 ± 3.5
Insulin (μU/mL)	15.4 ± 8.8	13.8 ± 8.3	17.1 ± 6.3	14.1 ± 7.5	15.7 ± 8.0	15.9 ± 5.1
HOMA-IR	3.6 ± 2.2	3.2 ± 2.1	4.0 ± 1.6	3.2 ± 1.8	3.6 ± 1.9	3.4 ± 1.0
Gas6 (ng/mL)	12.1 ± 3.3	14.1 ± 3.7	12.9 ± 3.7	12.6 ± 3.9	12.2 ± 3.1	11.8 ± 3.1

Data are expressed as mean ± SD.
BMI: body mass index; WC: waist circumference; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6.

in which the HOMA of insulin resistance (HOMA-IR) = fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5 [27].

Gas6 protein concentration was measured using a sandwich ELISA and a polyclonal mouse anti-human Gas6 antibody (R&D Systems, Lille, France) as a catcher and a biotinylated goat antiserum as a detector (R&D Systems), using previously described methods [28]. The technique has been validated by Food and Drug Administration guidelines in a previous study (intra- and interassay CVs of 6.5% and 8.5%, resp.; mean recovery on 10 patients of 97%; lower limit of quantification 0.26 ng/mL) [29].

2.5. DNA Extraction and Genotype Analysis. DNA was isolated from buffy coats using the QIAamp DNA blood kit and following the manufacturer's instruction (Qiagen, Valencia, CA, USA). The qualities of isolated genomic DNAs were quantified using agarose gel electrophoresis, and the quantities were determined using a spectrophotometer. Genotyping was performed using quantitative real-time PCR. The SNP selection and primer design are described in a previous study [30]. SNPs rs8191973 and rs8191974 in *GAS6*, as well as rs4802113 and rs2304232 in *AXL*, were genotyped using a TaqMan assay with allele-specific probes on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the standardized laboratory protocols [24].

2.6. Statistical Methods. Descriptive results of continuous variables were expressed as means ± SD. Prior to statistical analysis, the normal distribution and homogeneity of the variables were evaluated using the Levene test for quality of variance, and the variables were then given a base logarithmic transformation if necessary. The parameters HOMA-IR, triglycerides, hsCRP, IL-6, and TNF-α were analyzed and tested for significance using a log scale. The studied adolescents were categorized into subgroups based on their *GAS6* rs8191973 genotype (CC, CG, and GG), *GAS6* rs8191974 genotype (GG, GA, and AA), *AXL* rs4802113 genotype (CC, CT, and TT), and *AXL* rs2304232 (AA, AG, and GG) with gender specification. The differences between anthropometric and biochemistry data across genotypes were analyzed using a general linear model after adjusting for age, Tanner stages, smoking status, drinking status, and physical activity. Chi-square tests were used to determine the genotype distributions for the Hardy-Weinberg equilibrium and to compare the proportions of abnormal anthropometric and biochemistry variables across genotypes. We tested different genetic inheritance models, and a recessive model was applied in the final analyses for *GAS6* and *AXL*. To determine whether the *GAS6* and *AXL* SNPs are predictors of obesity and obesity-associated complications, logistic regression analysis was used to calculate the odds ratio (OR) and 95% confidence interval (CI) for each genotype and combined genotypes. A two-sided P-value of <0.05 was considered statistically significant. All statistical analyses were performed using PASW Statistics 18.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Subject Characteristics. In total, 727 adolescents (358 boys and 369 girls) were included in this study. The mean age of all adolescents in this study was 13.3 years (range, 12–15) and was similar between boys and girls. In general, boys had higher BMI (22.3 ± 4.0 versus 21.2 ± 3.3 kg/m^2), WC (80.0 ± 10.1 versus 75.1 ± 8.1 cm), hsCRP (0.9 ± 1.3 versus 0.6 ± 0.9 mg/L), and glucose levels (93.8 ± 6.3 versus 91.5 ± 6.5 mg/dL) than the girls (all $P < 0.001$). However, girls had higher Tanner stages (3.2 ± 0.5 versus 3.0 ± 0.4) than the boys ($P < 0.001$). There was no statistically significant difference in the ages, TNF-α, IL-6, and insulin levels, and HOMA-IR between the boys and girls.

3.2. Genotype and Allele Frequencies. The genotype frequencies of the 4 polymorphisms are presented in Tables 1–4. All genotype frequencies were found to be within the Hardy-Weinberg equilibrium. The allele frequency for the least frequent allele in boys was 12.6, 22.1, 41.9, and 29.7%,

Table 2: Anthropometric and biochemical data with different *GAS6* rs8191974 genotypes among boys and girls.

	Boys			Girls		
	GG ($n = 213$)	GA ($n = 132$)	AA ($n = 13$)	GG ($n = 242$)	GA ($n = 109$)	AA ($n = 18$)
BMI (kg/m^2)	22.5 ± 4.2[b]	22.0 ± 3.6[b]	20.7 ± 3.3[b]	21.2 ± 3.3	21.3 ± 3.4	20.3 ± 2.3
WC (cm)	80.6 ± 11.0[b]	79.3 ± 9.5[b]	76.7 ± 9.4[b]	75.8 ± 8.1	75.2 ± 8.2	74.6 ± 8.3
hsCRP (mg/L)	0.8 ± 1.2[a,b]	1.1 ± 1.6[a,b]	0.4 ± 0.2[a,b]	0.6 ± 1.0	0.5 ± 0.5	0.9 ± 1.3
TNF-α (pg/mL)	26.7 ± 8.2	25.5 ± 10.1	23.8 ± 4.6	24.5 ± 9.3	22.9 ± 6.5	27.9 ± 4.3
IL-6 (pg/mL)	3.4 ± 2.8	3.3 ± 3.2	2.5 ± 1.3	3.2 ± 2.8	3.1 ± 3.0	2.9 ± 0.7
Glucose (mg/dL)	94.0 ± 6.3	93.4 ± 6.4	95.3 ± 3.6	91.8 ± 6.7	90.6 ± 6.3	92.1 ± 5.7
Insulin (μU/mL)	14.9 ± 8.7	15.3 ± 9.0	15.0 ± 8.2	15.0 ± 8.1	13.9 ± 6.6	11.7 ± 4.2
HOMA-IR	3.5 ± 2.2	3.6 ± 2.2	3.5 ± 2.0	3.4 ± 2.0	3.1 ± 1.5	2.7 ± 1.1
Gas6 (ng/mL)	13.1 ± 3.7	13.2 ± 3.7	13.0 ± 3.1	11.8 ± 3.1	12.1 ± 3.0	1.7 ± 2.2

Data are expressed as mean ± SD.
BMI: body mass index; WC: waist circumference; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6.
[a]GG versus GA versus AA, $P < 0.05$; [b]GG versus (GA + AA), $P < 0.05$. All comparisons were analyzed using a general linear model after adjusting for age, Tanner stages, smoking status, drinking status, and physical activity.

Table 3: Anthropometric and biochemical data with different *AXL* rs4802113 genotypes among boys and girls.

	Boys			Girls		
	TT ($n = 123$)	TC ($n = 170$)	CC ($n = 65$)	TT ($n = 108$)	TC ($n = 181$)	CC ($n = 80$)
BMI (kg/m^2)	22.0 ± 3.7	22.6 ± 3.8	21.9 ± 4.7	21.4 ± 3.6	21.1 ± 3.2	21.2 ± 3.0
WC (cm)	79.4 ± 10.3	80.8 ± 10.0	79.0 ± 11.9	75.8 ± 9.0	75.2 ± 7.7	76.1 ± 7.9
hsCRP (mg/L)	0.8 ± 1.2[a]	1.0 ± 1.4[a]	0.8 ± 1.3[a]	0.6 ± 0.8	0.6 ± 0.9	0.6 ± 0.9
TNF-α (pg/mL)	24.8 ± 5.5	26.2 ± 6.5	28.2 ± 10.1	24.0 ± 7.3	25.7 ± 8.3	26.6 ± 8.3
IL-6 (pg/mL)	3.0 ± 2.8	3.4 ± 2.7	3.7 ± 3.5	3.5 ± 3.0	2.9 ± 2.0	3.3 ± 2.9
Glucose (mg/dL)	93.4 ± 6.1	94.3 ± 6.0	93.4 ± 7.1	92.0 ± 6.9	91.0 ± 6.5	91.8 ± 6.2
Insulin (μU/mL)	14.8 ± 8.1	15.6 ± 9.7	14.5 ± 7.9	15.2 ± 7.3	14.1 ± 7.8	14.7 ± 7.4
HOMA-IR	3.4 ± 2.0	3.7 ± 2.3	3.4 ± 2.0	3.5 ± 1.7	3.2 ± 1.9	3.3 ± 1.7

Data are expressed as mean ± SD.
BMI: body mass index; WC: waist circumference; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6.
[a]TT versus TC versus CC, $P < 0.05$. All comparisons were analyzed using a general linear model after adjusting for age, Tanner stages, smoking status, drinking status, and physical activity.

Table 4: Anthropometric and biochemical data with different *AXL* rs2304232 genotypes among boys and girls.

	Boys			Girls		
	AA ($n = 180$)	AG ($n = 143$)	GG ($n = 35$)	AA ($n = 165$)	AG ($n = 161$)	GG ($n = 43$)
BMI (kg/m^2)	22.3 ± 3.7	22.2 ± 3.9	22.4 ± 5.4	21.4 ± 3.5	21.0 ± 3.3	21.2 ± 2.5
WC (cm)	79.8 ± 10.1	80.3 ± 10.0	79.5 ± 13.8	75.7 ± 8.6	75.5 ± 7.9	75.0 ± 7.3
hsCRP (mg/L)	0.9 ± 1.5	0.8 ± 1.0	0.8 ± 1.5	0.5 ± 0.8	0.5 ± 0.8	0.8 ± 1.4
TNF-α (pg/mL)	26.9 ± 6.0	25.2 ± 7.0	24.7 ± 7.7	24.7 ± 7.5	25.4 ± 8.4	28.1± 9.5
IL-6 (pg/mL)	3.1 ± 3.0[a]	3.2 ± 3.1[a]	4.8 ± 4.0[a]	3.2 ± 3.1	3.0 ± 1.8	3.7 ± 3.5
Glucose (mg/dL)	93.7 ± 5.8	94.0 ± 6.2	93.4 ± 8.4	91.3 ± 6.5	91.5 ± 6.7	92.0 ± 6.0
Insulin (μU/mL)	15.4 ± 9.3[a]	14.5 ± 8.2[a]	15.9 ± 8.6[a]	15.1 ± 7.5	14.0 ± 7.9	14.1 ± 6.1
HOMA-IR	3.6 ± 2.3[a]	3.4 ± 2.0[a]	3.7 ± 2.3[a]	3.4 ± 1.8	3.2 ± 1.9	3.2 ± 1.4

Data are expressed as mean ± SD.
BMI: body mass index; WC: waist circumference; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6.
[a](AA + AG) versus GG, $P < 0.05$. All comparisons were analyzed using a general linear model after adjusting for age, Tanner stages, smoking status, drinking status, and physical activity.

Table 5: Logistic regression analyses of different *GAS6* SNP on abnormal variables among adolescents.

Variables[b]	*GAS6* rs8191973 GG OR (95% CI)[a]		*GAS6* rs8191974 GG OR (95% CI)[a]	
	Unadjusted	Adjusted[c]	Unadjusted	Adjusted[c]
Boys				
High BMI	1.12 (0.47–2.68)	1.26 (0.34–4.76)	1.40 (1.06–2.99)[d]	1.85 (1.04–3.23)[d]
High WC	0.82 (0.38–1.76)	1.11 (0.39–5.26)	1.58 (1.18–2.01)[d]	1.68 (1.08–3.25)[d]
High hsCRP	1.87 (1.25–2.87)[d]	2.53 (1.03–6.24)[d]	1.88 (0.68–3.25)	1.92 (0.93–3.96)
High TNF-α	4.26 (0.99–18.37)	3.34 (0.42–24.98)	0.93 (0.43–1.98)	0.99 (0.33–2.94)
High IL-6	2.09 (0.92–4.82)	2.27 (0.98–5.26)	2.68 (1.32–5.20)[d]	2.56 (1.33–5.00)[d]
High HOMA-IR	1.41 (0.56–3.52)	1.14 (0.31–4.17)	1.28 (0.61–2.67)	2.35 (0.73–7.69)
Girls				
High BMI	0.76 (0.36–1.61)	0.57 (0.22–1.45)	1.09 (0.53–2.26)	1.10 (0.43–2.86)
High WC	0.60 (0.30–1.23)	0.44 (0.17–1.11)	1.26 (0.60–2.64)	1.57 (0.55–4.55)
High hsCRP	0.90 (0.41–1.96)	1.04 (0.39–2.78)	1.19 (0.58–2.44)	1.05 (0.44–2.50)
High TNF-α	0.91 (0.41–2.04)	0.69 (0.24–1.96)	1.00 (0.47–2.13)	0.85 (0.31–2.33)
High IL-6	1.37 (0.61–3.09)	0.90 (0.34–2.50)	1.42 (0.69–2.95)	1.68 (0.06–4.55)
High HOMA-IR	0.76 (0.33–1.72)	0.69 (0.25–1.92)	1.48 (0.64–3.41)	2.20 (0.70–7.14)

[a]Under a recessive model (using heterozygotes and minor homozygotes as the reference for each SNP). [b]Abnormal variables were determined using an age- and gender-specific 90th percentile cut-off point.
[c]Adjusting for age, Tanner stage, cigarette smoking, alcohol drinking, and physical activity.
[d]$P < 0.05$.
OR: odds ratio; CI: confidence index; BMI: body mass index; WC: waist circumference; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6.

and 13.4, 19.6, 32.7, and 33.5% in girls for the *GAS6* rs8191973, *GAS6* rs8191974, *AXL* rs4802113, and *AXL* rs2304232 polymorphisms, respectively. There was no significant difference in allele or genotype distribution between boys and girls at the 4 polymorphisms.

3.3. Association of GAS6 Gene Polymorphisms with Adiposity, Inflammatory Markers, and HOMA-IR. No statistically significant association between anthropometric characteristics, biochemistry data, and the *GAS6* rs8191973 genotypes was observed in the boys and girls (Table 1). However, there were significantly different hsCRP levels between GG, GA, and AA genotypes of *GAS6* rs8191974 in boys, regardless of their age, Tanner stages, smoking status, drinking status, or physical activity (Table 2). Moreover, boys with the GG genotype of *GAS6* rs8191974 had significantly higher BMI, WC, and hsCRP levels than those carrying the A allele. The *GAS6* rs819174 genotypes were not significantly associated with any anthropometric characteristics and biochemistry in girls. The *P*-values of all comparisons between anthropometric and biochemistry data across *GAS6* genotypes were presented in Supplemental Tables 1 and 2 available online at http://dx.doi.org/10.1155/2014/674069.

In addition, the association between circulating Gas6 protein levels and *GAS6* polymorphisms was investigated. We found that the *GAS6* rs8191973 or rs8191974 genotypes were not significantly associated with circulating Gas6 protein levels in both the sexes.

3.4. Association of AXL Gene Polymorphisms with Adiposity, Inflammatory Markers, and HOMA-IR. There were significantly different hsCRP levels between TT, TC, and CC genotypes of *AXL* rs4802113 in boys, independent of their age, Tanner stages, smoking status, drinking status, or physical activity (Table 3). In addition, boys with the GG genotype of *AXL* rs2304232 had significantly higher IL-6 and insulin levels and increased HOMA-IR than those carrying the A allele (Table 4). However, in girls, *AXL* rs4802113 or rs2304232 polymorphisms were not significantly associated with any anthropometric characteristics or biochemistry (Tables 3 and 4). The *P*-values of all comparisons between anthropometric and biochemistry data across *AXL* genotypes were presented in Supplemental Tables 3 and 4.

3.5. Association of GAS6 and AXL Gene Polymorphisms with Elevated Adiposity, Inflammatory Markers Levels, and HOMA-IR. Boys with the GG genotype of *GAS6* rs8191973 were 1.87-fold more likely to have higher hsCRP levels than the C allele carriers. Even after adjusting for age, Tanner stage, smoking status, drinking status, and physical activity, a significant relationship between the GG genotype of *GAS6* rs8191973 and higher hsCRP levels was still observed in boys (Table 5). Moreover, boys with the GG genotype of *GAS6* rs8191974 exhibited a 1.40-fold greater risk for developing high BMI, a 1.58-fold greater risk for developing high WC, and a 2.68-fold greater risk to have higher IL-6 levels than the A allele carriers. Even after adjusting for all possible confounding factors including age, Tanner stage, smoking/drinking status, and physical activity, the relationship between the GG genotype of *GAS6* rs8191974, higher BMI/WC, and higher IL-6 levels still remained significant in boys. However, the *AXL*

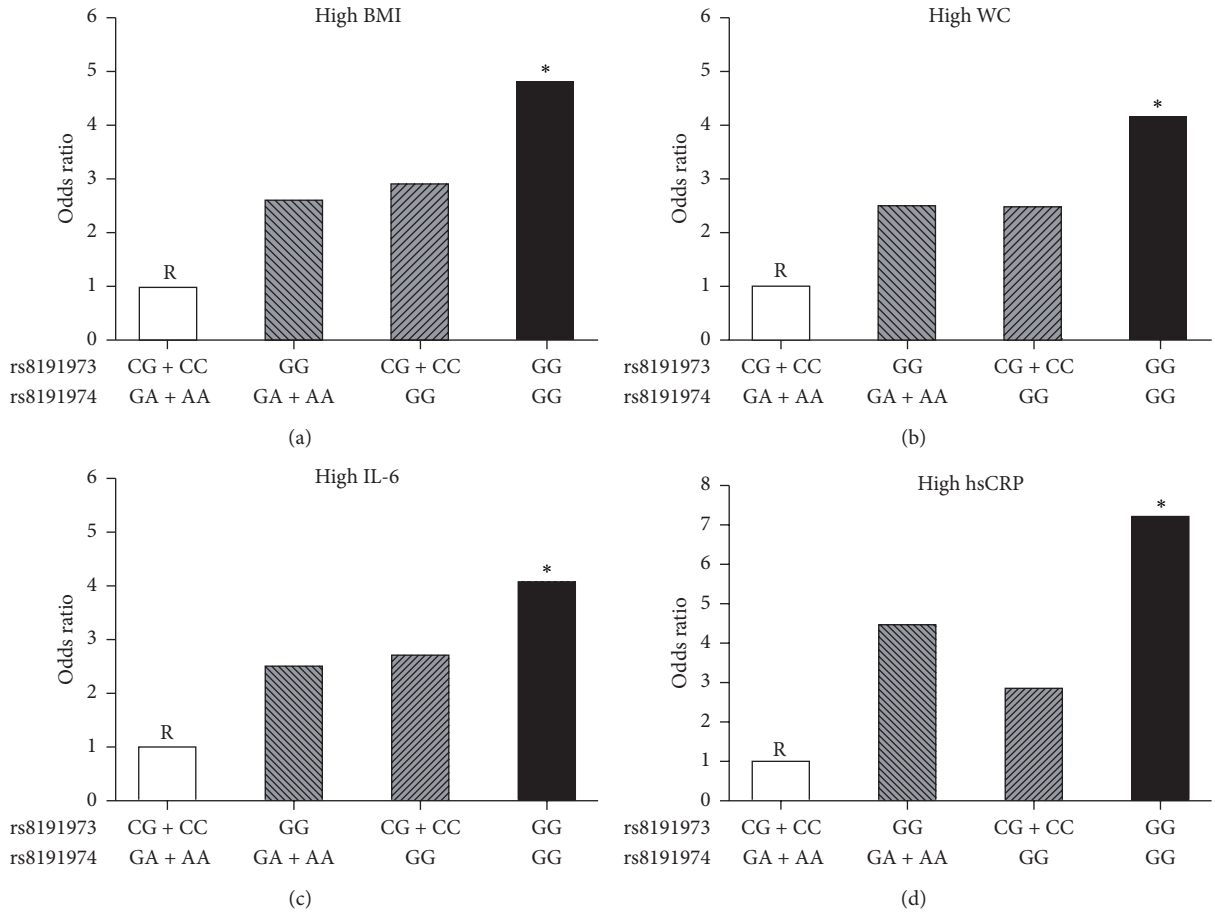


Figure 1: Combined effect of *GAS6* rs8191973 and rs8191974 polymorphisms on the risk of abnormal variables in boys. $^{*}P < 0.05$; R = reference group.

rs4802113 or rs2304232 polymorphisms showed no significant association with abnormal adiposity, inflammatory markers, and HOMA-IR in boys or girls (see Supplemental Table 5).

3.6. Combined Effect of the GAS6 and AXL Polymorphisms on High Adiposity, Inflammatory Marker Levels, and HOMA-IR. Logistic regression analyses were applied to evaluate whether the combination of the *GAS6* rs8191974 and rs8191973 polymorphisms is a stronger risk factor for high adiposity, inflammatory markers levels, and HOMA-IR than when alone. The combined effects of the 2 *GAS6* gene polymorphisms in the risk of high BMI, WC, IL-6, and hsCRP levels are shown in Figure 1. After adjusting for the relevant confounding factors, we still observed that boys with the GG genotype of *GAS6* rs8191973 and the GG genotype of *GAS6* rs8191974 exhibited a 4–7-fold higher risk of high BMI, WC, IL-6, and hsCRP levels than the individuals with both the C allele of the *GAS6* rs8191973 and the A allele of the *GAS6* rs8191974 did (OR = 4.92, 95% CI: 1.08–23.6, $P = 0.018$; OR = 4.18, 95% CI: 1.05–22.5, $P = 0.016$; OR = 4.08, 95% CI: 1.06–28.56, $P = 0.015$; OR = 7.22, 95% CI: 1.46–35.72, $P = 0.010$, resp.). However, for girls, there was no statistically significant association between the combination of the *GAS6* rs8191974 and rs8191973 polymorphisms and abnormal variables.

In addition, we evaluated the combined effect of the *GAS6* rs8191973 or rs8191974 marker with *AXL* gene polymorphisms and its association with risk of high adiposity, inflammatory marker, and HOMA-IR. However, combinations of *GAS6* markers with *AXL* gene polymorphisms were not found to be significantly associated with any abnormal variables in both boys and girls (data not shown).

4. Discussion

In this study, a strong association between *GAS6* and *AXL* polymorphisms with body adiposity, systemic inflammation, and insulin resistance was identified among boys. The risk of possessing high adiposity and inflammatory markers levels was higher in boys carrying the GG genotype with *GAS6* rs8191973 or rs8191974 than the noncarriers. Moreover, the combination of both *GAS6* polymorphisms had an additive effect on the development of obesity and obesity-associated inflammation in boys. These data strongly suggest that *GAS6* and *AXL* genes play a role in the pathogenesis of childhood obesity and its associated complications.

GAS6 was originally identified as a gene that is expressed in fibroblasts and increases with serum starvation and contact inhibition [6]; Gas6 is also a potential growth factor for fibroblasts [11]. Maquoi and colleagues demonstrated that when fed with a high-fat diet, *GAS6*-deficient mice had significantly less fat than their wild-type counterparts [9]. The authors also reported the expression of Gas6 and its 3 receptors (Tyro-3, Axl, Mer) in murine adipose tissues, thus suggesting that Gas6 may act in an autocrine and/or paracrine manner to promote murine adipose tissue development [9]. Previous experiments in transgenic mice demonstrate that Gas6 might also induce obesity-associated inflammation via recruiting immune cells into the adipose tissue to producing and secreting proinflammatory cytokines [8, 16, 17]. Our recent clinical study found that circulating Gas6 protein levels are associated with adiposity and inflammatory markers in overweight/obese adolescents [5]. In this study, *GAS6* is further implicated as a candidate susceptibility gene for obesity and systemic inflammation. However, the association between *GAS6* genotypes and circulating Gas6 protein levels was not observed among adolescents. We hypothesize that *GAS6* polymorphisms could affect the biology of the Gas6 protein itself rather than its transcription or process rate, thus influencing adiposity regulation and systemic inflammation. To validate this, further studies regarding the association between Gas6 protein biology and *GAS6* polymorphisms are required.

Recent studies demonstrated that Gas6/TAM signaling is involved in releasing inflammatory cytokines (such as IL-6 and hsCRP) in diverse human diseases [23, 31, 32]. In addition, the Gas6/TAM signaling is also known to be involved in several inflammation-related systems, including maturation of immune cells [33], endothelial activation [7], and immunoregulation [34]. Our present study found that the GG genotype of *GAS6* rs8191973 and the GG genotype of *GAS6* rs8191974 are strongly associated with higher circulating IL-6 and hsCRP levels in boys. Therefore, the *GAS6* polymorphisms presumably influence Gas6/TAM signaling and could further activate inflammatory reactions and result in releasing circulating IL-6 and hsCRP. However, the comprehensive effects of the *GAS6* polymorphisms in regulation of inflammatory cytokines still remain to be determined by more researches.

Interestingly, a previous study found that the A allele or the AA genotype of *GAS6* rs8191974 is associated with decreased risk of stroke [22]. Moreover, the A allele and the AA genotype are also thought to be related to a lower risk of developing acute coronary syndrome or type 2 diabetes, suggesting that this genotype exhibits protective activities against developing acute coronary syndrome and type 2 diabetes [23, 24]. We also observed similar results in those with the A allele or AA genotype of *GAS6* rs8191974. These subjects exhibited a lower risk for developing obesity and systemic inflammation than those with the GG genotypes. Together, these findings suggest that the *GAS6* rs8191974 polymorphisms play an important role in the development of obesity and obesity-associated complications (e.g., type 2 diabetes, cerebrovascular, and cardiovascular diseases). The protective role of the AA genotype of *GAS6* rs8191974 against the developing childhood obesity and obesity-associated complications requires further study.

The Axl protein is a membrane-bound receptor that belongs to the TAM family of receptor tyrosine kinases. Gas6 and protein S are the known ligands of the TAM receptor family [35]. Axl exhibits the highest affinity for Gas6 as compared to the other members of the TAM family, whereas protein S predominantly binds Mer and Tyro-3 [36]. Gas6/Axl signaling has been shown to be involved in the pathogenesis of obesity and systemic inflammation [13–15]. However, our study demonstrates that *AXL* polymorphisms are associated with systemic inflammation rather than childhood obesity. Moreover, the combination of *GAS6* and *AXL* gene polymorphisms is not significantly associated with any variables in adiposity among adolescents. Our findings indicated that *AXL* gene polymorphisms might not play a significant role in childhood obesity. Recently, Scroyen and colleagues [37] have published similar findings indicating that deficiency in a single Axl receptor did not significantly affect adipogenesis or adipose tissue development in mice. This is because an Axl deficiency can be partially compensated by other TAM family members (Tyro-3 and Mer) via Gas6 interaction. Axl may not be the only TAM receptor through which Gas6 could modulate adipogenesis. Further studies are needed to investigate the effect of Tyro-3 and Mer receptors on the development of childhood obesity.

In addition, our present study also indicates that gender disparity exists regarding the effects of the *GAS6* polymorphisms on anthropometric characteristics and inflammatory markers. We found no significant difference in genotype frequencies between boys and girls; however, the effects of the *GAS6* polymorphisms, individually or combined, only manifest in boys. The *GAS6* gene contains an estrogen response element in its promoter and is upregulated by estrogen via an activated estrogen receptor in mammary epithelial cells [38]. Moreover, androgen was reported to directly regulate *GAS6* transcription via the androgen receptor [39]. Therefore, we hypothesized that the gender-specific effect of the *GAS6* polymorphisms on childhood obesity might be due to a disparity in sex hormone distributions. This has been previously reported to be associated with *GAS6* expression and body composition [40, 41].

Despite these results, our study does have certain limitations. First, this was a cross-sectional study, as such we might not be able to assess *GAS6* polymorphisms on weight dynamics and the development of obesity-associated complications throughout life. Furthermore, longitudinal studies are required to confirm our results. Second, because of the limitations of our questionnaire, we were not able to comprehensively estimate every adolescent's daily intake. The impact of dietary energy intake on genetic susceptibility also requires further investigation to better understand any confounding effect.

In conclusion, we indicate an association between the *GAS6* and *AXL* polymorphisms with adiposity, circulating inflammatory markers, and insulin resistance of adolescents, especially in boys. Moreover, the GG genotype of *GAS6* rs8191973 or rs8191974 strongly correlates with susceptibility to develop obesity and systemic inflammation in boys.

Nonetheless, these results together with those from studies in cellular and animal models encourage the study of the Gas6/TAM system in childhood obesity and its potential complications and further support the hypothesis that modulation of Gas6 activity may indeed provide an important intervention point for future therapies.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank all adolescents, parents, and school staff who participated in this study. This study was supported by a Grant (NSC 102-2314-B-016-044-MY2) from the National Science Council, Grants (TSGH-C100-011-015-S04 and TSGH-C101-006-011-015-S04) from the Tri-Service General Hospital of the National Defense Medical Center, and a Grant (100swf02) from the Wan Fang Hospital of the Taipei Medical University, Taiwan.

References

[1] B. A. Swinburn, G. Sacks, K. D. Hall et al., "The global obesity pandemic: shaped by global drivers and local environments," *The Lancet*, vol. 378, no. 9793, pp. 804–814, 2011.

[2] J. Hebebrand, C. Sommerlad, F. Geller, T. Görg, and A. Hinney, "The genetics of obesity: practical implications," *International Journal of Obesity*, vol. 25, supplement 1, pp. S10–S18, 2001.

[3] I. S. Farooqi and S. O'Rahilly, "New advances in the genetics of early onset obesity," *International Journal of Obesity*, vol. 29, no. 10, pp. 1149–1152, 2005.

[4] G. Manfioletti, C. Brancolini, G. Avanzi, and C. Schneider, "The protein encoded by a growth arrest-specific gene (GAS6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade," *Molecular and Cellular Biology*, vol. 13, no. 8, pp. 4976–4985, 1993.

[5] F. C. Hsiao, Y. F. Lin, P. S. Hsieh et al., "Circulating growth arrest-specific 6 protein is associated with adiposity, systemic inflammation, and insulin resistance among overweight and obese adolescents," *The Journal of Clinical Endocrinology & Metabolism*, vol. 98, no. 2, pp. E267–E274, 2013.

[6] C. Schneider, R. M. King, and L. Philipson, "Genes specifically expressed at growth arrest of mammalian cells," *Cell*, vol. 54, no. 6, pp. 787–793, 1988.

[7] M. Tjwa, L. Bellido-Martin, Y. Lin et al., "Gas6 promotes inflammation by enhancing interactions between endothelial cells, platelets, and leukocytes," *Blood*, vol. 111, no. 8, pp. 4096–4105, 2008.

[8] E. Lutgens, M. Tjwa, P. G. De Frutos et al., "Genetic loss of GAS6 induces plaque stability in experimental atherosclerosis," *Journal of Pathology*, vol. 216, no. 1, pp. 55–63, 2008.

[9] E. Maquoi, G. Vörös, P. Carmeliet, D. Collen, and H. R. Lijnen, "Role of GAS-6 in adipogenesis and nutritionally induced adipose tissue development in mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 5, pp. 1002–1007, 2005.

[10] P. J. Godowski, M. R. Mark, J. Chen, M. D. Sadick, H. Raab, and R. G. Hammonds, "Reevaluation of the roles of protein S and GAS6 as ligands for the receptor tyrosine kinase Rse/Tyro 3," *Cell*, vol. 82, no. 3, pp. 355–358, 1995.

[11] S. Goruppi, E. Ruaro, and C. Schneider, "GAS6, the ligand of Axl tyrosine kinase receptor, has mitogenic and survival activities for serum starved NIH3T3 fibroblasts," *Oncogene*, vol. 12, no. 3, pp. 471–480, 1996.

[12] E. C. Shugart, A. S. Levenson, C. M. Constance, and R. M. Umek, "Differential expression of GAS and GADD genes at distinct growth arrest points during adipocyte development," *Cell Growth and Differentiation*, vol. 6, no. 12, pp. 1541–1547, 1995.

[13] H. R. Lijnen, V. Christiaens, and L. Scroyen, "Growth arrest-specific protein 6 receptor antagonism impairs adipocyte differentiation and adipose tissue development in mice," *Journal of Pharmacology and Experimental Therapeutics*, vol. 337, no. 2, pp. 457–464, 2011.

[14] K. A. Augustine, R. M. Rossi, G. Van et al., "Noninsulin-dependent diabetes mellitus occurs in mice ectopically expressing the human Axl tyrosine kinase receptor," *Journal of Cellular Physiology*, vol. 181, no. 3, pp. 433–447, 1999.

[15] M. Škopková, A. Penesová, H. Sell et al., "Protein array reveals differentially expressed proteins in subcutaneous adipose tissue in obesity," *Obesity*, vol. 15, no. 10, pp. 2396–2406, 2007.

[16] V. A. Korshunov, A. M. Mohan, M. A. Georger, and B. C. Berk, "Axl, a receptor tyrosine kinase, mediates flow-induced vascular remodeling," *Circulation Research*, vol. 98, no. 11, pp. 1446–1452, 2006.

[17] F. Lafdil, M.-N. Chobert, V. Deveaux et al., "Growth arrest-specific protein 6 deficiency impairs liver tissue repair after acute toxic hepatitis in mice," *Journal of Hepatology*, vol. 51, no. 1, pp. 55–66, 2009.

[18] S. Schenk, M. Saberi, and J. M. Olefsky, "Insulin sensitivity: modulation by nutrients and inflammation," *Journal of Clinical Investigation*, vol. 118, no. 9, pp. 2992–3002, 2008.

[19] C. Herder, S. Schneitler, W. Rathmann et al., "Low-grade inflammation, obesity, and insulin resistance in adolescents," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 12, pp. 4569–4574, 2007.

[20] C. N. Lumeng and A. R. Saltiel, "Inflammatory links between obesity and metabolic disease," *Journal of Clinical Investigation*, vol. 121, no. 6, pp. 2111–2117, 2011.

[21] C. S. Tam, K. Clément, L. A. Baur, and J. Tordjman, "Obesity and low-grade inflammation: a paediatric perspective," *Obesity Reviews*, vol. 11, no. 2, pp. 118–126, 2010.

[22] X. Muñoz, V. Obach, B. Hurtado, P. G. de Frutos, Á. Chamorro, and N. Sala, "Association of specific haplotypes of GAS6 gene with stroke," *Thrombosis and Haemostasis*, vol. 98, no. 2, pp. 406–412, 2007.

[23] L. Jiang, C. Y. Liu, Q. F. Yang, P. Wang, and W. Zhang, "Plasma level of growth arrest-specific 6 (GAS6) protein and genetic variations in the GAS6 gene in patients with acute coronary syndrome," *American Journal of Clinical Pathology*, vol. 131, no. 5, pp. 738–743, 2009.

[24] C.-H. Lee, N.-F. Chu, Y.-S. Shieh, and Y.-J. Hung, "The growth arrest-specific 6 (GAS6) gene polymorphism c.834+7G>A is associated with type 2 diabetes," *Diabetes Research and Clinical Practice*, vol. 95, no. 2, pp. 201–206, 2012.

[25] F.-H. Lin, N.-F. Chu, C.-H. Lee, Y.-J. Hung, and D.-M. Wu, "Combined effect of C-reactive protein gene SNP +2147 A/G

and interleukin-6 receptor gene SNP rs2229238 C/T on anthropometric characteristics among school children in Taiwan," *International Journal of Obesity*, vol. 35, no. 4, pp. 587–594, 2011.

[26] Y. Huang, "Body mass index reference for taiwanese children and adolescents," *Journal of Medical Sciences*, vol. 22, no. 5, pp. 221–226, 2002.

[27] R. Muniyappa, S. Lee, H. Chen, and M. J. Quon, "Current approaches for assessing insulin sensitivity and resistance *in vivo*: advantages, limitations, and appropriate usage," *American Journal of Physiology—Endocrinology and Metabolism*, vol. 294, no. 1, pp. E15–E26, 2008.

[28] Y.-J. Hung, C.-H. Lee, N.-F. Chu, and Y.-S. Shieh, "Plasma protein growth arrest-specific 6 levels are associated with altered glucose tolerance, inflammation, and endothelial dysfunction," *Diabetes Care*, vol. 33, no. 8, pp. 1840–1844, 2010.

[29] F. Alciato, P. P. Sainaghi, L. Castello, L. Bergamasco, S. Carnieletto, and G. C. Avanzi, "Development and validation of an ELISA method for detection of Growth Arrest Specific 6 (GAS6) protein in human plasma," *Journal of Immunoassay and Immunochemistry*, vol. 29, no. 2, pp. 167–180, 2008.

[30] B. Hurtado, N. Abasolo, X. Muñoz et al., "Association study between polymorphims in GAS6-TAM genes and carotid atherosclerosis," *Thrombosis and Haemostasis*, vol. 104, no. 3, pp. 592–598, 2010.

[31] C. Ekman, A. Linder, P. Åkesson, and B. Dahlbäck, "Plasma concentrations of GAS6 (growth arrest specific protein 6) and its soluble tyrosine kinase receptor sAxl in sepsis and systemic inflammatory response syndromes," *Critical Care*, vol. 14, no. 4, article R158, 2010.

[32] C. Ekman, A. Jonsen, G. Sturfelt, A. A. Bengtsson, and B. Dahlback:, "Plasma concentrations of GAS6 and sAxl correlate with disease activity in systemic lupus erythematosus," *Rheumatology*, vol. 50, no. 6, pp. 1064–1069, 2011.

[33] A. Caraux, Q. Lu, N. Fernandez et al., "Natural killer cell differentiation driven by Tyro3 receptor tyrosine kinases," *Nature Immunology*, vol. 7, no. 7, pp. 747–754, 2006.

[34] C. V. Rothlin, S. Ghosh, E. I. Zuniga, M. B. A. Oldstone, and G. Lemke, "TAM receptors are pleiotropic inhibitors of the innate immune response," *Cell*, vol. 131, no. 6, pp. 1124–1136, 2007.

[35] T. N. Stitt, G. Conn, M. Gore et al., "The anticoagulation factor protein S and its relative, GAS6, are ligands for the tyro 3/Axl family of receptor tyrosine kinases," *Cell*, vol. 80, no. 4, pp. 661–670, 1995.

[36] K. Nagata, K. Ohashi, T. Nakano et al., "Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases," *Journal of Biological Chemistry*, vol. 271, no. 47, pp. 30022–30027, 1996.

[37] I. Scroyen, L. Frederix, and H. R. Lijnen, "Axl deficiency does not affect adipogenesis or adipose tissue development," *Obesity*, vol. 20, no. 6, pp. 1168–1173, 2012.

[38] R. Mo, Y. T. Zhu, Z. Zhang, S. M. Rao, and Y.-J. Zhu, "GAS6 is an estrogen-inducible gene in mammary epithelial cells," *Biochemical and Biophysical Research Communications*, vol. 353, no. 1, pp. 189–194, 2007.

[39] B.-K. Son, M. Akishita, K. Iijima et al., "Androgen receptor-dependent transactivation of growth arrest-specific gene 6 mediates inhibitory effects of testosterone on vascular calcification," *Journal of Biological Chemistry*, vol. 285, no. 10, pp. 7537–7544, 2010.

[40] N. Abate, S. M. Haffner, A. Garg, R. M. Peshock, and S. M. Grundy, "Sex steroid hormones, upper body obesity, and insulin resistance," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 10, pp. 4522–4527, 2002.

[41] S. M. Haffner, "Sex hormones, obesity, fat distribution, type 2 diabetes and insulin resistance: epidemiological and clinical correlation," *International Journal of Obesity*, vol. 24, supplement 2, pp. S56–S58, 2000.