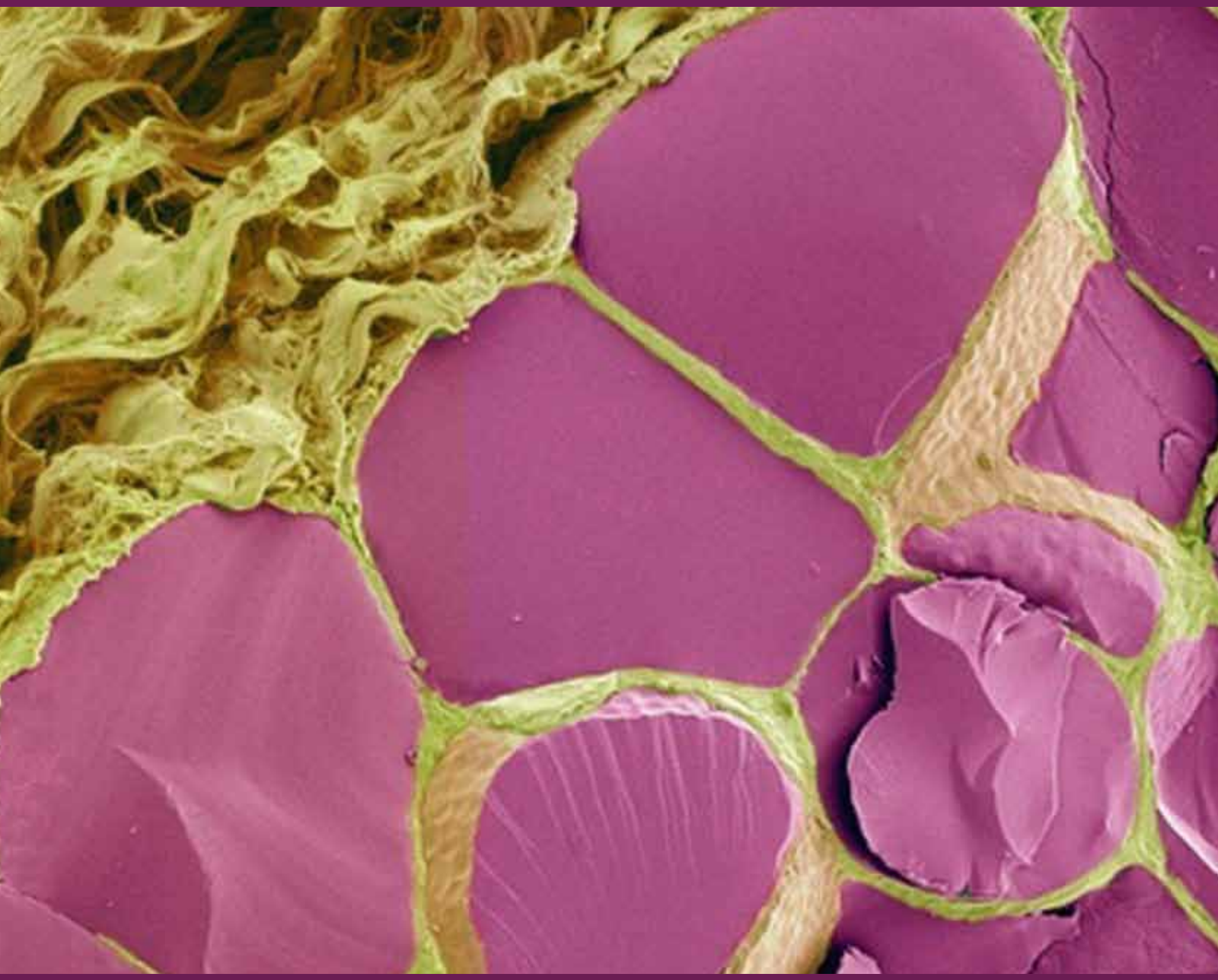
International Journal of Endocrinology

Obesity-Related Metabolic Syndrome and Vascular Complications

Guest Editors: Manfredi Tesauro, Micaela Iantorno, and Umberto Campia



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Editorial

Obesity-Related Metabolic Syndrome and Vascular Complications

Manfredi Tesauro,[1] Micaela Iantorno,[2] and Umberto Campia[3]

[1] *Dipartimento di Medicina dei Sistemi, Università di Tor Vergata, 00133 Rome, Italy*
[2] *Critical Care Medicine Department, National Institute of Health, Bethesda, MD 20852, USA*
[3] *MedStar Heart Institute and MedStar Cardiovascular Research Network, Washington, DC 20009, USA*

Correspondence should be addressed to Manfredi Tesauro; mtesauro@tiscali.it

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The epidemic of obesity, a condition defined as a body mass index (BMI) of 30.0 or more, has spread beyond the borders of high-income countries and is now dramatically on the rise also in low- and middle-income nations, particularly in urban settings [1]. One of the most concerning implications of this trend is the association between obesity and an increased risk of developing metabolic and cardiovascular disease in adults [2, 3] and in children [4–7]. Obesity, particularly when in a central body distribution, is frequently associated with the presence of the metabolic syndrome (MetS), a cluster of lipid abnormalities (i.e., elevated triglycerides and low HDL), hypertension, and impaired fasting glucose [8, 9]. Even if the MetS does not encompass risk factors that determine absolute risk (e.g., age, sex, cigarette smoking, and LDL cholesterol), it is associated with a two-fold higher risk of cardiovascular disease (CVD) at 5 to 10 years and a 5-fold increase in risk to develop type 2 diabetes mellitus (T2DM) [8]. However, the definition of MetS and the associated CVD risk have been validated for mostly white cohorts and they may not be valid for different populations [10].

Numerous pathophysiological mechanisms have been proposed to link central adiposity, MetS, T2DM, and cardiovascular risk. In particular, adipose tissue dysfunction, insulin resistance/hyperinsulinemia, sympathetic hyperactivity, and endothelial dysfunction have emerged as pivotal players [11]. Adipose tissue dysfunction is characterized by the presence of an inflammatory infiltrate, adipokine dysregulation, and release of inflammatory cytokines, which exert deleterious vascular effects in a systemic and paracrine fashion [12]. In turn, the abnormal vascular function may reduce skeletal muscle perfusion, thus worsening insulin sensitivity and establishing a self-perpetuating vascular-metabolic vicious cycle. However, our knowledge of the pathophysiology of vascular damage in obesity is still incomplete. A better mechanistic understanding could have important clinical implications, leading to the development of novel therapeutic strategies to reduce metabolic and cardiovascular risk in obesity. In this special issue, we have selected six papers investigating various aspects of the association between obesity-related metabolic syndrome and vascular complications.

To address the scarcity of studies that have examined the relation between obesity and cardiometabolic risk factors (CRFs) in population-based samples of children and adolescents, T. Lyngdoh et al. assessed the association between BMI, measured both at the age of 12–15 years (longitudinal analysis) and at the present time (cross sectional analysis), and several CRFs in 390 young adults aged 19-20 years in Seychelles (Indian Ocean, Africa). In this cohort, CRFs were strongly predicted by BMI levels at both ages. However, only BMI at age 19-20 years remained a strong predictor of CRFs in a regression model including both BMI at age 12–15 years and BMI at age 19-20 years. These data suggest that current BMI is a strong predictor of CRFs and indicate that weight control even at a later age may be effective in reducing CRFs in irrespective of past weight status.

Similar to other advanced countries, the prevalence of obesity in Turkey is higher than 30%. However, no population-specific cutoffs for waist circumference (WC) to use within the definition of MetS have been established. Therefore, A. Sonmez et al. investigated whether the World Health Organization's (WHO) sex specific WC cut-off points for the

Caucasian adults are appropriate for the Turkish adult population. Using a sample of over 4,200 Turkish men and women, the authors determined that the most appropriate WC cut-off levels to diagnose MetS are 90 cm and 80 cm, respectively, and suggest that these cut-off values should be used in Turkey and in areas with large Turkish immigrant populations.

One of the well-recognized abnormalities in obesity is the hyperactivity of the sympathetic nervous system (SNS). As detailed in the review by M. P. Canale et al., the SNS is activated by multiple features present in obese individuals, including insulin resistance and adipokine unbalance. Importantly, chronic SNS overactivity contributes to a further decline of insulin sensitivity and creates a vicious circle that may contribute to the development of vascular damage and CVD. Importantly, preliminary evidence appears to suggest that renal denervation may be a potential therapy to treat sympathetic hyperactivity and its effects on blood pressure and metabolism in obese individuals.

A variable amount of adipose tissue can be found around most vessels. Until recently, the vascular effects of perivascular adipose tissue- (PVAD-) derived factors on vascular function had remained mostly unrecognized. The structural and functional changes observed in the PVAD of obese individuals and their impact on vascular function are reviewed in detail by M. S. Fernández-Alfonso et al. As noted by these authors, further research is necessary to better understand the role of PVAT in the pathogenesis of CVD in obesity and MetS.

Recent evidence suggests that inhibition of the renin angiotensin system (RAS) may reduce the risk of T2DM. To understand the potential mechanisms underlying this observation, Z. Zhang et al. investigated the effects of angiotensin II type 1 receptor (AT1R) knockdown on glucose-stimulated insulin secretion in isolated islets of db/db mice. Their results suggest that intraislet AT1R is a physiological regulator of insulin sensitivity in β cell and a potential therapeutic target for the prevention of T2DM.

Finally, extensive calcification of the vessel wall is one of the notable features of vascular remodeling in patients with T2DM and end stage renal disease (ESRD). Given the high prevalence of impaired glucose metabolism and diabetes in patients with ESRD, K. Janda et al. investigated the association between a number of clinical and metabolic parameters and radial artery calcification in patients with ESRD. In their study population, impaired fasting glucose and T2DM were significant predictors of vascular calcification, suggesting an independent contribution of abnormal glucose metabolism in the vascular remodeling of patients with ESRD.

These papers represent a very small sample of the research work in the field. However, we are confident that they will convey to the readers the breadth of the ongoing epidemiological, clinical, and laboratory efforts to unravel the pathogenetic mechanisms underlying the association between obesity, MetS, T2DM, and cardiovascular disease.

Manfredi Tesauro
Micaela Iantorno
Umberto Campia

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Research Article

Impaired Fasting Glucose and Diabetes as Predictors for Radial Artery Calcification in End Stage Renal Disease Patients

Katarzyna Janda,[1] Marcin Krzanowski,[1] Mariusz Gajda,[2] Paulina Dumnicka,[3] Danuta Fedak,[4] Grzegorz J. Lis,[2] Piotr Jaśkowski,[1] Jan A. Litwin,[2] and Władysław Sułowicz[1]

[1] *Chair and Department of Nephrology, Jagiellonian University Medical College, 31-501 Krakow, Poland*
[2] *Chair and Department of Histology, Jagiellonian University Medical College, 31-501 Krakow, Poland*
[3] *Department of Medical Diagnostics, Jagiellonian University Medical College, 31-501 Krakow, Poland*
[4] *Chair of Clinical Biochemistry, Jagiellonian University Medical College, 31-501 Krakow, Poland*

Correspondence should be addressed to Władysław Sułowicz; wladsul@mp.pl

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Objective. The objective of the study was to assess the relationship between selected clinical and biochemical parameters of end stage renal disease (ESRD) patients and arterial calcification. *Materials and Methods.* The study comprised 59 stage 5 chronic kidney disease patients (36 hemodialyzed and 23 predialysis). The examined parameters included common carotid artery intima-media thickness (CCA-IMT), BMI, incidence of diabetes and impaired fasting glucose (IFG), dyslipidemia, hypertension, and 3-year mortality. Plasma levels asymmetric dimethylarginine (ADMA), osteopontin (OPN), osteoprotegerin (OPG), and osteocalcin (OC) were also measured. Fragments of radial artery obtained during creation of hemodialysis access were stained for calcifications using von Kossa method and alizarin red. *Results.* Calcification of radial artery was significantly associated with higher prevalence of IFG and diabetes ($P = 0.0004$) and older age ($P = 0.003$), as well as higher OPG ($P = 0.014$) and ADMA concentrations ($P = 0.022$). Fasting glucose > 5.6 mmol/l (IFG and diabetes) significantly predicted vascular calcification in multiple logistic regression. The calcification was also associated with higher CCA-IMT ($P = 0.006$) and mortality ($P = 0.004$; OR for death 5.39 [1.20–24.1] after adjustment for dialysis status and age). *Conclusion.* Combination of renal insufficiency and hyperglycemic conditions exerts a synergistic effect on vascular calcification and increases the risk of death.

1. Introduction

Vascular calcification is an active process similar to the mineralization that occurs in bone [1, 2]. Vascular smooth muscle cells undergo phenotypic differentiation into osteoblast-like or chondroblast-like cells and they synthesize calcification regulating proteins and matrix components typically found in bone and in cartilage [3, 4]. Calcification of the arterial media, observed even in small vessels (Mönckeberg's calcification), is common in uremic patients and seems to be less associated with inflammation as compared to intimal mineralization typical for atherosclerosis [5, 6].

Vascular mineralization advances with age and is intensified in diabetes, dyslipidemia, chronic kidney disease, and hypertension. In newly treated hemodialysis and peritoneal dialysis patients, diabetes, dialysis duration, and the previous presence of aortic arch calcification (AAC) accelerate further progression of AAC (an important risk factor for cardiovascular complications) [7]. Hyperinsulinemia and insulin resistance (clinical signs of type 2 diabetes) are positively correlated with the arterial calcification [8, 9]. Arterial medial calcifications often occur also in diabetic individuals as a component of the diabetic macroangiopathy. In the animal model, insulin resistance induced in rats by fructose feeding resulted in increased aortic calcium deposition, elevated calcium-phosphate index, and local hyperplastic changes in the aortic media [10].

Blood vessels obtained from end stage renal disease (ESRD) patients were often studied by various histological techniques to assess vascular calcification. They were

collected during renal transplantation (epigastric or iliac arteries) or by peripheral arterial biopsy (radial artery) [3, 11, 12].

In the present study we used small samples (otherwise routinely discarded) of radial arterial walls obtained during creation of arteriovenous fistula for hemodialysis access. The study was aimed at investigating the relationship between selected clinical and biochemical parameters, with special emphasis on diabetes markers, and the level of histologically assessed radial artery calcification in end stage renal disease patients.

2. Materials and Methods

2.1. Patients. The study population consisted of 59 patients (38 males, 21 female; mean age at the beginning of the study 61 ± 16 yrs), including 36 on maintenance hemodialysis (HD) and 23 on predialysis (stage 5 of CKD). The study was approved by the Bioethics Committee of the Jagiellonian University and all patients signed an informed consent for their participation. The data on mortality was collected over a period of three years. During this period, all the patients were treated at the Department of Nephrology, Jagiellonian University Hospital. The mortality data, including the causes of death, was based on the patients' records.

2.2. Laboratory Tests. In all patients, selected biochemical parameters were assessed: serum concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (TG), serum creatinine, peripheral blood counts, albumin, glucose, intact parathyroid hormone (iPTH), total calcium (Ca) and phosphate (Pi), high sensitive C-reactive protein (hsCRP), asymmetric dimethylarginine (ADMA), osteopontin (OPN), osteoprotegerin (OPG), and osteocalcin (OC). Homeostasis Model of Assessment-Insulin Resistance (HOMA-IR) was calculated by application of the international formula: fasting insulin (μIU/mL) × fasting glucose (mmol/L)/22.5 [13].

Routine biochemical tests were carried out using automatic biochemical analyzers: Hitachi 917 (Hitachi, Japan) and Modular P (Roche Diagnostics, Mannheim, Germany). Concentrations of hsCRP were measured using immunonephelometric method on Nephelometer BN II (Siemens Healthcare Diagnostics, Germany). Hematological parameters were measured using Sysmex XE 2100 Hematological Analyzer (Sysmex Corp., Japan).

OPN, OC, OPG, and ADMA were determined using ELISA microplate immunoassays and ELX808 automatic reader (BIO-TEK Instruments, Inc., Vermont, USA). The following kits of reagents were applied: OPN (R&D Systems), OC (METRA, Germany), OPG (QUIDEL, BioVendor, Czech Republic), and ADMA (Immundiagnostik, Germany).

The mean arterial pressure (MAP) was calculated according to the formula: MAP = DBP + 1/3(SBP-DBP), where SBP is systolic blood pressure and DBP is diastolic blood pressure.

The intima-media thickness of the common carotid artery trunk (CCA-IMT) was assessed by ultrasonography (B presentation, Acuson 128 XP/10 apparatus equipped with a linear head at 5/7 MHz). The measurements were performed bilaterally at 0.5 cm and 2 cm below the division of the common carotid artery during diastolic phase of the heart cycle. The results were expressed as the arithmetic means of the values obtained for the left and right arteries.

2.3. Histology. Fragments of radial artery, approx. 5 × 2 mm in size, were collected during the first creation of arteriovenous fistula for hemodialysis access. The samples were immediately immersed in 10% phosphate-buffered formalin and fixed overnight and then rinsed in PBS and soaked in 30% sucrose. The material was snap-frozen and tissue blocks were positioned in a cryostat for cutting sections in a plane encompassing the entire thickness of the vascular wall. Serial 10 μm-thick cryosections were cut and thaw-mounted on poly-L-lysine coated slides. Sections were stained routinely with Mayer's haematoxylin and eosin (HE), with von Kossa method and with alizarin red. The stained sections were examined using an Olympus BX-50 microscope (Olympus, Tokyo, Japan) in brightfield mode and images were registered using Olympus DP-71 digital CCD camera controlled by Olympus AnalySIS FIVE software. The advancement of vascular calcification was semiquantitatively evaluated in von Kossa and alizarin red-stained sections by two independent observers. The degree of mineralization was classified according to the following scale: 0: no mineral content, 1: a few small dispersed concretions, 2: numerous small dispersed concretions, 3: larger granular concretions, and 4: large areas occupied by fused mineral deposits. The reproducibility of the morphological analysis was confirmed by Bland-Altman method and by calculating intraclass correlation coefficient (ICC) which was 0.88.

2.4. Statistical Methods. The number of patients (percentage of the group) was reported for categorical variables and mean ± standard deviation or median (lower-upper quartile) for continuous variables, depending on distribution. The Shapiro-Wilk test was used to assess normality. Contingency tables were analyzed with Pearson chi-squared test. Student t-test or Mann-Whitney test was used for simple comparisons between the groups. Multiple logistic regression models were constructed using the variables that differed significantly between the groups in simple comparisons and/or predefined sets of confounders, as pointed in the results. Odds ratios (OR) for 1 unit increase with 95% confidence intervals (95% CI) being reported, unless otherwise stated. All tests were two-tailed and the results were considered statistically significant at $P \leq 0.05$. Statistica 10 software (StatSoft, Tulsa, OK, USA) was used for computations.

3. Results

3.1. Histology. Routine histology (HE) showed general morphology of the radial artery, with intimal thickening in the vast majority of the examined specimens (Figure 1(a)). Basophilic deposits were visible in arterial wall in cases of very intense calcification (Figure 1(b)). The preliminary comparison of two staining methods used for the assessment

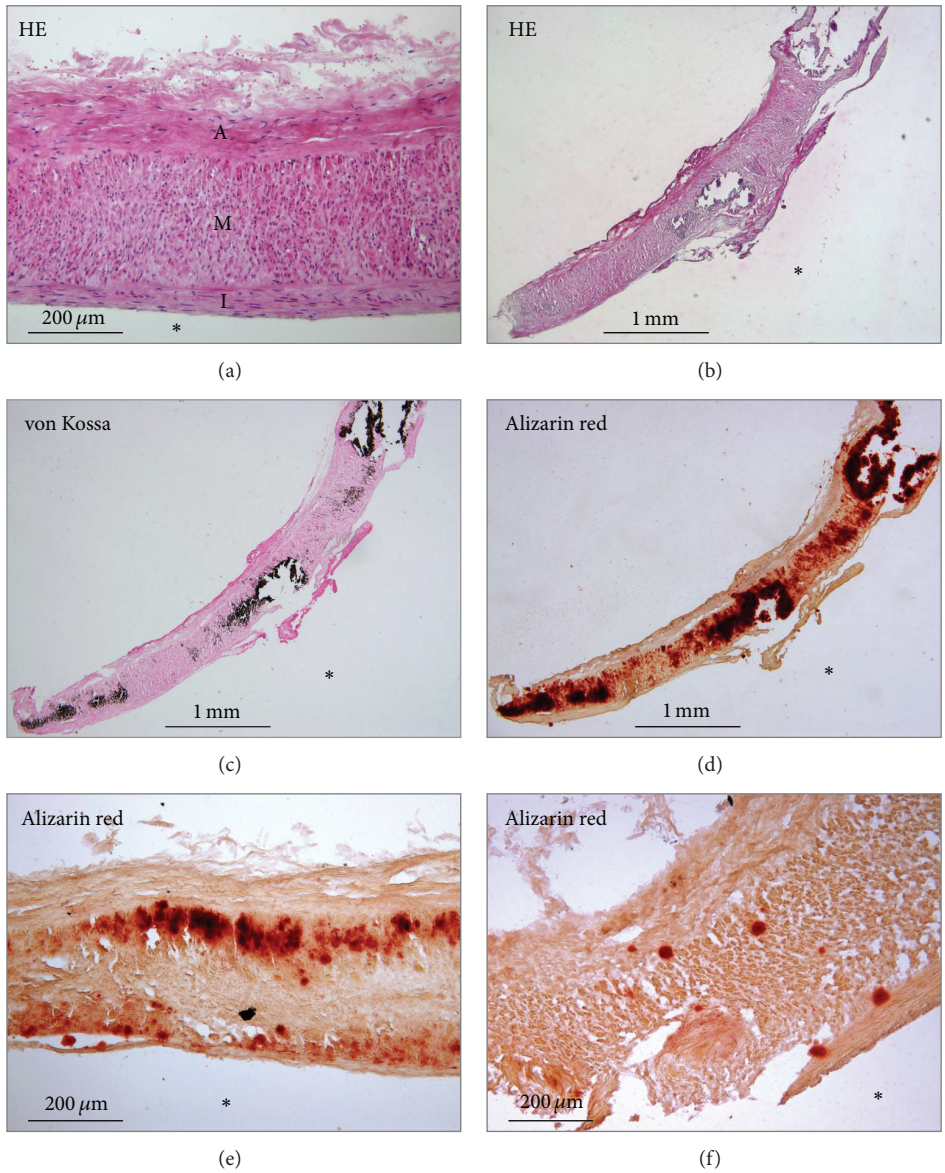

Figure 1: Histology of the radial artery samples. (a) Morphology of the routinely (HE) stained artery showing intimal thickening (I) and no mineral content. I: intima; M: media; A: adventitia. (b)–(d) Serial sections of the entire sample of the arterial wall demonstrating very advanced calcification (grade 4) stained with HE (b), von Kossa (c), and alizarin red (d). (e) Large granular calcifications (grade 3) localized on both sides of the media (alizarin red staining). (f) Small (grade 2) calcifications in the media (alizarin red staining). *Lumen of the vessel.

of the mineralization degree showed that von Kossa method was less sensitive; thus we employed alizarin red staining for further analysis and for correlation of the vascular calcification level with clinical and biochemical data.

Morphologically, mineral deposits were found in all layers of the arterial wall but they were most frequently localized in the media (Figures 1(b)–1(d)). In less advanced lesions, deposits were preferentially located close to the outer and inner elastic laminae (Figure 1(e)). Some deposits were fine and dispersed (Figure 1(f)), while others occupied larger areas and in the most advanced cases even the entire thickness of the media (Figures 1(b)–1(d)). Only very scanty mineral deposits were occasionally seen in the vascular intima.

Among 59 radial artery samples examined histologically, 34 showed positive alizarin red staining indicative of the calcification process. The proportion of samples with arterial wall mineralization (Table 1) as well as its advancement (Figure 2(a)) did not differ between HD and predialysis patients ($P = 0.6$). In further analysis, HD patients were studied together with predialysis patients; nevertheless all multiple regression models were adjusted for HD status.

3.2. Biochemical and Clinical Data. Table 1 summarizes differences in clinical and biochemical parameters between the groups with and without calcifications as assessed by alizarin

Table 1: Differences in clinical parameters between patients with and without vascular calcifications as assessed by alizarin red staining of the radial artery.

	Patients with vascular calcifications (grades 1–4, $N = 34$)	Patients without vascular calcifications (grades 0, $N = 25$)	P
Male gender, N (%)	23 (68%)	15 (60%)	NS
HD treatment, N (%)	21 (62%)	15 (60%)	NS
HD duration, months[a]	10 (3–36)	6 (1–38)	NS
Hemoglobin, g/dL	10.7 ± 1.6	11.5 ± 1.9	NS
Albumin, g/L	40.3 ± 4.2	41.5 ± 6.1	NS
Age, years	66 ± 15	54 ± 14	0.003
Active smoking, N (%)	10 (29%)	7 (28%)	NS
BMI, kg/m^2	26.1 ± 5.6	26.4 ± 6.1	NS
Diabetes, N (%)	18 (53%)	1 (4%)	<0.0001
Type 1 diabetes, N (%)	2 (6%)	0	NS
Type 2 diabetes, N (%)	16 (47%)	1 (4%)	0.0002
IFG, N (%)	3 (9%)	3 (12%)	NS
SBP, mmHg	138 ± 19	146 ± 18	NS
DBP, mmHg	82 ± 9	86 ± 11	NS
MAP, mmHg	101 ± 12	106 ± 12	0.045
Fasting glucose, mmol/L	5.7 (4.9–7.9)	4.8 (4.6–5.2)	0.022
Insulin, μU/mL[b]	7.60 (5.85–18.89)	9.88 (6.12–13.50)	NS
HOMA-IR[b]	1.68 (1.20–4.44)	1.89 (1.32–3.12)	NS
CRP, mg/L	8.81 (2.19–24.3)	4.86 (3.06–9.82)	NS
ADMA, μmol/L	0.86 ± 0.22	0.72 ± 0.16	0.022
Ca, mmol/L	2.18 ± 0.16	2.25 ± 0.27	NS
Pi, mmol/L	1.50 (1.34–1.79)	1.41 (1.21–1.86)	NS
Ca × Pi, mmol^2/L^2	3.15 (2.88–3.77)	3.51 (2.86–3.91)	NS
iPTH, pg/mL	213 (179–512)	290 (230–428)	NS
OPG, pmol/L	9.36 (5.93–12.38)	5.10 (2.40–7.70)	0.014
OPN, ng/mL	310 (208–559)	304 (217–377)	NS
OC, ng/mL	41.7 (31.7–69.5)	42.0 (23.9–56.0)	NS
CCA-IMT, mm	0.98 ± 0.13	0.86 ± 0.14	0.006
All-cause mortality, N (%)	16 (47%)	3 (12%)	0.004
Cardiovascular mortality, N (%)	13 (38%)	3 (12%)	0.025

[a]Data for the group of HD patients only (21 patients with calcifications and 15 patients without calcifications).
[b]Data for nondiabetic patients only (16 patients with calcifications and 24 patients without calcifications).

red staining of radial artery. Vascular calcification was associated with higher age of patients, higher glucose, and diabetes. MAP was slightly lower in patients with calcifications and the levels of ADMA and OPG were higher in this group.

Clinical criteria of the metabolic syndrome [14, 15] were compared between patients with and without vascular calcifications in radial artery (Table 2). Among patients with vascular calcifications, the number of individuals with fasting glucose level above 5.6 mmol/L, that is, patients with IFG (prediabetes) [16] and diabetes, was significantly higher ($P = 0.0004$). Moreover, vascular calcifications were more severe in the group of patients with IFG and diabetes (Figure 2(b)). Other criteria of the metabolic syndrome did not differ between the groups with or without calcifications.

The association of radial artery calcifications with IFG and diabetes was further confirmed by multiple logistic regression (Table 3). Three models were constructed, containing age and fasting glucose > 5.6 mmol/L as independent variables: the first one was adjusted for gender, HD status, and Ca × Pi product, and the second was additionally adjusted for dyslipidemia, hypertension, high BMI, and hsCRP. The third model, adjusted as the first one, included other variables that were significantly associated with arterial calcification in simple comparisons, that is, MAP, ADMA, and OPG. Fasting glucose > 5.6 mmol/L was the only variable independently associated with the vascular calcifications in all three models. The results were similar when diabetes was substituted for increased fasting glucose level in the models: OR = 17.2 (1.79–166); $P = 0.011$ in model 1; OR = 25.4 (1.74–372); $P = 0.015$ in model 2; OR = 24.6 (1.45–419); $P = 0.022$ in model 3.

Patients with calcifications in radial arteries presented higher CCA-IMT (Table 1). The relation between CCA-IMT

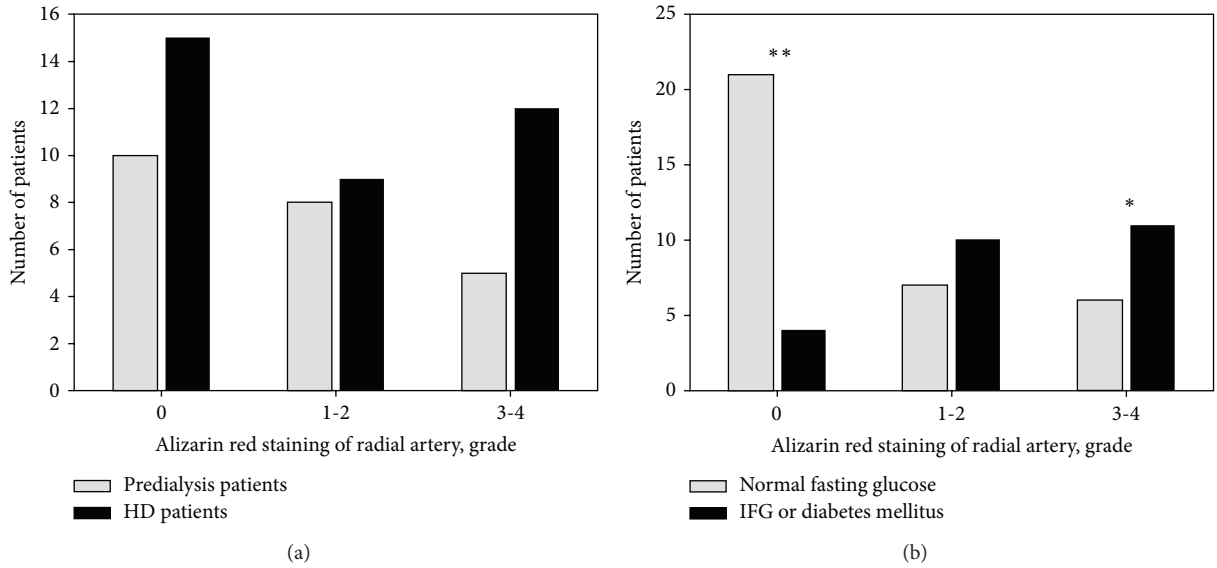

Figure 2: Advancement of vascular calcification as assessed by alizarin red staining in radial arteries: 0: no mineral deposits in arterial wall, 1-2: mild calcification, and 3-4: advanced calcification. (a) Calcification in predialysis patients versus HD patients ($P = 0.6$ in chi-squared test). (b) Calcification in patients with normal fasting glucose (<5.6 mmol/L) versus IFG (prediabetes) or diabetes ($P = 0.002$ in chi-squared test; $P = 0.004$ (**) and $P = 0.03$ (*) in post hoc tests). IFG: impaired fasting glucose.

and histologically detected vascular calcification remained significant after adjustment for dialysis status, gender, Ca × Pi, hsCRP, and the presence of metabolic syndrome (OR for vascular calcification 2.31 per 0.1 mm increase in CCA-IMT; 95% CI 1.18–4.52; $P = 0.011$) but not after adjustment for age. CCA-IMT was significantly higher in patients with IFG and diabetes (0.98 ± 0.13 versus 0.88 ± 0.15 mm; $P = 0.007$).

Sixteen patients with calcifications in the radial artery (47%) died during 3 years of the followup, while in the group without calcifications the mortality was lower: 3 deaths (12%). All except 3 deaths occurred due to cardiovascular causes (Table 1). Vascular calcification was significantly associated with patients' mortality in simple analysis ($P = 0.004$) and after adjustment for HD status and age (OR for death 5.39; 95% CI 1.20–24.1; $P = 0.024$).

4. Discussion

This study presents a comprehensive comparison of biochemical and clinical data with the calcification status assessed histologically in the peripheral arteries of ESRD patients. Biopsies of radial artery collected during the creation of vascular access for hemodialysis were used previously by other authors to study calcification. However, in that study, the surgical anastomosis was performed in an end-to-end fashion; thus a sample encompassing the entire circumference of the artery could be excised and used for further analysis, allowing for a more reliable assessment of the calcification extent in the arterial wall [12].

In the study mentioned above, the authors found mineral deposits in 37% of the examined radial arteries. After adopting stringent morphological criteria to include even the finest calcifications and using more sensitive alizarin red staining for calcium detection we found arterial calcification in 57% of cases.

Calcification can develop in two distinct layers of the artery: intima and media [17]. Intimal calcification occurs in advanced atherosclerotic lesions and is associated with lipid accumulation and infiltration of the inflammatory cells, such as macrophages and T cells. Medial arterial calcification (MAC) displays features very similar to those of physiological calcification in bone [18]. MAC develops independently of atherosclerosis and is a commonly observed pathology in diabetes, ESRD, and ageing [17].

The present study revealed a significant association of arterial medial calcifications with impaired fasting glucose (IFG, prediabetes) and diabetes but not with other criteria of the metabolic syndrome including overweight. Our results are in accordance with the study of Lim et al. [19] demonstrating the relationship between anthropometric parameters, metabolic profiles, and coronary artery calcium scoring (CACS). Subjects with IFG or diabetes had higher CACS and more advanced coronary stenosis than normal subjects. Moreover, several studies confirmed that fasting plasma glucose is a better independent determinant of the progression of coronary artery calcification than the other metabolic syndrome risk factors [20–22].

The above mentioned papers presented a relationship between hyperglycemia and vascular calcification based on noninvasive imaging of blood vessels. In our study, this relationship was analyzed for the first time using histologically examined samples of peripheral arteries.

Hyperglycemia is an established risk factor for cardiovascular disease. Our study showed that fasting hyperglycemia, mostly associated with type 2 diabetes, was the only significant predictor of vascular calcifications in ESRD patients. Consistently, type 2 diabetes was associated with more severe

Table 2: Differences in clinical criteria of the metabolic syndrome between patients with and without vascular calcifications as assessed by alizarin red staining of the radial artery.

	Patients with vascular calcifications (grades 1–4, $N = 34$)	Patients without vascular calcifications (grades 0, $N = 25$)	P
BMI > 25 kg/m^2 (overweight or obesity), N (%)	18 (53%)	14 (56%)	NS
Fasting glucose > 5.6 mmol/L (IFG or diabetes), N (%)	21 (62%)	4 (16%)	0.0004
Hypertension, N (%)	28 (82%)	23 (92%)	NS
Low HDL[a], N (%)	13 (38%)	7 (28%)	NS
High TG (>1.7 mmol/L), N (%)	15 (44%)	12 (48%)	NS
Three or more of above criteria present, N (%)	20 (59%)	9 (36%)	NS

[a]Low HDL cholesterol (<1.0 mmol/L in men, <1.3 mmol/L in women).

Table 3: Multiple logistic regression models to study the associations of the selected variables with vascular calcifications as assessed by alizarin red staining of the radial artery.

	Model 1[a]		Model 2[b]		Model 3[a]	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age, years	1.05 (0.99–1.10)	NS	1.05 (0.99–1.11)	NS	1.00 (0.93–1.08)	NS
Fasting glucose > 5.6 mmol/L[c]	8.24 (1.66–40.9)	**0.008**	14.8 (1.68–130)	**0.012**	23.8 (1.84–309)	**0.012**
MAP, mmHg	—	—	—	—	0.93 (0.86–1.02)	NS
ADMA, 0.1 μmol/L	—	—	—	—	1.66 (0.70–3.92)	NS
OPG, pmol/L	—	—	—	—	1.16 (0.92–1.45)	NS

Odds ratios for positive staining are presented.
[a]Adjusted for gender, dialysis status of patients, and Ca × Pi.
[b]Adjusted for the parameters of the metabolic syndrome: low HDL cholesterol (<1.0 mmol/L in men, <1.3 mmol/L in women), high triglycerides (>1.7 mmol/L), and high BMI (≥25 kg/m^2), as well as hypertension, CRP, gender, dialysis status of patients, and Ca × Pi.
[c]Includes patients with IFG and patients with diabetes.
Bold font: statically significant data.

calcification. Recent evidence suggests that medial calcification in diabetes is an active, cell-mediated process, similar to that observed in patients with end stage renal disease [23, 24].

Vascular calcifications and atherosclerosis are frequent in patients with ESRD and they are associated with increased cardiovascular morbidity [25]. Coronary artery calcification (CAC) was found in 70.2% of dialysis patients and was significantly associated with CCA-IMT and the thickness of atherosclerotic plaques [26]. These results indicate that both, medial calcification and atherosclerotic lesions, frequently coexist in patients with ESRD and that CCA-IMT, increased in patients with calcifications examined in this study, may serve as a surrogate marker of vascular calcification.

The mechanisms responsible for vascular calcification include inflammation and oxidative stress, as well as bone and mineral metabolism disturbances. In our study higher ADMA and OPG levels were associated with vascular calcification. High ADMA levels are associated with endothelial dysfunction and cardiovascular damage [27]. Serum levels of ADMA in chronic kidney disease increase due to its defective inactivation and excretion. Coen at al. [28] postulated that ADMA may play a role in the pathogenesis of vascular calcification in dialysis patients. Increased serum OPG is associated with type 2 diabetes, chronic kidney disease, and the severity of vascular calcification and coronary artery disease [29–31]. It could represent a compensatory mechanism for vascular damage, also showing a protective effect against vascular calcification [32, 33].

Arterial calcification was associated with higher mortality in ESRD patients. According to our knowledge, the relationship between vascular calcification assessed histologically and the long-term mortality in chronic kidney disease patients has not yet been studied. Ogawa et al. [34] examined the effect of CT-assessed aortic arch calcification on mortality in the 401 hemodialysis patients during 4-year follow-up period and demonstrated that cardiovascular mortality was significantly higher in patients with calcification. In our study, employing a different assessment model (radial artery and histology) this effect of arterial calcification on mortality in ESRD patients has been confirmed.

5. Conclusions

Small samples of radial artery obtained during creation of vascular access for hemodialysis may successfully serve as source of the material for histological assessment of vascular mineralization. In end stage renal disease patients, impaired fasting glucose (prediabetes) and diabetes predict vascular calcification which is significantly associated with higher mortality. These results indicate that combination of

renal insufficiency and hyperglycemic conditions exerts a synergistic effect on vascular calcification and increases the risk of death.

Conflict of Interests

The authors have no conflict of interests to declare.

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Clinical Study

Waist Circumference Cutoff Points to Predict Obesity, Metabolic Syndrome, and Cardiovascular Risk in Turkish Adults

Alper Sonmez,[1] Fahri Bayram,[2] Cem Barcin,[3] Muge Ozsan,[4] Ahmet Kaya,[5] and Vedia Gedik[6]

[1] *Department of Endocrinology and Metabolism, School of Medicine, Gulhane Military Medical Academy, 06018 Etlik Ankara, Turkey*
[2] *Department of Endocrinology and Metabolism, School of Medicine, Erciyes University, Talas, 38039 Kayseri, Turkey*
[3] *Department of Cardiology, School of Medicine, Gulhane Military Medical Academy, 06018 Etlik Ankara, Turkey*
[4] *Department of Endocrinology and Metabolism, Nigde State Hospital, 51100 Nigde, Turkey*
[5] *Department of Endocrinology and Metabolism, School of Medicine, Necmettin Erbakan University, Meram, 42080 Konya, Turkey*
[6] *Department of Endocrinology and Metabolism, School of Medicine, Ankara University, Sıhhıye, 060100 Ankara, Turkey*

Correspondence should be addressed to Alper Sonmez; alpersonmez@yahoo.com

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Objective. The waist circumference (WC) cutoff levels defined for the Caucasian people may not be representative for different ethnic groups. We determined sex specific WC cutoff points to predict obesity, metabolic syndrome, and cardiovascular risk in Turkish adults. *Design and Methods*. The demographic characteristics of 1898 adult males and 2308 nonpregnant females from 24 provinces of 7 different regions of Turkey (mean age 47 ± 14 yrs) were evaluated. *Results*. The WC levels of 90 cm and 100 cm define overweight and obese males while the levels of 80 cm and 90 cm define overweight and obese females. With these cutoff values, 239 additional males (12.6%) are diagnosed as overweight and 148 additional males (7.8%) as obese. Instead, 120 females (5.1%) are free of being labeled as obese. *Conclusions*. This is the first nationwide study to show the action levels of WC for overweight and obese Turkish adults. The ideal cutoff levels of WC to predict metabolic syndrome are 90 cm and 80 cm for Turkish adult men and women, respectively. These values are easy to implement and suggested to be used by the physicians dealing with cardiometabolic disorders in Turkey.

1. Introduction

Obesity is a global epidemic affecting more than one third of the adult population in Turkey [1, 2] as in the rest of the world [3–5]. Although the rise in obesity prevalence brings about a significantly increased mortality risk [6, 7], it is not practical to manage all the obese subjects with a global program. Therefore, subjects with the significantly increased risk of morbidity and mortality should be identified and prioritized for any intervention. For this, an appropriate and accurate measure of obesity should be at hand.

The body mass index (BMI) is a convenient and widespread measure of obesity. However, it is just a crude proportion, which does not take into account the amount of abdominal fat mass [8]. Waist circumference (WC), on the other hand, is an easy and reliable measure of visceral adipose tissue and a simple index of cardiovascular risk [9, 10]. The World Health Organization (WHO) reported sex specific WC cutoff values for the Caucasian people, named as action levels 1 and 2, to define the overweight or obese people [8]. These cutoff values, originally established in a Dutch population [11, 12], were later adopted by several medical organizations in order to define Metabolic Syndrome [13, 14]. However, these cutoff levels may not necessarily represent the characteristics of the other populations. Therefore, it is recommended that the sex specific WC cutoff points should be established for different ethnic groups [8, 13].

Many reports have been published so far to describe the WC cutoff points of different populations. However, most of these reports show discrepant results even within the same

ethnic groups [15, 16]. The main reason of the discrepancies is the use of different methodologies to establish the WC cutoff points. Likewise, different reports show different WC cutoff points in Turkish adult men and women [17–20]. Also, the WC cutoff values do not define overweight and obesity in these studies by using the criteria in the reference studies [11, 12] adored by WHO [8]. Therefore, we aimed to investigate whether the suggested sex specific WC cutoff points for the Caucasian adults are appropriate for the Turkish adult population. The secondary aim of the study is to search for better WC cutoff points to predict obesity, metabolic syndrome, and increased cardiovascular risk in Turkish adult men and women.

2. Methods and Procedures

2.1. Study Population and Sampling. This cross-sectional study was conducted by the *Obesity Hypertension and Lipid Study Group of the Turkish Society of Endocrinology and Metabolism* in 24 provinces from the 7 regions of Turkey. The detailed description of the study protocol is given elsewhere [1]. The multistage probability sampling design was used to select the cases. In brief, Turkish adult males and nonpregnant females were randomly enrolled from both the provincial district centers and villages, considering the demographics, economic, social, and geographical statuses. Approval was obtained from the Ethical Committee of the Ministry of Health and the household identification form (HIF) data were obtained from the Primary Health Care Centers of the Provincial Health Directorates affiliated to the Ministry of Health. The study was employed in accordance with the Declaration of Helsinki. The informed consents were obtained from all participants, before the enrolment.

At least 3 provinces were selected from each region by a random sampling method. The populations of these 7 regions were obtained from the records of the 2000 census. The study sample included males and nonpregnant females aged between 20 and 83 years. The populations of city centers, districts, and villages were classified by using the stratified sampling method and then were selected from the HIF data by a random sampling method. The geography of Turkey was classified into three groups according to altitude. Sea level was accepted as zero. 0–300 m was taken as coastal, 300–900 m as moderate elevation and 900 m and above as high elevation.

The medical histories and demographical data of the participants were obtained and structured questionnaires were completed with face-to-face interviews. The personal and family histories of hypertension, diabetes mellitus, cardiovascular diseases, and other chronic diseases were obtained. Heights and weights of participants were measured. When the subjects were weighed, they were asked to take off their shoes and any other belongings that could possibly add extra weight. BMI was calculated by dividing the weight (in kg) by the height in meters squared. WC of the participants were measured at the level of the iliac processes and the umbilicus with a soft tape measure to evaluate abdominal obesity. The same definitions for overweight and obese people as in the original Dutch study [8] were used. Accordingly, the participants with either the BMI $\geq 25\,kg/m^2$ or BMI $< 25\,kg/m^2$ but WHR > 0.95 in men or >0.80 in women were defined as overweight. Subjects with the BMI $\geq 30\,kg/m^2$ in both sexes were considered obese. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice in the sitting position, with an interval of 15 min between the measurements, after a rest period for 30 min. The mean of these two measurements obtained by a standard sphygmomanometer was recorded as final value of blood pressure. The participants whose SBP $\geq$ 130 mmHg and/or DBP $\geq$ 90 mmHg as well as the ones who were on antihypertensive medications were diagnosed to have hypertension. Metabolic syndrome was diagnosed as the presence of at least 3 of the following parameters, according to Adult Treatment Panel III criteria: abdominal obesity (WC > 102 cm for males and >88 cm for females), hypertension (SBP > 130 mmHg and/or DBP > 85 mmHg) or history of antihypertensive usage, hypertriglyceridemia (≥150 mg/dL) or presence of treatment for this disorder, low HDL-C (<40 mg/dL in males and <50 mg/dL in females), and high fasting plasma glucose (≥100 mg/dL) or presence of diagnosis of type 2 diabetes [14].

The venous blood samples were taken between 7:00 and 10:00 a.m after an overnight fasting. Blood samples were centrifuged at room temperature for 10 min at 3000 rpm and the sera were stored in ice bags and placed into deep freezers at −70°C on the same day. Glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels were measured by the enzymatic spectrophotometric method with the Kone Lab Auto Analyzer (Thermo Clinical Labsystems Oy Vantaa, Finland). Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald formula (in those with a triglyceride level of below 400 mg/dL). Diabetes mellitus was diagnosed according to the American Diabetes Association (ADA) criteria. Accordingly, single fasting blood glucose of above 126 mg/dL was considered to be the evidence of diabetes mellitus. Those with a previous diagnosis of diabetes mellitus and using oral antidiabetics and/or insulin were also considered as diabetics. Impaired fasting glucose was defined as fasting blood glucose levels between 100 and 126 mg/dL.

2.1.1. Statistical Analysis. Continuous variables were shown as mean ± SD. and categorical variables as percentage. Student's t-test was used to compare continuous variables.

Action level 1 and 2 were calculated as WC values to predict overweight and obese individuals, respectively. Receiver operating curve analysis was used to calculate sensitivity and specificity. The level of WC where the sum of the sensitivity and specificity is the highest was considered as the cutoff point.

Positive prediction for the Metabolic Syndrome was calculated as the percentage of subjects with a WC above the action level who have two or more risk factors other than WC criterion (considering the individual would have at least 3 of 5 metabolic syndrome criteria after adding the WC). Negative prediction for the Metabolic Syndrome was calculated as the percentage of subjects with a WC below the action level and with two or less Metabolic syndrome risk factors.

All statistical analyses were calculated using SPSS version 15.0 (Chicago, IL). Two-tailed P-values of <0.05 were considered to be statistically significant.

3. Results

The demographic characteristics of the study population are given in Table 1. According to the data, 37.3% of the adult population is overweight and 36% is obese. The prevalence of obesity in Turkish women (42.5%) was significantly higher than that of the Turkish men (28.0%) ($P < 0.001$). On the other hand, the male population had significantly higher number of smokers, higher serum triglyceride and total cholesterol, and lower HDL cholesterol levels ($P < 0.001$ for all). The plot graphs giving the distribution of the WC levels for females and males related to BMI divided by the proposed action levels are given in Figure 1.

The sensitivity and specificity of the WC cutoff levels recommended by the WHO and the levels calculated from the Turkish population in the present study are given in Figure 2. The cutoff level for the action level 1 to predict the overweight men is calculated as 90 cm, and the cutoff level for the action level 2 to predict the obese men is calculated as 100 cm. These cutoff values for the Turkish males, are lower than those proposed by the WHO (94 cm and 102 cm resp.) and have higher sensitivities and lower specificities. On the other hand, the cutoff level for the action level 1 to predict the overweight women is the same with the level proposed by the WHO (80 cm). But, the cutoff level for the action level 2 to predict obese Turkish women (90 cm) is higher than that of the level of the WHO (88 cm). Consequently, the action level 2 to detect obese Turkish women has decreased sensitivity and increased specificity when compared to the level proposed by the WHO (Figure 2).

The positive and negative predictive values of these cutoff values for the diagnosis of metabolic syndrome are mentioned in Table 2. These cutoff levels are also calculated for each of the components of metabolic syndrome and are given in Table 2.

The 10-year Framingham cardiovascular risk ratios of the participants categorized according to different WC cutoff values are given in Table 3. The 10-year cardiovascular risk ratios are significantly different in each category, either calculated according to the WHO criteria or according to the proposed cutoff levels in this study. When the WC levels of 90 cm and 100 cm are used instead of 94 cm and 102 cm for the diagnosis of action levels of the male population, 239 additional subjects (12.6%) are detected as overweight and 148 additional subjects (7.8%) are diagnosed as obese. Moreover, when the WC level of 90 cm is used instead of 88 cm for the diagnosis of action level 2 in the female population, 120 subjects (5.1%) can be free of being labeled as obese.

4. Discussion

The WC cutoff levels calculated in this study are not the same but rather close to those levels recommended for the Caucasian adults [8] or the values established in the previous

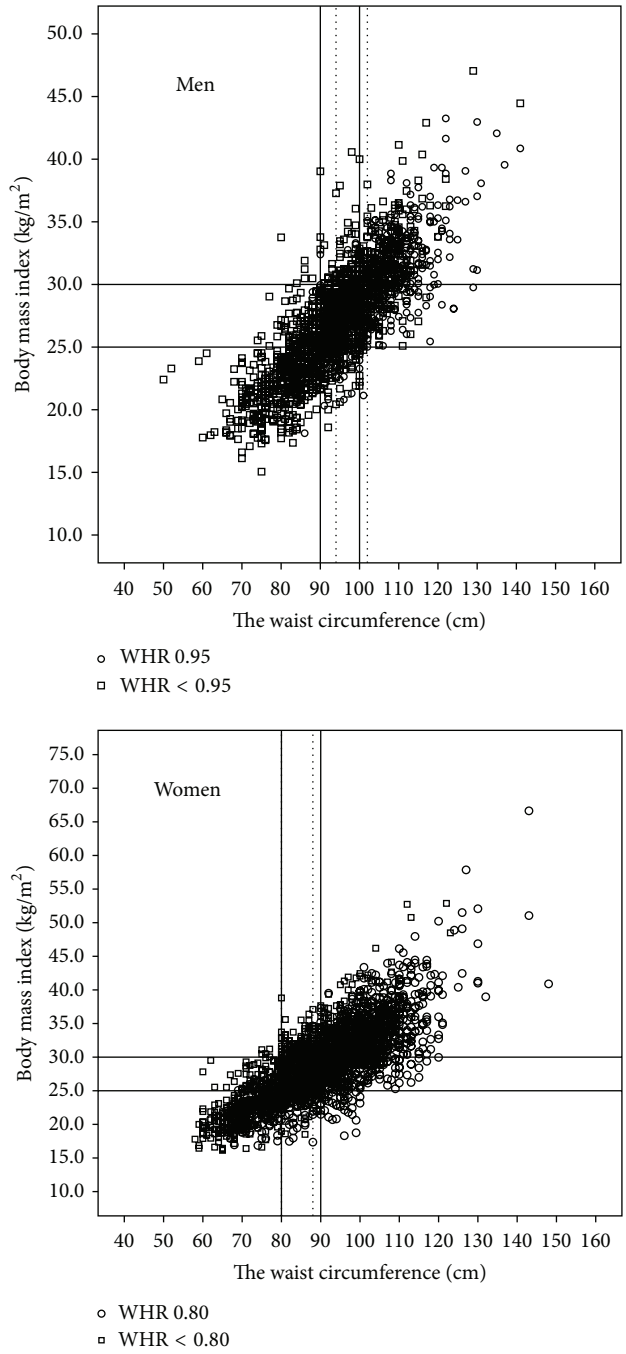

Figure 1: The relationship of waist circumference (WC) and body mass index in Turkish adults and cutoff values of WC both recommended by World Health Organization and calculated in the present study. The relation between WC levels and BMI in men and women and the two action levels of WC that identify subjects with BMI $\geq$ 25 kg/m^2 or $\geq$30 kg/m^2 and with WHR $\geq$ 0.95 for men and $\geq$0.80 for women. Dotted lines show action levels 1 and 2 recommended by the WHO. Solid lines show the action levels determined by the present study. $\circ$ indicates the individuals with WHR $\geq$ 0.95 (in men) and $\geq$0.80 (in women); false negative in upper left quadrant. $\square$ indicates the individuals with WHR < 0.95 for men and <0.80 for women; false positive in lower right quadrant. Linear Regression: BMI = (WC $\times$ 0.297) $-$ 0.975 in men; BMI = (WC $\times$ 0.338) $-$ 1.32 in women.

Table 1: The demographic characteristics of the Turkish adult population.

	General population (n = 4206)	Males (n = 1898)	Females (n = 2308)	P
Age [years]	47.0 ± 14.8	48.9 ± 14.6	45.3 ± 14.7	<**0.001**
SBP [mm Hg]	133.1 ± 26.4	132.5 ± 25.6	133.5 ± 27.1	0.24
DBP [mm Hg]	81.2 ± 14.8	81.0 ± 15.0	81.4 ± 14.7	0.44
BMI [kg/m^2]	28.4 ± 5.2	27.4 ± 4.4	29.1 ± 5.7	<**0.001**
WC [cm]	92.6 ± 12.8	95.5 ± 11.8	90.3 ± 13.1	<**0.001**
WHR	0.87 ± 0.9	0.91 ± 0.07	0.83 ± 0.08	<**0.001**
Fasting BG [mg/dL]	103.1 ± 45.2	102.8 ± 42.0	104.7 ± 47.6	0.19
Triglycerides [mg/dL]	145.2 ± 96.9	152.6 ± 104.0	139.2 ± 90.1	<**0.001**
Total chol. [mg/dL]	194.7 ± 47.5	191.7 ± 47.3	197.1 ± 47.6	<**0.001**
HDL-C [mg/dL]	49.7 ± 15.3	46.2 ± 14.4	52.6 ± 15.4	<**0.001**
LDL-C [mg/dL]	117.7 ± 41.0	117.1 ± 40.7	118.1 ± 41.2	0.45
Smoking [n (%)]	1024 (24.3)	749 (39.5)	275 (11.9)	<**0.001***
Overweight [n (%)]	1569 (37.3)	797 (42.0)	772 (33.4)	<**0.001**
Obesity [n (%)]	1513 (36.0)	531 (28.0)	982 (42.5)	<**0.001**

The comparisons were done by independent samples t test. *Chi-Square test. SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, WC: Waist circumference, WHR: Waist to hip ratio, fasting BG: Fasting blood glucose. The results are given as mean ± SD.

Table 2: The positive and negative predictive values of different waist circumference cutoff points for metabolic syndrome and its individual components.

	Males								Females					
	Action Level 1				Action Level 2				Action level 1*		Action level 2			
WC cutoff levels (cm)	WHO (94)		Turkish (90)		WHO (102)		Turkish (98)		WHO/Turkish (80)		WHO (88)		Turkish (90)	
Predictive values (%)	P	N	P	N	P	N	P	N	P	N	P	N	P	N
Metabolic syndrome	64	87	62	91	70	82	69	84	62	92	67	86	69	85
Hyperglycemia	39	72	38	74	44	70	43	71	37	82	39	76	40	76
Hypertriglyceridemia	46	73	44	77	49	67	47	68	38	85	42	80	43	79
Low HDL	39	67	39	69	40	65	40	66	49	62	51	59	52	60
Hypertension	68	53	67	60	73	47	71	48	70	68	74	56	75	54

P: Positive predictive value, N: Negative predictive value.
*The cut off levels for the action level 1 to predict the overweight women according to the WHO or Turkish criteria are the same (80 cm).

Table 3: The 10-year Framingham cardiovascular risk ratios of the Turkish adults, calculated according to the different waist circumference categories using the thresholds proposed by the world health organization and the data of the present study.

	The values for the male population (n = 1898)				The values for the female population (n = 2308)			
	Cutoff (WHO)	10 years risk ratio	Cutoff (Turkish)	10 years risk ratio	Cutoff (WHO)	10 years risk ratio	Cutoff (Turkish)	10 years risk ratio
Normal	≤94 cm (n = 783) (41.8%)	9.0 ± 9.3	≤90 cm (n = 544) (28.7%)	7.6 ± 8.2	≤80 cm (n = 465) (20.1%)	2.5 ± 3.7	≤80 cm (n = 465) (20.1%)	2.5 ± 3.7
Overweight	94–102 cm (n = 551) (29.0%)	12.8 ± 11.4	90–100 cm (n = 642) (33.8%)	12.1 ± 10.7	80–88 cm (n = 470) (20.3%)	5.2 ± 6.5	80–90 cm (n = 590) (25.6%)	5.4 ± 6.4
Obese	≥102 cm (n = 564) (29.7%)	15.1 ± 12.2	≥100 cm (n = 712) (37.5%)	15.1 ± 12.4	88 cm (n = 1373) (59.5%)	8.9 ± 8.3	≥90 cm (n = 1253) (54.3%)	9.1 ± 8.5
P^*	<0.001		<0.001		<0.001		<0.001	

*The comparisons of the 10 yrs risk ratios in each category, performed by the ANOVA.

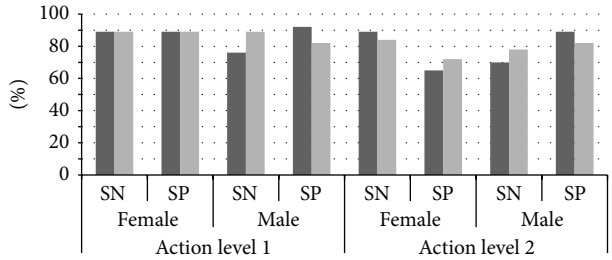

Figure 2: The sensitivity and specificity of the waist circumference cutoff levels recommended by the WHO and the levels calculated from the Turkish population. SN: sensitivity, SP: specificity, bold bars: the criteria of the WHO, The light Bars: The Criteria measured for the Turkish adult population.

reports for the Turkish adult population [17–20]. The cutoff levels, which we recommend according to the present study, are practical and have several advantages on the previously recommended values. By reducing the previously defined action levels of ≥94 cm and ≥102 cm to ≥90 cm and ≥100 cm for the Turkish adult men, 12.6% more overweight subjects and 7.8% more obese subjects can be detected. Again, by taking the action level 2 as 90 cm instead of 88 cm defined for the Caucasian women, 5.1% of Turkish women can be free of being labeled as obese. The results also show that the cutoff values of 80 cm for the females and 90 cm for the males are appropriate for the diagnosis of Metabolic Syndrome in this specific population. The reasons for the discrepancies from the previous reports and the clinical implications of the present findings are addressed below.

Visceral adipose tissue is no longer regarded as a depot but an active endocrine organ coordinating a variety of biological processes including energy metabolism, neuroendocrine, and immune functions [21]. WC is an easy and reliable surrogate marker of the visceral adipose tissue mass and a simple index of cardiovascular risk [9, 10]. Visceral obesity, determined by increased WC, is a significant risk for the cardiovascular morbidity and mortality and is one of the diagnostic criteria of the Metabolic Syndrome [13]. However, it is well reported that distinct ethnic groups may have significantly different visceral adipose tissue distributions and different cardiometabolic risk profiles [16, 22, 23]. Therefore, the identification of risk by using WC is population specific and depends on levels of obesity and other risk factors for cardiovascular disease and type 2 diabetes mellitus [8]. The WC cut points generally recommended for the Caucasian adults [8, 13] are inferred from a Dutch population [11, 12]. The demographic characteristics of the Dutch people are not likely to represent those of the Turkish adults. Therefore, the WC cutoff values, established in this study, should be used to better estimate the presence of metabolic syndrome and the risk of cardiovascular diseases.

Many research papers have been published so far in order to report specific WC cutoff points in different populations. However, the results of these studies significantly differ, even within the same ethnic groups [15, 16]. Using different methods to measure WC and taking distinct health outcome measures to establish cutoff levels are among the reasons of the discrepancies between the studies. The widely accepted sex specific WC cutoff values for the Caucasian adults were initially adopted by the WHO [8]. These cutoff values were originally calculated in a Dutch population to establish the overweight (BMI > 25 kg/m^2 or WHR > 0.95 in men or >0.80 in women) and obese (BMI > 30 kg/m^2 or WHR > 0.95 in men or >0.80 in women) adults [11]. Given the names of *"action level 1"* (means no further weight gain) and *"action level 2"* (*should reduce weight*), these cutoff values point out significant increases in the cardiovascular event risk [12]. The parameters to define the increased cardiovascular risk in the Dutch study were high blood pressure, high levels of total cholesterol and low levels of HDL cholesterol [12]. However, different outcome measures were used to calculate the cutoff values in the subsequent studies, including hyperglycemia (either impaired fasting glucose, impaired glucose tolerance, or diabetes mellitus), high LDL cholesterol, Triglycerides, Metabolic syndrome, Coronary heart disease, cardiovascular disease, and overall mortality [15]. Using different outcome measures in calculation, for sure causes different results for the cutoff values. Another significant reason for the discrepant cutoff values is the use of different methods of optimizing sensitivity and specificity [15]. Published studies have either taken the WC where the sum of the sensitivity and the specificity is the highest or the point where the sensitivity and the specificity are nearly equal. Some authors on the other hand, have used the point on the ROC curve where the distance to the upper left corner is the shortest. Of note, the method regarding to this issue was not given in some studies. In our study we have chosen the cutoff values, where the sum of sensitivity and specificity is the highest. This detection limit allowed us to cover an optimal number of overweight or obese adults when compared to the current criteria recommended for the Caucasian adults.

Few studies reported sex specific WC cutoff levels of the Turkish adults [17–20]. All these studies used different methods to establish the cutoff limits. A study reported the WC cutoff levels for the prediction of the increased insulin resistance, as 93 cm for men and 83 cm for women. These thresholds, however, do not label any specific BMI limit or any significant increase in the cardiovascular risk [19] Onat et al. mentioned distinct WC action levels as 87 cm and 95 cm for the Turkish men [17] and 83 cm and 91 cm for the Turkish women [18]. These action levels do not show any specific BMI or WHR limits, but point out to increased risk of dyslipidemia, hypertension and Metabolic Syndrome in action 1 and the increased risk of coronary heart disease and type 2 diabetes in action 2. It is for sure, important to establish cutoff levels to predict the increased risk of metabolic disorders and coronary heart diseases. However, in order to compare the demographic properties and the cardiometabolic risk states of the different populations, the action levels should be defined by using similar criteria. Therefore, the cutoff levels in our study were calculated by using the methods of the original studies [11, 12]. Namely, the action level 1 in our study defines the point of BMI > 25 kg/m^2

or WHR > 0.95 in men or >0.80 in women. The action level 2 on the other hand defines the BMI > 30 kg/m^2. Although one study has used the above criteria to establish WC cutoff limits so far, this study was performed in an obesity outpatient clinic of a university hospital and reported only the WC cutoff points for women in Turkey [20]. Still and interestingly, the action levels 1 and 2 for the women participated in this study were 81 cm and 90 cm, respectively, very similar to our results.

The WC cutoff levels, given in the present study, are appropriate to identify the overweight and obese Turkish adults and have reasonable power to establish people with increased cardiometabolic risk. The Framingham risk ratios of the subjects below the action level 1 significantly disperses from those above the action level 2. Moreover, the high negative predictive values for the diagnosis of metabolic syndrome indicate that it is very unlikely to have metabolic syndrome if the WC of a Turkish adult is below the action level 1. Therefore, it would be wiser to take the cutoff values 90 cm and 80 cm for action level 1 for the diagnosis of Metabolic Syndrome in Turkish men and women. Although calculated by different methods, these cutoff values are somewhat close to the previously reported cut points for the Turkish men [17, 19] and women [18–20]. Using the action levels given in our study has two significant advantages. Firstly these levels reflect the cutoff values of the Turkish adult population, calculated by the generally recognized methods. Secondly, these cutoff levels are very easy to recall and implement which would be practical for the family physicians and internists dealing with obesity.

This study however has several limitations. Due to the cross sectional design, the action levels only give us the risk estimates, not the outcomes. Therefore, using the cutoff points inferred from the outcome studies may still be advantageous. Another limitation may be the substantially low 10-year cardiovascular event risk calculated according to Framingham risk score. Even the subjects having WC above the action level 2 have moderate calculated cardiovascular risk. Because the Turkish population is regarded to have high cardiovascular risk, the validity of the Framingham risk score to predict hard clinical end points in Turkish population may be questioned.

In conclusion, this is the first nationwide study to show the action levels of WC to predict overweight and obese Turkish adults using the same criteria of the original Dutch study. These levels, however, are not very much different from the previous values recommended for the Caucasian men and women. The WC cutoff levels to diagnose metabolic syndrome can be established as 90 cm and 80 cm for Turkish adult men and women respectively. Finally, it would be pragmatic for the physicians to accept these cutoff values in Turkey, and in areas with large Turkish immigrant populations.

Conflict of Interests

There is no potential conflict of interests relevant to this paper.

Authors' Contribution

Alper Sonmez wrote the paper and researched data, Fahri Bayram designed the study and edited the paper, Cem Barcin reviewed the data, made the statistical analyses, and contributed to discussion. Muge Ozsan performed the field research. Ahmet Kaya and Vedia Gedik contributed to discussion, reviewed the manuscript.

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Research Article

Improved Glucose-Stimulated Insulin Secretion by Selective Intraislet Inhibition of Angiotensin II Type 1 Receptor Expression in Isolated Islets of db/db Mice

Zhen Zhang,[1] Chunyan Liu,[1] Zhenhua Gan,[1] Xinyi Wang,[1] Qiuyan Yi,[1] Yanqing Liu,[1] Yingzhijie Wang,[1] Bin Lu,[1] Hong Du,[1] Jiaqing Shao,[1] and Jun Wang[2]

[1] *Department of Endocrinology, Jinling Hospital, Southern Medical University, 305 Zhongshan East Road, Nanjing, Jiangsu Province 210002, China*
[2] *Department of Cardiology, Jinling Hospital, Southern Medical University, 305 Zhongshan East Road, Nanjing, Jiangsu Province 210002, China*

Correspondence should be addressed to Jiaqing Shao; shaojiaq@hotmail.com and Jun Wang; zero991127@hotmail.com

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Recent evidence supported the presence of a local renin-angiotensin system (RAS) in the pancreas, which is implicated in many physiological and pathophysiological processes. We utilized small interfering RNA (siRNA) to investigate the effects of angiotensin II type 1 receptor (AT1R) knockdown on glucose-stimulated insulin secretion (GSIS) in isolated islets of db/db mice and to explore the potential mechanisms involved. We found that Ad-siAT1R treatment resulted in a significant decrease both in AT1R mRNA level and in AT1R protein expression level. With downexpression of AT1R, notable increased insulin secretion and decreased glucagon secretion levels were found by perifusion. Simultaneously, significant increased protein levels of IRS-1 (by 85%), IRS-2 (by 95%), PI3K(85) (by 112.5%), and p-Akt2 (by 164%) were found by western blot. And upregulation of both GLUT-2 (by 190%) and GCK (by 121%) was achieved after AT1R inhibition by Ad-siAT1R. Intraislet AT1R expression level is a crucial physiological regulator of insulin sensitivity of β cell itself and thus affects glucose-induced insulin and glucagon release. Therefore, the characteristics of AT1R inhibitors could make it a potential novel therapeutics for prevention and treatment of type 2 diabetes.

1. Introduction

Recent decades have seen a dramatic rise in the incidence and prevalence of type 2 diabetes mellitus (T2DM) throughout the world. The damage of pancreatic islet function plays a crucial role in the pathogenesis and progression of T2DM. However, current treatment of T2DM can not provide effective protection against islet failure. Interestingly, recent clinical researches had shown that RAS blockade by ACE inhibitor (ACEI) or angiotensin receptor blocker (ARB) could reduce the onset of diabetes in people at high risk by 14%–34% [1–4]. The mechanisms underlying this protective effect appear to be complex and may involve improvement of both insulin sensitivity and insulin secretion. However, the detailed mechanisms are still unknown.

RAS components, such as angiotensinogen (AGT), angiotensin converting enzyme (ACE), angiotensin II (AngII) and type 1 and type 2 angiotensin II receptors (AT1R and AT2R), had been found in islets [5, 6]. Evidence suggests that the local pancreatic islet RAS performs multifactorial activities in structure and function of islet, including cell proliferation, apoptosis, oxidative stress, inflammatory responses, and glucose-stimulated insulin secretion, and these regulatory functions are probably mediated via AT1R [7]. Such a local islet RAS is subject to overactivation by diabetes and thus drives islet fibrosis and reduces islet blood flow, oxygen tension, and insulin biosynthesis. Kampf et al. demonstrated that endogenous levels of Ang II exerted detrimental effects on islet blood perfusion in transplanted mouse islets [8]. Moreover, overactivation of an islet RAS may accelerate the synthesis of reactive oxygen species, aggravate oxidative stress-induced β-cell dysfunction, and apoptosis and thus contribute to the islet failure seen in type 2 diabetes [9]. Our

previous studies found that candesartan treatment could improve glucose tolerance with the preservation of β-cell mass and morphology in db/db mice [10, 11]. However, it is unclear whether or not these benefits are dependent on the changes of circulating RAS components. So selective inhibition of AT1R expression in islet could reveal the definite role of intraislet RAS in glucose homeostasis.

The insulin receptors and their substrates (IRS-1 and IRS-2) have been proved to be expressed in the β-cell [12–14]. By the insulin signaling pathway, these molecules could impact GSIS of β-cell [15, 16]. Accumulated data have demonstrated that local RAS expressed in peripheral tissues is overactivated in the state of insulin resistance, and the effects of RAS blockade on insulin resistance have been proved [17, 18]. As there is evidence for insulin signaling molecules expression in islets itself and because RAS is implicated in the pathogenesis of insulin resistance, it is possible that local RAS expressed in islets may affect GSIS through insulin signaling pathway of β-cell.

In the present study, we aim to explore whether intrinsic RAS in islet is involved in glucose-stimulated insulin secretion by affecting insulin sensitivity of β-cell itself via RNA interference technique which can inhibit the expression of intraislet AT1R effectively and specifically.

2. Materials and Methods

2.1. Islet Isolation and Culture. Five eight-week old female db/db mice and five age and gender matched nondiabetic littermates db/m mice were obtained from the Animal Experiment Center of Jingling Hospital. Mice were anesthetized with pentobarbital (Nembutal, Abbot Laboratories). After clamping the common bile duct at a point close to the duodenal outlet, pancreas was injected through the pancreatic duct with 2 mL Krebs-Ringer bicarbonate buffer (KRBB: 129 mmol/L NaCl, 5 mmol/L NaHCO3, 4.8 mmol/L KCl, 1.2 mmol/L KH2PO4, 1.2 mmol/L MgSO4, 0.2% BSA, 10 mmol/L Hepes, 2.5 mmol/L CaCl2, and 2.8 mmol/L glucose at pH 7.4) containing 1.5 mg/mL of collagenase (Worthington, Biochemical Co., St. Louis, Missouri). The swollen pancreas was removed and incubated at 37°C for 40 min. The digested pancreas was shaken and washed with ice-cold HBSS four times, and islets were handpicked under a stereomicroscope cultured in RPMI 1640 medium at 37°C in a humidified atmosphere (5% CO2, 95% air). For both batch incubation and perifusion studies, the islets were pre-incubated for 30 min in KRBB (1.4 mmol/L glucose) at 37°C, 5% CO2, and saturated humidity.

2.2. Isolation of Total RNA and Real-Time Reverse Transcription PCR of AT1R. Total RNA was extracted from islets by the TRIzol reagent according to the manufacturer's protocol (Invitrogen). For quantitative real-time PCR, the first strand cDNA was synthesized from 300 ng of total RNA using the oligo (dT) primer and MMLV reverse transcriptase (Invitrogen). Samples were subjected to quantitative amplification using the TaqMan probe and primer sets for mice AT1R. PCR amplification was performed in a total volume of 10 μL containing 30 ng of cDNA, 900 nM of each primer, 250 nM of the respective probe, and 6 μL of Taq Man Universal PCR Master Mix. Real-time PCRs (95°C for 15 s, 55°C for 20 s, and 72°C for 20 s × 35 cycles) were performed in an ABI-Prism 7700 sequence detector system (Applied Biosystems, Foster City, CA). Different cDNA samples were normalized using primer sets to the housekeeping gene β-actin. Primers were as follows: AT1R, 5′-AGCTACAACAAGGCAAGG-3′ and 3′-TAGAAGGCACAGTCGAGG-5′; β-actin, 5′-TGTTGTCCCTGTATGCCTCTGGTC-3′ and 3′-ATGTCACGCACGATTTCCCTCTCA-5′. The fold changes were calculated by using the comparative threshold cycle method.

2.3. Cell Culture. INS-1 cells were grown in monolayer cultures in RPMI 1640 complete medium at 11.1 mmol/L glucose supplemented with 10% (w/v) fetal bovine serum, 10 mmol/L HEPES, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, and 50 μmol/L β-mercaptoethanol at 37°C in a humidified atmosphere (5% CO2, 95% air). For transfection experiments, the cells were seeded in 75-cm^2 flasks at 4×10^6 cells 2 days prior to transfection and were at 60–70% confluency at the time of the transfection.

2.4. Short Hairpin RNA-Mediated Gene Suppression. shRNAs directed against mice AR1R (GenBank accession number NM_030985) were designed according to Ambion (Austin, TX) siRNA design guidelines. Briefly, from 5′- to 3′-end, the whole length of shRNA was sequentially composed of the BamHI restriction site, the 19-nucleotide antisense gene-targeting sequence, TTT TTT ending transcription sequence, and HindIII restriction site. Three different shRNAs candidates (siAT1R1, siAT1R2, and siAT1R3) were designed and tested for their potency to decrease the targeted gene expression. A duplex with no known target (GAG ACC CTA TCC GTG ATT A) was used as control (siControl). All of the oligonucleotides were synthesized and purified. After annealing, the double-stranded oligonucleotides were ligated by self-formed restriction sites for BamHI and HindIII in pSilencer 2.0 vector (Ambion). The siRNAs were transfected into INS-1 cells using Lipofectamine PLUS at a concentration of 5 μg of DNA for 6×10^6 cells. Three days after transfection, INS-1 cells were harvested for RNA and protein. AT1R expression was confirmed by qRT-PCR and immunoblot analysis as described above. The most efficient one (siAT1R2) was chosen for subsequent experiments. Relative to the start codon, the 5′ ends of the target correspond to mice AT1R nucleotide 540 (GCGTCTTTCTTCTCAATCT).

2.5. Recombinant Adenovirus Construction. Recombinant adenoviruses containing the siATR12 (Ad-siATR1) or the siControl (Ad-siControl) sequences described above were constructed using AdEasy System. Briefly, for each pSilencer-based clone, the siRNA expression cassette was excised and ligated into linearized adenoviral shuttle vector pAdTrack. Subsequently, 1 μg recombinant PmeI-linearized pAd-siATR1 was transfected into *Escherichia coli* BJ5183 cells with an adenoviral backbone plasmid, pAdEasy-1. Recombinants were selected and successful recombination was determined by

restriction endonuclease analysis. The linearized recombinant adenoviral construct was transfected into 293 cells and high-titer viral stocks were prepared. Viral titers were determined by plaque assay and expressed as plaque-forming units per mL (pfu/mL). The viral titers of Ad-siATR1 and Ad-siControl were 3.6×10^9 pfu/mL and 2.9×10^9 pfu/mL, respectively.

2.6. Gene Silencing in Islets of Langerhans. Islets of db/db mice and db/m mice in aliquots of 50 islets per well of a six-well plate in 2 mL medium were divided into three groups: (1) Ad-siAT1R group, islets were treated with Ad-siAT1R for 20 h at 2,000 plaque-forming units/islet; (2) Ad-siControl group, islets were treated with Ad-siControl; (3) Control group, Mock transduced islets. Mock infected islets were not exposed to virus during the incubation period and were not incubated with any vectors. After removal of virus-containing medium, islets were cultured for an additional 72 h with medium changes every 24 h. GSIS was measured. Subsequently, islets were collected and lysed for analysis of AT1R expression as described above.

2.7. Islet Perifusion. Kinetics of insulin release in vitro was studied by the perifusion system. Size-matched 50 islets were placed in each column. Then the columns were gently closed with the top adaptors, immersed in vertical position and controlled temperature in the water bath at 37°C. The perifusion medium was maintained at 37°C in a water bath. And all columns were perfused in parallel at a flow rate of 0.5 mL/min with KRB (2.8 mmol/L glucose) at 37°C. After 60 min static incubation with KRB (2.8 mmol/L glucose), the islets were stimulated in the continuous presence of a high concentration of 16.7 mmol/L glucose. Samples were collected every 20 second until 2 min, every 1 min until 5 min, and thereafter every 5 min until 30 min. Samples were immediately stocked at −80°C until further analysis.

2.8. Western Blot Analysis of AT1R, IRS-1, IRS-2, PI3-K p85, p-Akt2, GLUT-2, and GCK in Islets. Isolated islets were dissolved in lysis buffer (25 mmol/L HEPES, 50 mmol/L KCl, 6% glycerol, 5 mmol/L EDTA, 5 mmol/L EGTA, 0.5% Triton-X100, 50 μmol/L NaF, 40 mmol/L glycerophosphate, and 25 mmol/L sodium pyrophosphate with proteinase inhibitors). Total protein was measured (BCA protein assay, Pierce, Rockford, IL), and 50 μg protein were fractionated by SDS-PAGE and electrophoretically transferred onto nitrocellulose membranes (Invitrogen). Membranes were incubated in blocking buffer (1TBS, 0.1% Tween 20, and 5% nonfat dry milk) for 1 h at room temperature. The following primary antibodies were used: rabbit anti-AT1R antibody, anti-insulin receptor substrate 1 antibody, anti-insulin receptor substrate 2 antibody, anti-PI3-kinase p85 α, anti-phospho-Akt2, anti-glucokinase antibody, anti-glucose transporter- 2 (GLUT-2) antibody, and anti-β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA). After three washes in TBS/0.1% Tween 20, the membranes were hybridized with a horseradish peroxidase-conjugated anti-rabbit immunoglobulin G prepared in goat (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at room temperature. After three washes in TBS/0.1% Tween 20, the bands were visualized by enhanced chemiluminescence (Super Signal West Femto; Pierce, Rockford, IL). The intensities of blots were quantified by scanning densitometry and normalized to the values for actin.

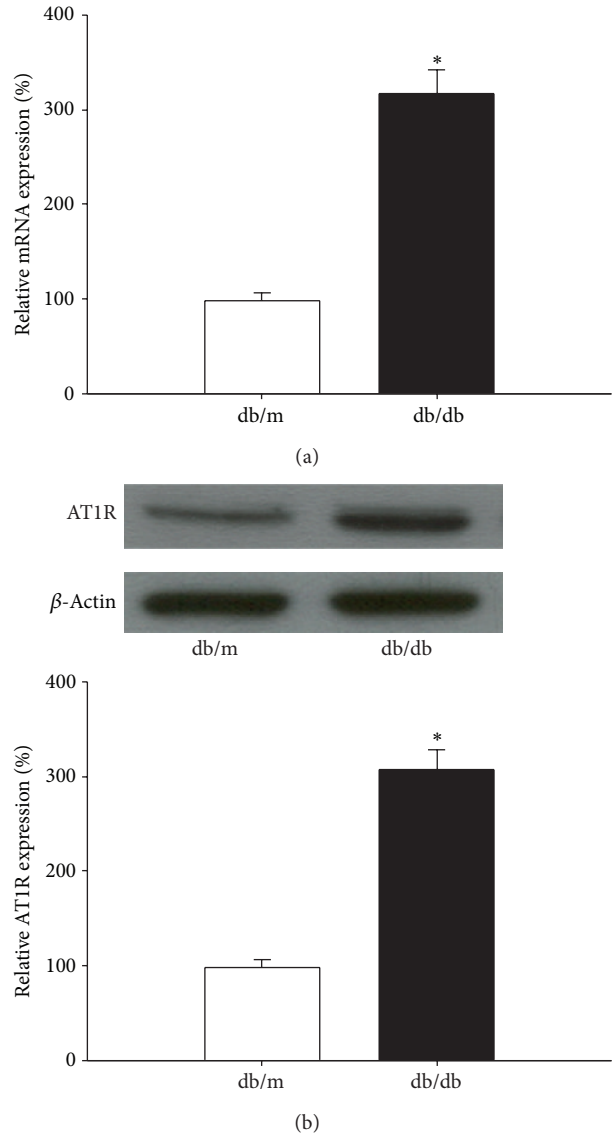

Figure 1: Expression in islet of AT1R in db/m mice and db/db mice. (a) Expression in islets of AT1R mRNA in db/m mice or db/db mice. Gene expression was measured by quantitative RT-PCR. Graphical presentation shows the relative AT1R mRNA abundance after normalization to actin. Data are presented as mean ± SD, $^*P < 0.05$ versus db/m group, $n = 5$. (b) Expression of AT1R protein in islets from db/m mice or db/db mice. Lysates from freshly isolated islets were analyzed for AT1R expression by western blot analysis. Results represent the mean ± SD. Blots from one representative experiments are shown. $^*P < 0.05$ versus db/m group, $n = 5$.

2.9. Statistical Analysis. Data are expressed as means ± SD. Statistical analysis was performed with SPSS XI. Data were

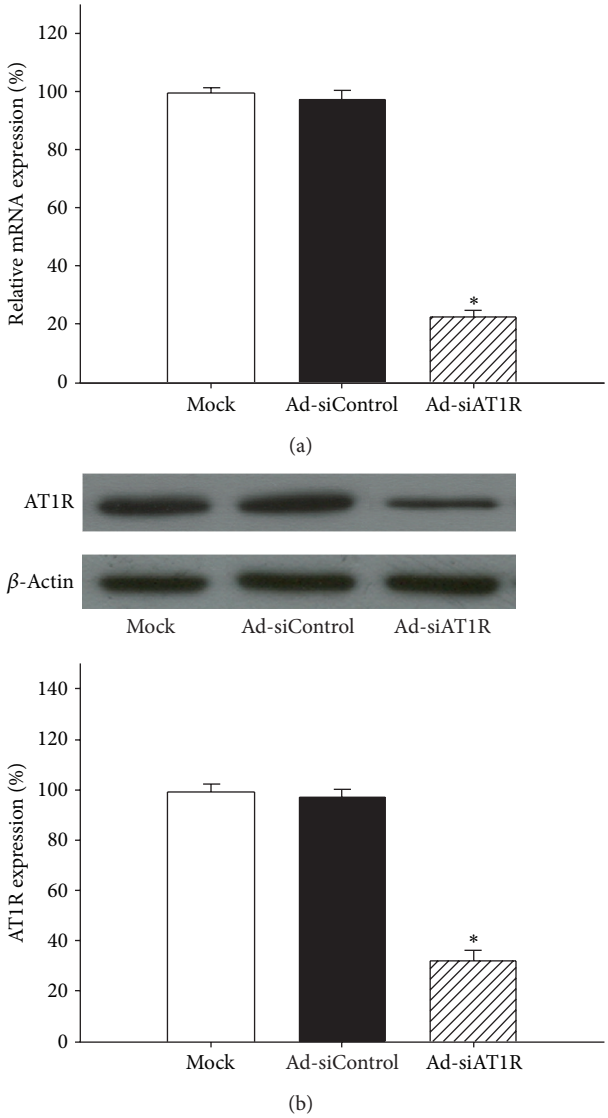

Figure 2: Effects of AT1R silencing on AT1R expression. (a) Islets of db/db mice were transfected with Ad-si AT1R, Ad-siControl, or Mock and cells were cultured for 72 h prior to quantitative RT-PCR. Results are represented as mean ± SD for six independent experiments. $^{*}P < 0.05$ versus Ad-siControl. (b) Islets of db/db mice were transfected with Ad-si AT1R, Ad-siControl, or Mock and cells were cultured for 72 h prior to quantitative immunoblot evaluation. Results are represented as mean ± SD for six independent experiments. $^{*}P < 0.05$ versus Ad-siControl.

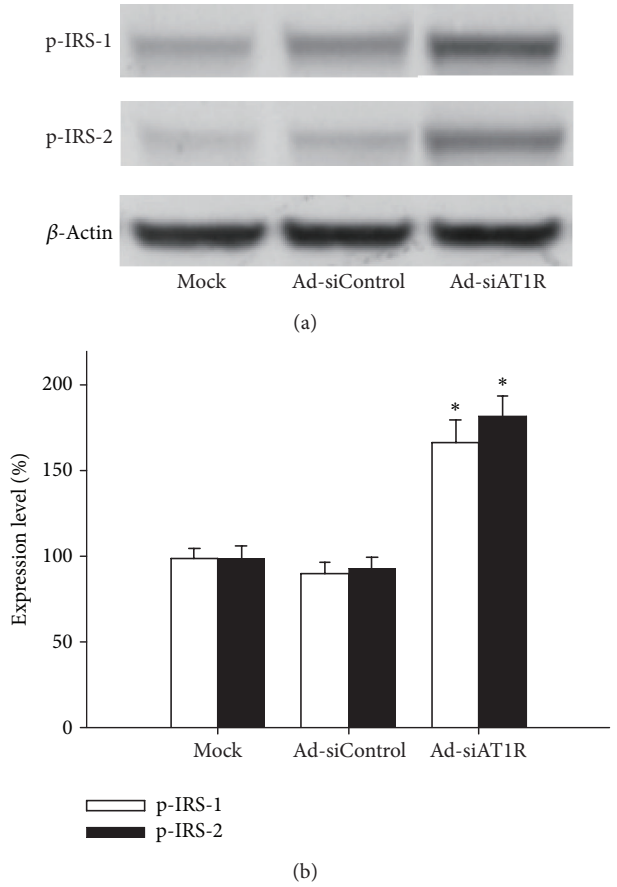

Figure 3: Effects of AT1R silencing on IRS-1 and IRS-2 expression. Islets of db/db mice were transfected with Ad-si AT1R, Ad-siControl, or Mock and cells were cultured for 72 h prior to quantitative immunoblot evaluation. Results are represented as mean ± SD for six independent experiments. $^{*}P < 0.05$ versus Ad-siControl.

grouped according to treatment and analyzed by an independent sample t-test or a one-way ANOVA. A value of $P < 0.05$ is considered statistically significant for all comparisons.

3. Results

3.1. AT1R Expression in Islets of db/db and db/m Mice. The expression level of AT1R, both in mRNA and in protein, in isolated islets of db/db mice was nearly two times higher than that of db/m mice ($P < 0.05$), indicating that AT1R was overexpressed in diabetic pancreatic islets (Figure 1).

3.2. Reduction of AT1R by Ad-shRNA-AT1R Treatment. The islets treated with Ad-siAT1R exhibited a 75% reduction in AT1R mRNA compared with ones treated with Ad-siControl ($P < 0.05$). Moreover, immunoblot analysis demonstrated a 65% decrease in AT1R immunoreactivity in the total extract ($P < 0.05$) (Figure 2). Altogether, these data validated that the RNA interference (RNAi) strategy was effective to suppress the expression of intraislet AT1R.

3.3. Improved Insulin Sensitivity in β-Cells by Ad-siAT1R Treatment in Islets of db/db Mice. Western blot showed that islets treated with Ad-siAT1R manifested significant increased protein levels of IRS-1 (by 85%), IRS-2 (by 95%), PI3-K(85) (by 112.5%), and p-Akt2 (by 164%) when compared with ones treated with Ad-siControl ($P < 0.05$) (Figures 3 and 4), which indicated that inhibition of AT1R by RNAi improved insulin sensitivity of β-cells.

3.4. Improved GSIS by Ad-siAT1R Treatment in Islets of db/db Mice. The insulin secretion stimulated by lower (2.8 mM) or higher (16.7 mM) glucose concentration was measured in

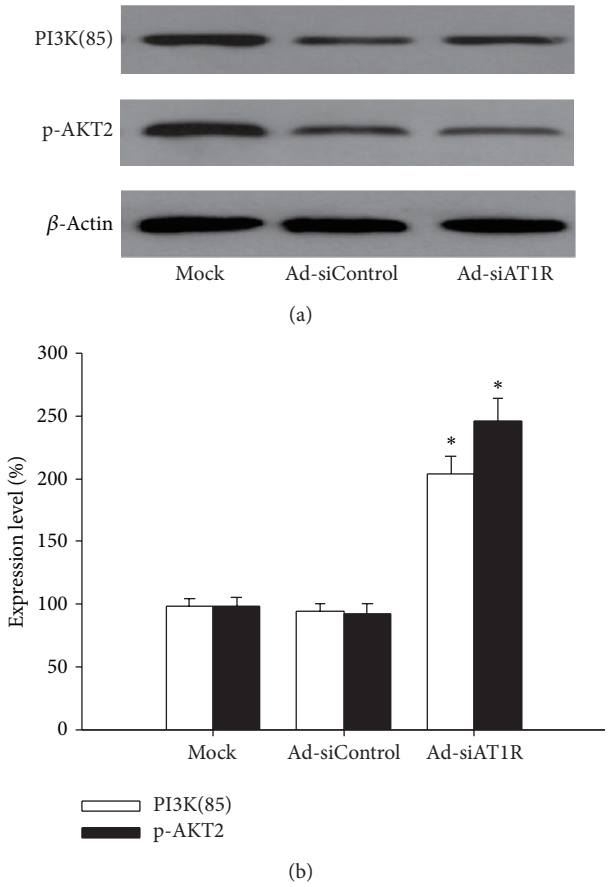

Figure 4: Effects of AT1R silencing on PI3-K(85) and p-Akt2 expression. Islets of db/db mice were transfected with Ad-si AT1R, Ad-siControl, or Mock and cells were cultured for 72 h prior to quantitative immunoblot evaluation. Results are represented as mean ± SD for six independent experiments. $^{*}P < 0.05$ versus Ad-siControl.

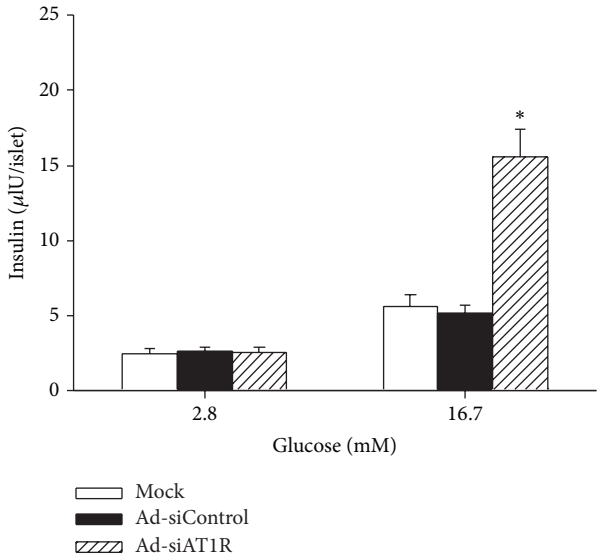

Figure 5: Effect of AT1R silencing on insulin secretion in islets. Islets were treated with Ad-siAT1R, Ad-siControl, or Mock, and 72 h later, insulin secretion was measured at basal and stimulatory glucose. Results are represented as mean ± SD for six independent experiments. $^{*}P < 0.05$ versus Ad-siControl.

uninfected cells and cells infected with Ad-siAT1R and Ad-siControl. No significant differences were observed between Ad-siAT1R group and Ad-siControl group at 2.8 mM glucose. Contrarily, there was a significant improvement of insulin secretion response at 16.7 mM glucose (SR 6.1 ± 1.0) in Ad-siAT1R group compared with Mock group (SR 2.3 ± 0.6) or Ad-siControl group (SR 2.0 ± 0.4) ($P < 0.01$) (Figure 5).

Perifusion is a golden method to evaluate the first-phase insulin secretion of islet in vitro. Islets of db/db mice manifested only a slight elevation of insulin secretion, while islets treated with Ad-siAT1R showed a pronounced increase in insulin peak at 1 minute after 16.7 mM glucose loaded ($P < 0.05$) (Figure 6).

3.5. Reduction of Glucagon Secretion by by Ad-siAT1R Treatment in Islets of db/db Mice. The Glucagon secretion was assayed in vitro by islet perifusion. Persistently elevated levels of glucagon were observed in Ad-siControl group, while Ad-siAT1R group showed a significant reduction of glucagon secretion since being stimulated by 16.7 mM glucose solution compared with Ad-siControl group ($P < 0.05$) (Figure 7).

3.6. Improved Glucose-Sensing Apparatus in β-Cells. Glucose transporter-2 (GLUT-2) and glucokinase (GCK) have been considered main components of β-cell glucose-sensing apparatus. Thus, we further investigated the expression levels of GLUT-2 and GCK in islets. By Western blot, significant decrease in both GLUT-2 (by 65.8%) and GCK (by 62.7%) was found in db/db mice when compared with db/m mice ($P < 0.05$) (Figure 8). In parallel with the reduction of AT1R expression, the expression of GLUT-2 and GCK increased by 190% and 121%, respectively, in islets treated with Ad-siAT1R, compared with ones treated with Ad-siControl ($P < 0.05$) (Figure 9).

4. Discussion

RAS components have long been known to express locally in rodent and human islets. The role of local RAS in islet function has become the focus of recent research, ever since Chappell MC and his colleagues discovered intrinsic angiotensin system in dog exocrine pancreas approximately 20 years ago [19]. The local pancreatic RAS has been shown to be upregulated in diabetic animals, whereas treatment with RAS blockade can improve β-cell function and glucose tolerance in variety of studies [20–22]. But the common RAS blockade usually inhibits the RAS systemically rather than locally. So we can not exclude the possibility that these benefits were dependent on the changes of systemic circulating RAS components due to the defects of previous experiment design. In the present study, we indeed inhibited the expression of intraislet AT1R by means of RNAi, a specific and efficient way for gene silencing. We successfully downregulated the expression of AT1R in islet and found not only notable improvement

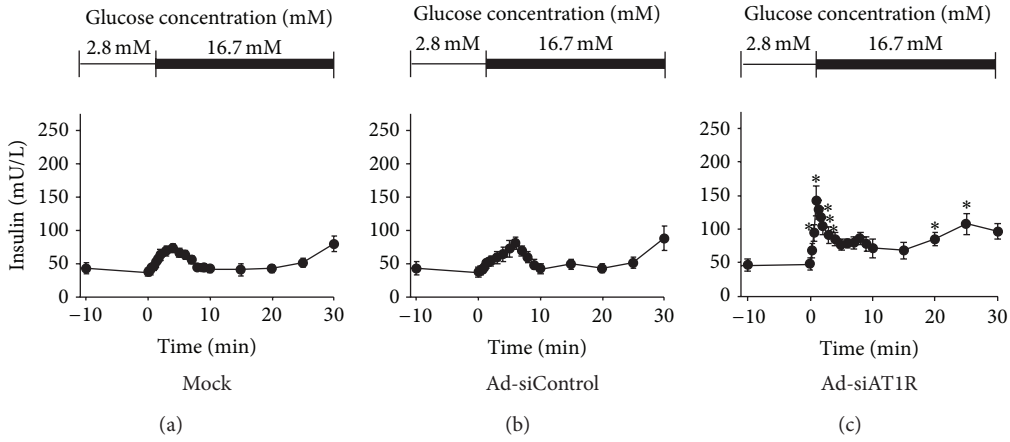

Figure 6: Insulin secretion by islet perifusion in Ad-siAT1R, Ad-siControl and Mock group. Results are represented as mean ± SD for three duplicate experiments. $^{*}P < 0.05$ versus Ad-siControl.

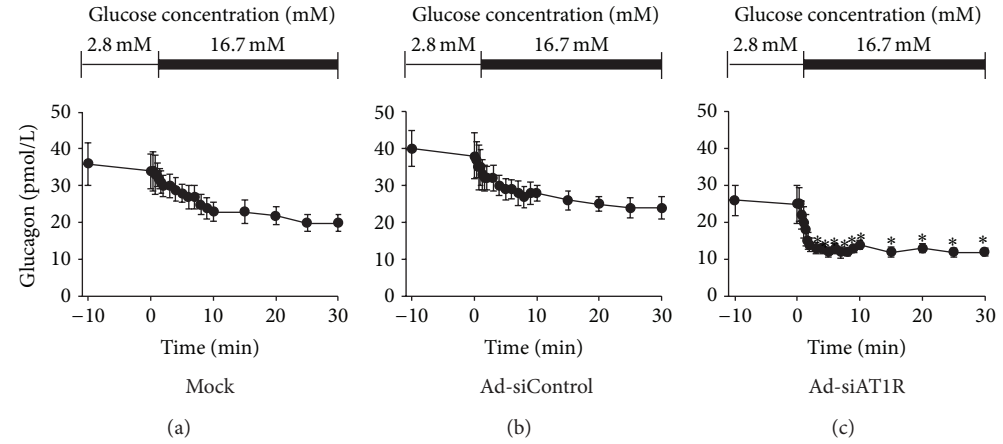

Figure 7: Glucagon secretion by islet perifusion in Ad-siAT1R, Ad-siControl, and Mock group. Results are represented as mean ± SD for three duplicate experiments. $^{*}P < 0.05$ versus Ad-siControl.

of first-phase insulin secretion but also significant reduction of glucagon secretion in Ad-siAT1R group. What is more, our results showed that improvement of islet function by blocking intraislet AT1R is associated with a detectable increased IRS-1, IRS-2, PI3-kinase p85, and phospho-Akt2 expression levels, as well as increased activities of glucose-sensing apparatus such as GLUT-2 and GCK in pancreatic islet.

The mechanisms of the protective action of RAS blockade on islet function are diverse and complicated. A study found that RAS blockade could improve microvessel density of islets and their function, suggesting that the improvement of blood supply of islets may be a crucial mechanism by which RAS blockade protects islet function [23]. Tikellis et al. [20] showed that chronic (10 weeks) RAS inhibition starting at the age of 10 weeks attenuated disordered islet architecture in Zucker diabetic fatty rats; these beneficial effects were partly attributed to decreased intraislet fibrosis, apoptosis, and oxidative stress. Meanwhile, Kwan and Leung [21] indicated that islet AT1R activation in young diabetic mice could mediate progressive islet-cell failure through UCP-driven oxidative damage. In addition, a recent finding indicated the effects of high glucose levels on islet function might be mediated by local islet RAS, partially AT2 receptors, via the alteration of β-cell potassium channels [24]. However, how RAS exerts these effects is less well documented and remains to be clarified.

Several recent studies have indicated that β-cells express components of insulin signaling systems including insulin receptors, insulin receptor substrates (IRS-1 and IRS-2), phosphatidylinositol 3-kinase (PI3-K), and protein kinase B [25–27]. IRS-1 and IRS-2 molecules are key mediators in insulin and IGF-1 signaling and their importance in β-cell physiology has been proven by several studies. Firstly, insulin binds to receptors on the surfaces of β-cells and causes tyrosine phosphorylation of the insulin receptor, IRS-1, and PI3-K. Such activation of PI3-K leads to production of PIP3. Secondly, PIP3 binds with PH domain of Akt and promotes its activation, which stimulates GLUT-2 translocation and glucose uptake into β-cells. Finally, increased intracellular glucose leads to increased production of ATP by the catalyze of GCK. The increased ATP/ADP ratio leads to closing of the potassium channel and depolarization of β-cells which leads the open of calcium channel and insulin secretion [28].

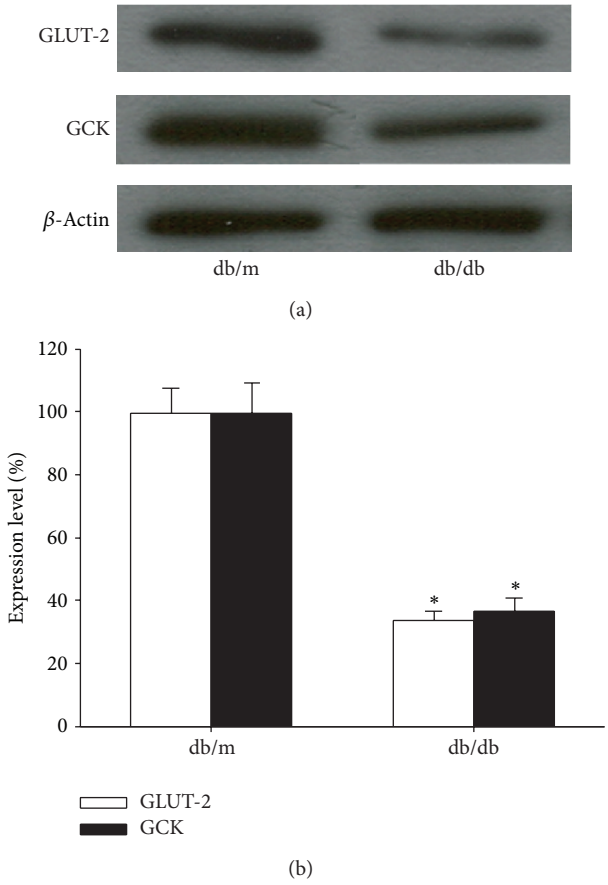

Figure 8: Expression of GLUT-2 and GCK protein in islets from db/m mice or db/db mice. Lysates from freshly isolated islets were analyzed for GLUT-2 and GCK expression by Western blot analysis. Results represent the mean ± SD. Blots from one representative experiments are shown. $^{*}P < 0.05$ versus db/m group, $n = 5$.

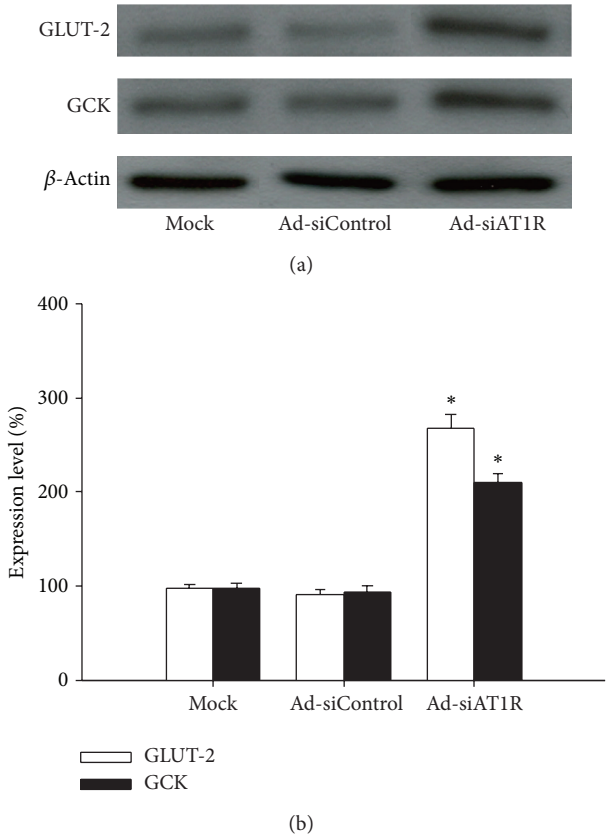

Figure 9: Effects of AT1R silencing on GLUT-2 and GCK expression. Islets of db/db mice were transfected with Ad-si AT1R, Ad-siControl, or Mock and cells were cultured for 72 h prior to immunoblot evaluation of GLUT-2 and GCK. Results are represented as mean ± SD for six independent experiments. $^{*}P < 0.05$ versus Ad-siControl.

Therefore, as insulin signal moleculars, IRS-1, PI3-K, and Akt, all play important role in GSIS. Decreased expression of IRSs or derangement in their signal transduction pathway leads to impaired insulin secretion similar to that seen in type 2 diabetes. In this study, we found that inhibition of intraislet AT1R expression resulted in notable improvement of first-phase insulin secretion with significantly increased protein levels of IRS-1, IRS-2, PI3-K p85, and phosphorylated Akt. Such result suggests that IR/IRSs/PI3-K/Akt may act as a potential link between intraislet RAS activity and GSIS.

As mentioned above, both GLUT-2 and GCK are glucose-sensing apparatus of β-cell acting as important roles in GSIS. GSIS is initiated by the uptake of glucose by the translocation of glucose transporter GLUT-2 in pancreatic beta cell. It is suggested that GLUT-2-null mice are hyperglycemic and hypoinsulinemic with a loss of first-phase glucose-stimulated insulin secretion and die within the first 3 weeks of life [29]. Furthermore, GCK is the rate-limiting step in glucose metabolism by β-cells, and it therefore has a high control strength over the entire process of glucose utilization, glucose oxidation, and insulin secretion. The discovery that GCK gene mutations account for many of the cases of maturity-onset diabetes of youth (MODY) has pointed out the pivotal role played by this enzyme in glucose homeostasis [30, 31]. Im Walde et al. have found that the GCK mRNA decreased by 50% in pancreas of diabetic mice compared with normal mice [32]. Here we show that GLUT-2 and GCK expression in db/db mice are significantly lower than that in db/m mice, while being restored after AT1R inhibition by Ad-siAT1R. In summary, we can conclude that AT1R inhibition improves GSIS by restoring β-cell insulin sensitivity and downstream glucose-sensing apparatus, while the detailed mechanism remains to be further investigated in future work.

A few researches have revealed the mechanisms of AngII-mediated insulin resistance. AngII could exert its influence on insulin sensitivity via the AT1 receptor in at least three ways. Firstly, AngII directly inhibits tyrosine-phosphorylation of IRS-1 and increases serine phosphorylation of IRS-1 and PI3-K p85 regulatory subunit [33]. Secondly, AngII indirectly dephosphorylates IR and IRS-1 by activating TNF-α and protein tyrosine phosphatase (PTP)-1B [34]. Furthermore, AngII-induced oxidative stress impairs activity of PI3-K and its downstream signaling, including AKT2-mediated

GLUTs translocation and expression levels of other glucose-sensing apparatus [35]. Therefore, in our study, AT1R inhibition induced increased tyrosine phosphorylation of IRS-1 and PI3-K p85 regulatory subunit may contribute to the improvement of β-cell insulin sensitivity. And since our previous study showed the expression levels of oxidative stress markers in islet of db/db mice decreased with candesartan treatment, the alleviation of oxidative stress may be also an impact factor of β-cell insulin sensitivity and downstream glucose-sensing apparatus expression.

For the first time, we evaluated glucagon dynamic secretion by perifusion and found significantly reduction of glucagon secretion in Ad-siAT1R group. Glucagon is the principal counterregulatory hormone that opposes insulin action leading to coordinate bihormonal control of glucose homeostasis. Increasing evidence has suggested that increased glucagon secretion is implicated in the development of T2DM [36]. A study just published in July showed that enalapril appeared to reduce hyperglucagonemia in high fat diet-induced insulin resistant mice [37]. But the mechanism by which glucagon secretion is inhibited by RAS block is far from clear. Consistent with an important role for insulin in the β-cell, insulin receptor and the insulin-signaling molecules are expressed highly in pancreatic α-cells and play an important role in modulating α-cell function [38–42]. The insulin receptor defects in the insulin-signaling pathway of pancreatic α-cell may contribute to the development of diabetic hyperglucagonemia, manifesting as blunted insulin-stimulated Akt phosphorylation and insulin-suppressed glucagon secretion [43]. Therefore, it is conceivable that AT1R block could attenuate hyperglucagonemia by restoring insulin suppression of glucagon secretion driven by insulin, mainly through improved first-phase insulin secretion (indirect) and insulin sensitivity of α-cell (direct). Yet the detailed mechanisms remain to be further explored in future research using islet α-cell strain.

In conclusion, our study suggests that intraislet AT1R is a crucial physiological regulator of insulin sensitivity of β-cell and thus affects glucose-induced insulin release. We also found that intraislet inhibition of AT1R expression led to downregulation of glucagon secretion from isolated islets of db/db mice. The characteristics of AT1R inhibitors in both insulin and glucagon secretion could make it a potential novel therapeutics for the prevention and treatment of type 2 diabetes.

Abbreviations

AT1R: Angiotensin II type 1 receptor
GSIS: Glucose-stimulated insulin secretion
IRS: Insulin receptor substrates
GCK: Glucokinase
GLUT-2: Glucose transporter-2
ACEI: ACE inhibitor
ARB: Angiotensin receptor blocker
AGT: Angiotenogen
PI3-K: Phosphatidylinositol 3-kinase
Akt: Protein kinase B
PDK-1: Phosphoinositide-dependent kinase-1.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

Authors' Contribution

Zhen Zhang and Chunyan Liu contributed equally to this study.

Acknowledgments

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Review Article

Mechanisms of Perivascular Adipose Tissue Dysfunction in Obesity

Maria S. Fernández-Alfonso,[1] Marta Gil-Ortega,[2] Concha F. García-Prieto,[2] Isabel Aranguez,[1] Mariano Ruiz-Gayo,[2] and Beatriz Somoza[2]

[1] *Instituto Pluridisciplinar and Facultad de Farmacia, Universidad Complutense, Juan XXIII 1, 28040 Madrid, Spain*
[2] *Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad CEU-San Pablo, 28660 Madrid, Spain*

Correspondence should be addressed to Maria S. Fernández-Alfonso; marisolf@farm.ucm.es

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Most blood vessels are surrounded by adipose tissue. Similarly to the adventitia, perivascular adipose tissue (PVAT) was considered only as a passive structural support for the vasculature, and it was routinely removed for isolated blood vessel studies. In 1991, Soltis and Cassis demonstrated for the first time that PVAT reduced contractions to noradrenaline in rat aorta. Since then, an important number of adipocyte-derived factors with physiological and pathophysiological paracrine vasoactive effects have been identified. PVAT undergoes structural and functional changes in obesity. During early diet-induced obesity, an adaptative overproduction of vasodilator factors occurs in PVAT, probably aimed at protecting vascular function. However, in established obesity, PVAT loses its anticontractile properties by an increase of contractile, oxidative, and inflammatory factors, leading to endothelial dysfunction and vascular disease. The aim of this review is to focus on PVAT dysfunction mechanisms in obesity.

1. Introduction

Obesity is an independent risk factor for the development of endothelial dysfunction and vascular disease, hypertension, myocardial infarction, and stroke [1]. The probability of suffering from vascular diseases is four times higher in obese (body mass index >30 kg/m^2) than in normal-weight people (body mass index ≤25 kg/m^2) [2]. It is not exclusively the excess of body weight, but more precisely how this excess is distributed, which correlates with cardiovascular risk. In fact, abdominally obese individuals, that is, with an excess of visceral adipose tissue, tend to have higher blood pressure values than individuals with a peripheral body fat distribution [3].

Adipose tissue acts as an endocrine organ by secreting various signaling cytokines, called adipokines, which affect energy metabolism, insulin sensitivity, inflammatory response, and blood flow. Perivascular adipose tissue (PVAT) is the adipose tissue surrounding blood vessels. It was considered until recently only as a passive structural support for the blood vessel, and it was routinely removed for isolated blood vessel studies. Soltis and Cassis [4] demonstrated in 1991 for the first time that PVAT reduced contractions to noradrenaline in rat aorta. This initial description of the anticontractile effect of PVAT was unnoticed until 2002, when Löhn et al. [5] reapproached this issue. These investigators described the inhibitory action of PVAT on aortic contractions to a variety of vasoconstrictors and demonstrated in an elegant bioassay approach that the anticontractile action was induced by a transferable protein factor released by adipocytes [5]. The authors called it adipocyte-derived relaxing factor (ADRF) in analogy to the endothelium-derived relaxing factor (EDRF) described in the 1980s [6].

PVAT is now considered a highly active endocrine organ that releases a variety of adipokines, inflammatory cytokines, and other factors which influence vascular tone in a paracrine way [7–9]. Under physiological conditions, PVAT releases a number of vasoactive substances, such as ADRF [5, 10–13] adiponectin [11], angiotensin-(1–7) [14], H_2O_2 [15], leptin [16], and nitric oxide (NO) [17], that elicit a net beneficial anticontractile effect on vascular function and are essential for the maintenance of vascular resistance [7–9].

Table 1: Mechanisms of PVAT dysfunction.

(i) Increase in adipocyte size and PVAT amount
(ii) Hypoxia
(iii) Aging
(iv) Leptin/adiponectin dysregulation
(v) Loss of anticontractile properties
(vi) Loss of eNOS and NO
(vii) Increase in oxidative stress
(viii) Increase in inflammatory response

Since the anticontractile influence of PVAT is directly dependent on its amount [12, 13, 15] and this increases throughout the vasculature in obesity [18–21], it would be conceivable to think that the anticontractile effect of PVAT would be increased in these circumstances. However, it is now known that obesity triggers both structural and functional changes in PVAT which seem to be related to endothelial dysfunction and vascular damage. In fact, in obese patients and animal models of obesity, alterations in the amount and expression pattern of adipokines have been described causing an unbalance in favour of vasoconstrictor and pro-inflammatory substances. It was an approach as simple as leaving the artery intact with its surrounding adipose tissue which has opened a new research line. The aim of this review is to focus on the mechanisms leading to PVAT dysfunction and alteration of its paracrine role in obesity (Table 1).

2. The Anticontractile Effect of PVAT Is Lost in Obesity

The loss of the anticontractile effect of PVAT in obesity has been described in several models and conditions (Table 2). Gao et al. [22] demonstrated that the anticontractile effect of PVAT was lost in an animal model of obesity despite higher amounts of perivascular fat. Similarly, New Zealand obese (NZO) mice which have a severe metabolic syndrome and a higher amount of perivascular fat show a reduced anticontractile effect of PVAT [11]. These studies suggest that in obesity, besides to an increased amount of PVAT, there might be changes in the expression pattern of PVAT-derived factors responsible for alterations in vascular function. In this context, a recent study in obese Ossabaw swine [23] described an alteration in the proteomic profile of 186 proteins which correlate with an augmented contractile effect of coronary PVAT and underlying increases in vascular smooth muscle Ca^{2+} handling via $Ca_V1.2$ channels, H_2O_2-sensitive K^+ channels, and Rho-dependent signaling [23]. The mechanisms involved in increased contractility are reviewed in the following paragraphs.

3. Role of ADRF in Obesity

In several studies in obese models [8, 11, 22] and patients [20], the loss of the anticontractile effect of PVAT has been attributed to the downregulation of ADRF. The anticontractile effect of ADRF in physiological conditions has been described in several species [5, 7–13, 24–26] and is mediated by different mechanisms depending on the vascular bed. Both an endothelium-dependent [15] and -independent relaxation [5, 15] have been reported in rat aorta. Endothelium-dependent relaxation is mediated through nitric oxide (NO) release and subsequent calcium-dependent K^+ channel activation [15], whereas endothelium-independent dilatation is mediated by either the activation of tyrosine kinase pathways and opening of ATP-dependent K^+ (K_{ATP}) channels [5] or by H_2O_2 formation [15]. In contrast, ADRF induces an endothelium-independent relaxation through the activation of voltage-dependent K^+ channels (Kv) in rat mesenteric arteries [12, 13].

An essential question that remains to be answered concerns the nature of ADRF. The identity of ADRF with adiponectin (see below) is controversial. Löhn et al. [5] excluded this possibility, since the anticontractile effect of PVAT is still present in adiponectin knock-out mice (APN −/−) [12]. However, in a recent study, β_3-adrenoceptor stimulation of PVAT under basal, noncontracted conditions releases an adipocyte-derived hyperpolarizing factor which is probably adiponectin [27]. This factor activates AMPK to indirectly open myocyte BK_{Ca} and TRPM4 channels. Additionally, it also liberates NO which also contributes to PVAT-dependent myocyte hyperpolarization [27]. A recent study proposes palmitic acid methyl ester (PAME) as a candidate for ADRF since it also elicits vasorelaxation by opening voltage-dependent K^+ channels on smooth muscle cells [26]. The identity of ADRF with leptin (see below) has also been discarded, since the lack of functional leptin receptors in the Zucker fa/fa rats did not modify the anticontractile effect of PVAT [5].

4. Nitric Oxide and Hydrogen Sulfide: Two Gases in PVAT

Nitric oxide (NO) release in mesenteric PVAT from C56/Bl6 mice contributes to the enhancement of vasodilator responses [17]. In obese mice, after 32 weeks of HFD, eNOS and NO production in PVAT are downregulated to undetectable levels [28]. Moreover, ob/ob mice lacking leptin do not exhibit NO production in perivascular adipocytes. Interestingly, this is restored in PVAT after 2-week subcutaneous leptin infusion, suggesting that NO release in PVAT seems to be mediated by leptin [17] (Figure 1).

Hydrogen sulfide (H_2S) production has been demonstrated in rodent aortic and mesenteric PVAT [29, 30] and has been proposed to be ADRF [31]. This gas is synthesized in the cytosol from L-cysteine and is enzymatically oxidized in mitochondria (for a review, see [31]). The effect of H_2S on blood vessels is dual depending on its concentration, although its net effect seems to be antihypertensive. H_2S elicits vasoconstriction at low and vasodilation at higher concentrations [32]. Although PVAT-derived H_2S production has not been studied in obesity, treatment with atorvastatin increases its production preventing mitochondrial oxidation and increasing the anticontractile effect of PVAT [33]. Further research will be necessary to assess the role of H_2S in obesity.

Table 2: Loss of anticontractile effect of PVAT in obesity.

Study reference	Vessel	Type of PVAT	Species	Obesity
[20]	Small arteries (100–150 μm)	Subcutaneous gluteal	Human	Obese patients
[28]	Mesenteric arteries	Mesenteric	C57BL6 mice	DIO (32 w)
[11]	Mesenteric arteries	Mesenteric	NZO mice	
[53]	Mesenteric arteries		NZO mice	
[19]	Mesenteric arteries	Mesenteric	Rat	DIO (6 mo)
[19]	Aorta	Periaortic	Rat	DIO (6 mo)
[22]	Aorta	Periaortic	Rat	Perinatal nicotine adm
[42]	Coronary	Epicardial	Ossabaw obese swine	DIO (20 w)
[23]	Coronary	Epicardial	Ossabaw obese swine	DIO (6–12 mo)

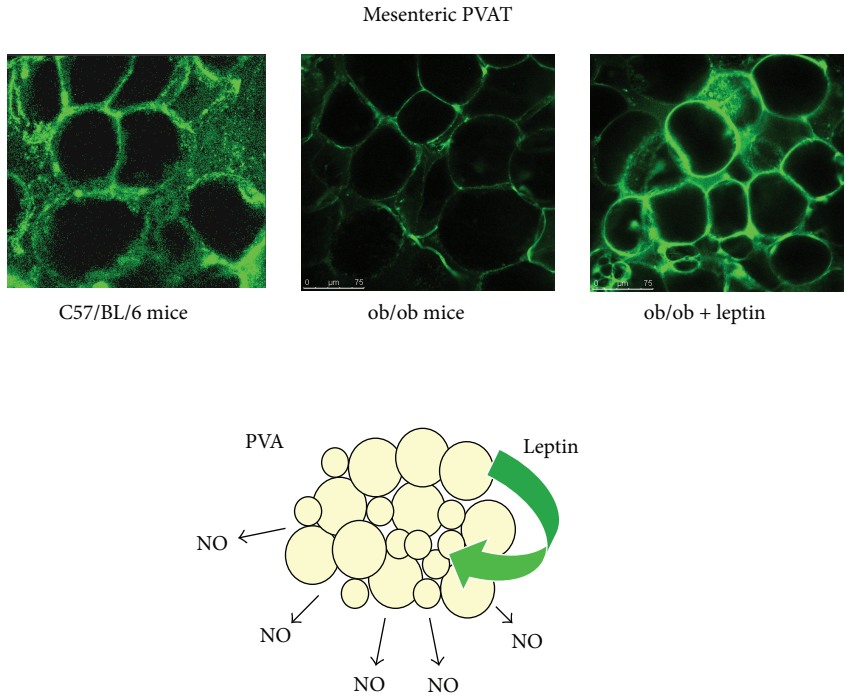

Figure 1: Leptin stimulates nitric oxide (NO) release in perivascular adipose tissue (PVAT). Mice lacking leptin (ob/ob) do not exhibit NO production in perivascular adipocytes. NO release is restored in PVAT after 2-week subcutaneous leptin infusion. Data from [17].

5. PVAT-Derived Adipokine Dysregulation in Obesity

Leptin participates in the regulation of vascular tone (for a review, see [34]). Vascular effects of leptin seem to be the net result of two different actions: (i) a direct vasodilatation depending on an intact and functional endothelium through mechanisms that vary between different vascular beds and (ii) an indirect vasoconstriction through stimulation of sympathetic activity at hypothalamic level [35]. Leptin activates endothelial nitric oxide synthase (eNOS) in aorta [36, 37], whereas it induces the release of endothelium-derived hyperpolarizing factor in mesenteric arteries [37]. *In vivo* experiments have revealed that leptin infusion reduces arterial pressure by increasing NO release [38]. Moreover, leptin exerts an endothelium-independent anticontractile effect on angiotensin II-induced contractions [39].

Leptin released from PVAT has a paracrine role in the regulation of vascular tone. PVAT surrounding rat aorta and mesenteric arteries, as well as the human saphenous vein [40], releases leptin at active concentrations which elicits an anticontractile effect [12, 16]. Moreover, elevated leptin levels in PVAT promote neointima formation independent of obesity and systemic hyperleptinemia [41]. In Ossabaw swine with metabolic syndrome, epicardial PVAT-derived leptin enhancement aggravates endothelial dysfunction via a PKC-β-dependent pathway [42]. Similarly, an upregulation of leptin in aortic PVAT is paralleled by a reduced anticontractile effect of PVAT. In a mice model of diet-induced obesity, the increase in leptin levels correlates with a loss in PVAT-derived NO and eNOS [28]. It has to be elucidated, however, if this is

a result of a deleterious effect of high leptin levels or of leptin resistance.

Adiponectin induces vasodilatation in rat aorta and in mouse mesenteric arteries through an endothelium independent mechanism involving the activation of Kv channels [11]. Other authors have shown that adiponectin increases NO release from vascular endothelial cells in culture [43, 44]. Moreover, adiponectin seems to preserve endothelial function through inhibition of endothelial cell activation [45] and synthesis of inflammatory markers [46]. The first evidence for the paracrine vasodilator effect of PVAT-derived adiponectin was demonstrated by Greenstein et al. [20]. Incubation with an adiponectin type I-receptor blocking peptide entirely abolished the anticontractile properties of PVAT in human subcutaneous and in rat arteries, suggesting that adiponectin is a physiological modulator of local vascular tone by increasing NO bioavailability.

Since eNOS is the final step for leptin- and adiponectin-induced NO release, alterations in this enzyme might be related with obesity-related endothelial dysfunction. In this context, an impairment of eNOS-mediated vasodilatation through a downregulation of the AMPK/mTOR pathway in obese rats has been shown [19]. Thus, vascular dysfunction in obesity is both the result of adipokine disbalance as well as of a downregulation of their signalling pathways.

6. Oxidative Stress and Inflammation in PVAT Are Increased in Obesity

One proposed mechanism to explain the loss in the anticontractile effect of PVAT is the increase in oxidative stress observed in obesity. Reactive oxygen species (ROS), such as superoxide anion or hydrogen peroxide (H_2O_2), play an important role in PVAT-mediated modulation of vessel function. NADPH oxidase, which represents the major source of superoxide anion in the vasculature, is also expressed in PVAT of rat mesenteric arteries [15]. In these vessels, PVAT-derived superoxide anion enhances the arterial contractile response to perivascular nerve stimulation involving activation of tyrosine kinase and MAPK/ERK pathway. Superoxide anions are rapidly converted by superoxide dismutases (SODs) to H_2O_2, which is a cell-permeant and highly stable ROS [47]. Expression of the three SOD isoforms, the copper-zinc SOD (Cu/Zn-SOD), the manganese SOD (Mn-SOD), and the extracellular form of Cu/Zn-SOD (ec-SOD), has been detected in mice mesenteric PVAT [28].

H_2O_2 has been shown to be a vasoactive substance that induces both contractile and relaxant responses on blood vessels by different mechanisms depending on the vessels type, the contractile status, its concentration, and the animal species [47]. Contractile effect mediated by H_2O_2 is due to direct cyclooxygenase activation and to an increase of intracellular Ca^{2+} [48, 49]. Interestingly, it has recently been suggested that extracellular H_2O_2 can enter vascular smooth muscle cells stimulating Nox1 oxidase and superoxide anion production [50]. H_2O_2 also induces endothelium-dependent relaxation as a result of an increased NO release secondary to endothelial K^+ channel activation [48]. Moreover, H_2O_2 induces endothelium-independent relaxation through (i) direct opening of smooth muscle K^+ channels by oxidation of their cysteine residues, as well as (ii) by direct activation of smooth muscle soluble guanylate cyclase (sGC) [15, 51].

Since superoxide anion promotes vessel contraction, while H_2O_2 induces its relaxation, the final outcome will depend on their relative PVAT production/release balance, and activity of SODs in PVAT might be crucial [28]. Ketonen et al. [52] showed in C57/Bl6 mice fed a very high fat diet (60% cal from fat) for 8 weeks that endothelial-dependent relaxation was due to an increase in PVAT-derived oxidative stress characterized by the enhanced production of superoxide anion and hydrogen peroxide. Another study in mice fed a long-term high fat diet (45% cal from fat 32 weeks) showed that mesenteric bed endothelial dysfunction was aggravated in the presence of PVAT [28]. An increase in NADPH oxidase activity and in superoxide anion production, together with a decrease in ecSOD expression and total SOD activity, was found in PVAT. These changes were accompanied by a decrease in eNOS expression and NO production in PVAT from these obese mice [28]. Similarly, in NZO mice, an impaired H_2O_2 production in PVAT, as a consequence of increased $\cdot O_2^-$ formation and decreased SOD expression, contributes to vascular dysfunction through reduced anticontractile effects [53]. Ossabaw obese swine, H_2O_2-mediated vasodilatation was markedly attenuated by the presence of coronary PVAT [24].

An interesting issue which deserves future investigation is the impact of the fatty acid (FA) composition in the diet to the increase in oxidative stress in PVAT. A fructose-rich diet decreases polyunsaturated FA, increasing saturated and monounsaturated FA in PVAT [54]. These changes in FA composition are paralleled by a decrease in antioxidant enzymes, a reduction in glutathione content, and alterations in vascular function.

Obesity is also associated with a state of chronic low-grade inflammation that can be detected both systemically and within specific tissues. An increase in inflammatory cytokines has been shown in PVAT surrounding small arteries from obese subjects with metabolic syndrome correlating with an elevated oxidative stress [20]. High-fat diet-induced obesity promotes a marked proinflammatory shift in the profile of secreted cytokines and chemokines which is associated with oxidative stress in PVAT [55]. PVAT of NZO mice also exhibits inflammation and an increase in oxidative stress, leading to endothelial dysfunction, the latter as a result of decreased NO and enhanced superoxide generated by uncoupled endothelial NO synthase [53].

Moreover, HFD causes an increase in expression of leptin and MIP1alfa correlating with a decrease in adiponectin, PPARy, and FABP4 [56]. This is paralleled by an enhanced CD3 expression in PVAT with no changes in CD68 levels [56]. In another study, diet-induced obesity increased mesenteric PVAT macrophage content and vascular oxidative stress in mice [57]. Human adipocytes show an increase in proinflammatory state (IL-6, IL-8, and MCP-1) and reduced adipocytic differentiation [56]. These data suggest that inflammatory cytokine release by PVAT could attract macrophages to the depot further aggravating inflammation

Table 3: Increase in PVAT amount and adipocyte size in different models of obesity.

Species	Model	PVAT	Adipocyte size	Study reference
Wistar rat	HFD (6 mo)	Aortic	Increase	[19]
Wistar Kyoto rat	HFD (3 mo—60% cal from fat)	Aortic	Increase	[63]
Zucker fa/fa rat		Aortic	Increase	[63]
C57Bl6 mice	HFD (2 w—42% cal from fat)	Aortic	Increase	[56]
C57Bl6 mice	HFD (13–20 w—45% cal from fat)	Thoracic aortic	No change	[58]
C57Bl6 mice	HFD (8 w—45% cal from fat)	Mesenteric	Increase	[17]
C57Bl6 mice	HFD (32 w—45% cal from fat)	Mesenteric	Increase	[28]

HFD: high fat diet; mo: month; w: weeks.

and PVAT dysfunction. The key role of inflammatory cells in PVAT-aggravated endothelial dysfunction was demonstrated in mice lacking P-selectin glycoprotein ligand-1 (Psgl-1), an inflammatory adhesion molecule enabling the recruitment of leukocytes to the endothelium. Psgl-1 deficiency prevented PVAT inflammation and endothelial dysfunction [57]. In contrast to the abovementioned findings, Fitzgibbons et al. [58] have proposed that mice thoracic PVAT shows a very low inflammation after 13 wk of HFD, probably due to its similarity with the brown adipose tissue phenotype. This study suggests the attractive possibility of promoting a BAT phenotype in PVAT which might have preventive effects in vascular disease development.

7. Role of Hypoxia in Obese PVAT

It is well known that the increase of the adipocyte area and mass that occurs in obesity leads to hypoxia [59–61] (Table 3). However, there are controversial results about the role of hypoxia in PVAT. Maenhaut et al. [61] demonstrated that hypoxia enhances relaxation of mouse aorta in presence of PVAT independently of the vasoconstrictor agent. This effect is mediated by a basally released factor which meets the criteria for ADRF [7, 10]; that is, (i) it acts through K_{ATP} channels, (ii) it is independent of NO and a functional endothelium, and (iii) its similarity with lactate, CO, H_2S, prostanoids, adenosine, leptin, or adiponectin has been excluded. In obese patients, however, hypoxia abolishes the anticontractile effect of PVAT and has been linked to oxidative stress and increased inflammation in PVAT [20].

8. Role of Aging in Obese PVAT

Obesity in elderly individuals is increasing at alarming rates, and there is evidence suggesting that this population is more vulnerable to the deleterious cardiovascular effects of obesity than younger individuals [62]. The paracrine effect of young and aged adipocytes on vascular smooth muscle cell proliferation has been described by Barandier et al. [63]. These authors nicely showed that proliferative effects of adipocyte-conditioned medium were significantly increased in 24-month-old and in HFD WKY rats versus young 3-month-old animals. Aging also exacerbates endothelial dysfunction and vascular inflammation in HFD mice due to an increase in oxidative stress, inflammation, and macrophage infiltration in periaortic adipose tissue [55]. These studies suggest that that there is a synergy between age and obesity-related alterations in PVAT damaging the vascular wall. However, more studies will be necessary to deeper characterize the mechanisms and impact of aging on the paracrine effects of PVAT in obesity.

9. Impact of Weight Loss on PVAT Dysfunction

Weight loss by different approaches significantly correlates with improvements in blood pressure levels, left ventricular mass, exercise capacity, and glucose tolerance [64]. Bariatric surgery by gastric bypass has been shown to reduce white adipose tissue inflammation by inducing significant reductions in macrophage content, MCP-1, and hypoxia inducible factor-1α [65]. Interestingly, bariatric surgery also reverses the obesity-induced damage to PVAT anticontractile function by reducing adipocyte hypertrophy, PVAT inflammation and increasing both PVAT-derived NO and adiponectin availability [66]. This study opens a new approach for the management of vascular damage associated to PVAT dysfunction.

10. Overweight versus Obesity: Adaptative Changes in PVAT

After onset of high fat, feeding alterations in PVAT develop in a sequential manner. Early stages of diet-induced obesity (DIO) are characterized by increased adiposity (overweight rather than obesity) and moderate hyperleptinemia but preserving peripheral responsiveness to leptin, as well as normal postprandial values of adiponectin, insulin, glucose, triglycerides, and free-fatty acids [67]. It is well known that fat-enriched diets trigger initial compensatory mechanisms in adipose tissue aimed at preventing organ damage and lipotoxicity [68]. In this context, our group has recently shown that a moderate enlargement of PVAT during early DIO correlates with NO overproduction in this tissue and an improvement of vascular function [28].

We thus suggest that the consequence of PVAT enlargement in response to a HFD might be dual (Figure 2). Initially, a moderate PVAT enlargement might be beneficial, being an adaptative response probably aimed at protecting vascular function in situations such as moderate overweight, pregnancy, or hibernation. In established obesity, however, changes in PVAT amount and in the expression pattern

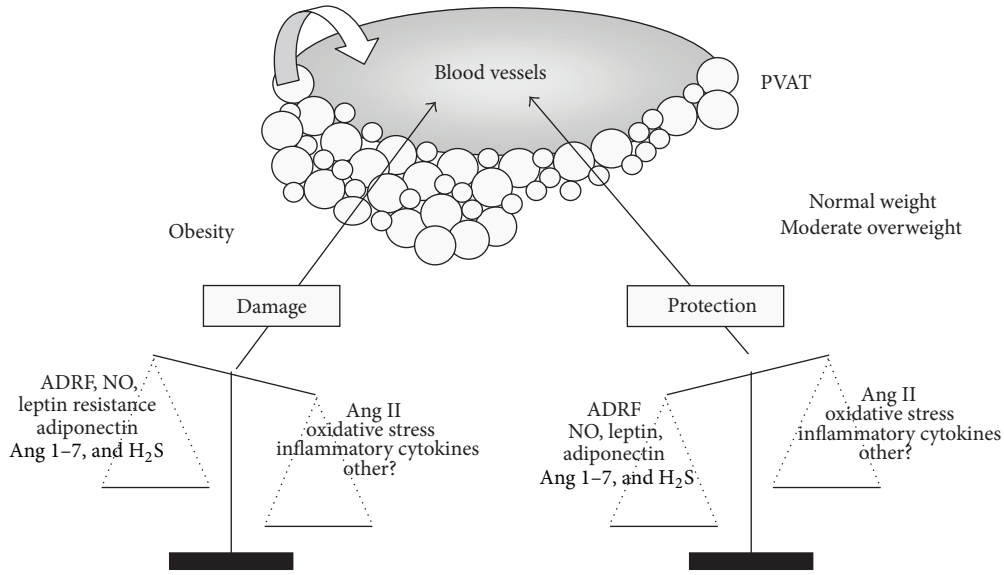

Figure 2: Hypothesis of the role of PVAT and PVAT-derived adipokines in health and obesity.

of adipokines and other PVAT-derived factors might shift the paracrine influence of PVAT from a net anticontractile effect to a prooxidant, proinflammatory, and contractile environment. This unbalance towards a predominance of vasoconstrictor and inflammatory factors in obesity could provide the link between obesity, cardiovascular functional, and structural alterations and cardiovascular diseases.

Several questions regarding PVAT dysfunction need to be addressed in the next years: (1) to identify new PVAT-derived factors that reach the vascular wall, (2) to determine the time point when the balance shifts from a protective to a deleterious paracrine effect of PVAT during weight gain, (3) to characterize the interplay between different adipokines (actions and time-course), and (4) to assess the effects of dietary or pharmacological interventions on PVAT-derived adipokine expression profile and vascular function. A better understanding of PVAT dysfunction may lead to new approaches in the management of cardiovascular risk prevention in obesity.

Conflict of Interests

The authors declare that there is no conflict of interests.

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Review Article

Obesity-Related Metabolic Syndrome: Mechanisms of Sympathetic Overactivity

Maria Paola Canale,[1] Simone Manca di Villahermosa,[1] Giuliana Martino,[1] Valentina Rovella,[1] Annalisa Noce,[1] Antonino De Lorenzo,[2] and Nicola Di Daniele[1]

[1] *Division of Hypertension and Nephrology, Department of System Medicine, University of Rome Tor Vergata, Rome, Italy*
[2] *Division of Clinical Nutrition and Nutrigenomic, Department of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy*

Correspondence should be addressed to Nicola Di Daniele; didaniele@med.uniroma2.it

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The prevalence of the metabolic syndrome has increased worldwide over the past few years. Sympathetic nervous system overactivity is a key mechanism leading to hypertension in patients with the metabolic syndrome. Sympathetic activation can be triggered by reflex mechanisms as arterial baroreceptor impairment, by metabolic factors as insulin resistance, and by dysregulated adipokine production and secretion from visceral fat with a mainly permissive role of leptin and antagonist role of adiponectin. Chronic sympathetic nervous system overactivity contributes to a further decline of insulin sensitivity and creates a vicious circle that may contribute to the development of hypertension and of the metabolic syndrome and favor cardiovascular and kidney disease. Selective renal denervation is an emerging area of interest in the clinical management of obesity-related hypertension. This review focuses on current understanding of some mechanisms through which sympathetic overactivity may be interlaced to the metabolic syndrome, with particular regard to the role of insulin resistance and of some adipokines.

1. Introduction

The metabolic syndrome (MetS) is a cluster of abnormalities that include diabetes mellitus (DM), morbid obesity, dyslipidemia, and hypertension (HT) that are all risk factors for the development of cardiovascular disease (CVD) and chronic kidney disease (CKD) [1]. The National Cholesterol Education Program's Adult Treatment Panel III report (ATP III) identified six components of the MetS that relate to CVD: abdominal obesity, atherogenic dyslipidemia, raised blood pressure (BP), insulin resistance (I.R.)/glucose intolerance, and proinflammatory and prothrombotic state [2]. A major problem concerning the WHO and NCEP ATPIII definitions was their applicability to different ethnic groups, especially when obesity cutoffs were to be defined. This is particularly evident for the risk of type II DM, which may be associated with much lower levels of obesity in Asians compared to Caucasians. The International Diabetes Federation has then proposed a new set of criteria with ethnic/racial specific cutoffs [3]. The MetS central feature is obesity, and the MetS is a growing epidemic in the United States and throughout the world [4, 5]. Approximately 1 adult in 4 or 5, depending on the country, has the MetS. Incidence increases with age; it has been estimated that in people over 50 years of age, the MetS affects more than 40% of the population in the United States and nearly 30% in Europe [6, 7]. Whether the effects of the MetS are due to a sum of comorbidities or to individual features is still a matter of debate; however, there is sufficient data to support an increased risk of CVD in people affected by the MetS in the absence of other baseline risk factors [8–10]. Central obesity is an independent risk factor for CVD and is associated with MetS [11]. Central obesity predisposes to diabetic nephropathy, hypertensive nephrosclerosis, and focal segmental glomerulosclerosis and represents an independent risk factor for the development and progression of CKD [12]. Obesity and the development of I.R. are thought to be a central feature, contributing to the significant morbidity and mortality associated with

the MetS and development of a particular resistant form of HT [13–15]. The development of resistant HT in individuals with MetS can be attributed to a number of factors including proinflammatory cytokines, inappropriate activation of the renin-angiotensin system (RAAS), vasoconstriction from increased sympathetic nervous system (SNS) activation, and dysregulation in adipokines production and secretion [16]. Several components of the MetS are associated with indirect or direct markers of adrenergic overdrive [17]. This review will focus on current understanding of the mechanisms through which sympathetic overactivity may be interlaced to the metabolic syndrome, with particular regard to the role of insulin resistance and of some adipokines.

2. Pathophysiology of the Mets

In 1988, Reaven first postulated "the syndrome X," which is now named "Metabolic Syndrome" (MetS) [14]. Reaven noticed the frequent association of factors leading to the development of CVD: glucose intolerance, hyperinsulinemia, high serum triglycerides, low serum high-density lipoprotein cholesterol, and HT. I.R. was proposed as the "driving force" of the syndrome [14, 18]. Subsequently, other abnormalities, in particular prothrombotic and chronic proinflammatory states, were added to the definition of the MetS. Later on, abdominal obesity became the "core" of the syndrome [19–21]. Since metabolic abnormalities linked to I.R. are usually found in patients with abdominal obesity [22, 23] I.R. is considered to be the "core" of the MetS and central obesity its most important clinical clue [24].

3. Metabolic Syndrome and Sympathetic Overactivity

As BP and thermogenesis are both under adrenergic control, an alteration in the SNS could be part of the pathophysiology of the MetS. Also, alterations in the sympathetic control of heart rate (HR), cardiac output, peripheral vascular resistance, and renal sodium handling may promote, alone or in combination, the development and progression of HT [25, 26]. Actually, sympathetic overdrive occurs in MetS. Many components of the MetS are characterized by an increased adrenergic activity. Interestingly, sympathetic overdrive is detectable in obese patients prone to MetS before HT occurs. Also, when obesity and HT are both present in the same patient the degree of sympathetic activation is much greater than in those with either condition separately [27]. Individuals with central obesity show increased sympathetic nervous activity (SNA) when compared to individuals with subcutaneous form of obesity [28]. Increased sympathetic outflow has been reported in obese nonhypertensive individuals with the determination of circulating catecholamines, urinary norepinephrine (NE), muscle sympathetic nerve activity (MSNA) recordings of postganglionic sympathetic nerve fibers, and renal NE spillover [29], suggesting that vasoconstriction and renal mechanisms are both involved [30]. There is also evidence that SNS overactivity is not generalized in obesity [31]. Obesity causes differential activation of tissue SNS activity. Increase in HR results from a decrease in parasympathetic activity rather than an increase in sympathetic activity. On the contrary, SNA increases both in the kidney and skeletal muscles of obese hypertensive subjects. Increased SNA does not directly determine vasoconstriction but instead stimulates renin secretion and increases renal sodium reabsorption [15]. Interestingly, Kalil and Haynes showed that the presence of misleading inferences from multifiber recordings of MSNA involved vascular tone in obesity and also that increased SNA does not necessarily translate into increased vascular tone [30]. It is worth noting that MSNA was mainly measured rather than renal SNS activity, the most important pathway for SNS to cause chronic HT [15]. So MSNA activity may not reflect renal sympathetic nervous activity. Renal sympathetic nerves mediate most, if not all, of the chronic effects of SNA on BP in obesity. In obese dogs fed with a high-fat diet, bilateral renal denervation greatly attenuates sodium retention and HT. Thus, obesity may increase renal sodium reabsorption and cause HT mainly by increasing renal sympathetic activity [15].

4. Mechanisms of Sympathetic Overactivity in the Metabolic Syndrome

Among the different hypotheses which have been proposed to explain the cause of obesity and obesity-related metabolic disturbances, SNS activation plays thus a pivotal role. A large body of evidence clearly shows that sympathetic activity is increased in human obesity [32, 33]. In 1986, Landsberg suggested that sympathetic activation could represent an insulin-mediated adaptative response to overeating promoting thermogenesis and acting as a buffer against weight gain [34]. Reaven first proposed I.R. as the key abnormality leading to hyperinsulinemia, sympathetic activation, and HT [14]. Later on, other investigators stressed the important role of I.R. and hyperinsulinemia and their relationship to SNS activation [35, 36].

Interestingly, Julius et al. proposed increased sympathetic activity as the primary defect leading to I.R. and weight gain [35]. Whether SNS activation is the cause or the consequence of obesity is still matter of debate. SNS activation results in the release of norepinephrine which stimulates adrenergic receptors. Physiological responses depend upon the receptors present in the target organs, the fasting state, and the rate of neuronal firing [37]. Cardiovascular, renal, and metabolic effects of chronic and sustained SNS activation may contribute to HT and the development of I.R. over a prolonged period of time [37]. The pathophysiological mechanisms linking SNS overactivity and obesity-related MetS are complex and still need to be fully elucidated.

Multiple neurohumoral mechanisms can activate the SNS in patients with the MetS. Neural mechanisms include direct activation of the SNS in response to the activation of higher cerebral nuclei by hunger or feeding and renal afferent nerve activation mediated by perirenal fat accumulation and kidney compression [15]. Sympathetic activation can also be triggered by reflex mechanisms (arterial baroreceptor impairment), psychological stress, oxidative stress, obstructive sleep apnea, inflammation, and metabolic factors as I.R.

Table 1: Effects of insulin resistance, ghrelin, and some adipokines on endocrine and metabolic functions in the pathogenesis of the MetS.

	General effects	Effects on sympathetic nervous system	*In vitro*/animal studies (references)	Human studies (references)
Insulin resistance	Direct antinatriuretic action	(i) Its intracerebral administration increases sympathetic outflow (ii) Induces sympathetic overactivity (iii) Stimulates SNS to increase cardiac output	[46–50]	[39, 49, 56–58]
Leptin	(i) Levels correlate with adipose tissue mass (ii) Satiating factor decreases food intake (iii) Physiological regulation of feeding behavior trough hypothalamic receptors	Vasocontractile effect related to SNS activation	[90, 92, 95, 97, 103, 104, 109, 110]	[90, 91, 99, 100, 102, 107, 111]
NEFAs	Levels are increased in obesity and inversely correlated with insulin sensitivity	Induce a central activation of MNSA in lean subjects	[115–118]	[114, 119, 122]
Adiponectin	(i) Levels are inversely related to obesity, DM, and insulin resistant states (ii) Ameliorates obesity-related hypertension		[127, 128, 132, 133]	[129, 131]
Ghrelin	(i) Its infusion decreases blood pressure and HR (ii) Improves endothelial function (iii) Promotes weight gain and increases appetite	Its infusion increases SNS activity	[136, 137, 141, 143]	[140, 142, 144]

and dysregulated production and secretion of adipokines from visceral fat with a particular important role of leptin.

Table 1 summarizes the possible causes and clinical effects of the MetS. We will first focus on the role of I.R./hyperinsulinemia, the key metabolic alteration in MetS, and then discuss the role of adipokines in the activation of SNS and their interplay with insulin. Figure 1 summarizes these interactions.

5. Hyperinsulinemia and Sympathetic Overactivity

Hence, insulin plays a pivotal role in the development of DM, HT, and the MetS [38]. Insulin stimulates SNS to increase cardiac output and the delivery and enhances the utilization of glucose in the peripheral tissues [39]. I.R. is the inability of insulin to produce its numerous actions, in spite of its normal secretion from the pancreatic beta cells [15, 40]. Insulin elicits its various biological responses by binding to a specific receptor [41, 42]. The ability of insulin receptor to autophosphorylate and then phosphorylate intracellular substrates is crucial for the complex cellular responses to insulin [41–43]. The two major signaling pathways activated by insulin binding to its receptor, the phosphatidylinositol-3′-kinase (PI3K) pathway and the mitogenic-activated protein kinase (MAPK) pathway, among their effects, play a role in vasodilatation and in the decrease in nitric oxide (NO) production, respectively, [43, 44]. In the MetS I.R. mainly results from an impairment in the cellular events distal to the interaction insulin/insulin surface receptor [14]. Selective I.R., located primarily in the muscle and the adipose tissue, causes compensatory hyperinsulinemia which has an adverse impact on insulin-sensitive tissues [41, 42]. I.R. arises due to various genetic and acquired factors, including obesity [45]. The effects of insulin on BP are multifactorial, including sympathetic activation and direct antinatriuretic action [43]. In animal studies insulin increases sympathetic outflow via intracerebral administration [46, 47]. Recent investigations from Cassaglia and coworkers identify the arcuate nucleus, via the paraventricular nucleus of the hypothalamus, as the central site of action of insulin in the increase of SNS activity and in the sympathetic baroreflex gain [48]. Insulin receptors in the hypothalamus coactivate the SNS through a transport-mediated uptake of peripheral insulin across the blood-brain barrier [49]. Also, the presence of highly permeable capillaries in the arcuate nucleus (AN) allows insulin to activate receptors without a specific transport mechanism [50, 51]. In 1991, Anderson and coworkers showed that hyperinsulinemia causes sympathetic activation in humans [52]. Sympathetic overactivity occurring in the MetS is dependent on hyperinsulinemia and related I.R. [53]. Human studies suggest that hyperinsulinemia may contribute to the increased SNS activity observed in the obese MetS patients. Insulin secretion following a meal [54–56] or during a hyperinsulinemic euglycemic clamp [52, 57–60] determines an increase in MSNA

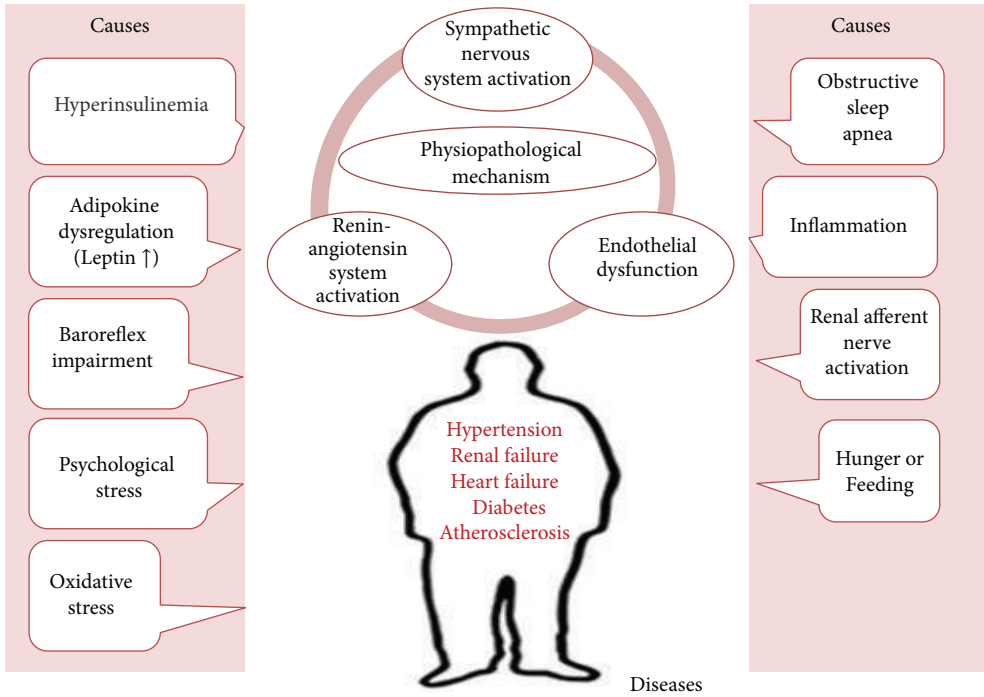

Figure 1: In obesity, infiltration of inflammatory cells in the white adipose tissue disturbs the secretion of adipokines and increases the activity of adipocyte renin-angiotensin system. Increased secretion of leptin and proinflammatory cytokines and decreased amounts of adiponectin contribute to the development of obesity-related hypertension.

and enhances the arterial baroreflex gain of SNA. Interestingly, chronic SNS overactivity contributes to a further decline of insulin sensitivity. Furthermore, SNS coactivation by the hypothalamic-pituitary-adrenal axis may also occur in the hyperinsulinemic state secondary to obesity [29]. In addition to the neural SNS overactivity at rest, obese subjects demonstrate baroreflex impairment and blunted responses to sympathoexcitatory manoeuvres. The exact cause of changes in baroreflex function in obese patients is not entirely clear. Changes in baroreceptor signalling may be a contributing factor to sympathetic overactivity as well as reduced baroreflex responsiveness [29]. Following hypocaloric diet, obese subjects showed a reduction in MSNA and whole body NE spillover rate [61]. However, during weight maintenance period following weight loss MSNA rebounded while NE spillover was preserved [29]. Recent findings from Straznicky et al. on obese MetS patients demonstrated that the progression to type II DM is associated with increased central sympathetic drive, blunted sympathetic responsiveness, and altered NE disposition [62]. Many studies showed that perturbed autonomic nervous system function in the MetS may be reversible. Recently, caloric restriction inhibited SNA via an antioxidant mechanism in the rostral ventrolateral medulla in obesity-induced hypertensive rats [63]. Also, data from Lambert et al. showed that in MetS patients dietary weight loss decreased sympathetic nerve firing and improved hemodynamic and metabolic parameters [64]. It is worth noting that in a very recent longitudinal investigation from Licht and coworkers, a dysregulation of autonomic nervous system in subjects under stress predicted 2-year development of the MetS [65]. However, other studies call into question the role of hyperinsulinemia in the determination of sympathetic overactivity. Obese subjects do not appear to retain their sensitivity to the stimulatory effects of insulin on SNS [29]. A greater increase in MSNA activity in response to euglycemic hyperinsulinemia was observed in lean subjects compared to obese by Vollenweider and coworkers [66]. Later on, similar results were obtained by Straznicky et al. in patients with I.R. and MetS, who exhibited a blunted sympathetic response to increased plasma insulin following a glucose load [67] consistent with central I.R. Certain adipokines expressed in central obesity may contribute to determining sympathetic overdrive and in case of overexpression may contribute to I.R., resulting in hyperinsulinemic state with greater sympathetic outflow [29].

Among the factors promoting the development of I.R. and progression of MetS, reactive oxygen species (ROS) play an important role. In obesity ROS are increased and ROS can be reduced with weight loss in humans [68]. In rats, obesity induced by a high-fat diet resulted in enhanced oxidative stress [69]. Ogihara and coworkers reported that ROS overproduction may cause insulin resistance in AngII-infused rats [70]. Blendea and coworkers confirmed these data and showed that insulin resistance was ameliorated by tempol in TG(mREN-2)27 (Ren-2) transgenic rats, which have a stimulated renin-angiotensin system [71]. Thus, ROS overproduction results in I.R. In mice, ROS overproduction in target organs of insulin, like adipose tissue and liver, preceded the onset of obesity and I.R. [72]. In a recent investigation, Yubero-Serrano and coworkers showed that

the higher the number of MetS components, the greater the degree of oxidative stress, leading to increased plasma superoxide dismutase and glutathione peroxidase activities, plasma H_2O_2, lipid peroxidation products and sVCAM-1, and as well to decreased postischemic reactive hyperemia and total plasma nitrites. Also, oxidative stress increase was associated with higher body mass index, waist circumference, diastolic blood pressure, HOMAIR and triglycerides, glucose and insulin, and lower HDL-cholesterol and HOMAb and QUICKI indexes [73], confirming previous results from Fujita and coworkers [74].

6. Visceral Adiposity and MetS

Although adiposity is defined as an increase in total body mass, visceral fat expansion correlates with a cluster of metabolic abnormalities observed in the MetS [75]. Visceral fat represents a metabolically active organ and has been strongly related to insulin sensitivity [76, 77] and CVD both in humans and animals [77]. Subcutaneous fat, characterized by insulin-sensitive adipocytes, is mainly a fat depot. Instead, visceral fat adipocytes are insulin-resistant cells within a network of blood capillaries and infiltrating inflammatory cells [40]. Inflammatory cells within the visceral fat may play a role in adipocyte behaviour as a source of hormones and cytokines, called adipokines, with proinflammatory and proatherogenic action. Circulating cytokines including resistin and leptin are generally increased in obese subjects and in patients with DM [76, 78–80]. On the other hand, circulating adiponectin is decreased. Adiponectin is a tissue-specific circulating hormone with insulin-sensitizing and antiatherogenic properties. Also, adiponectin stimulates glucose and fatty acid oxidation in the muscle, enhances insulin sensitivity in the liver, increases free fatty acid oxidation, reduces hepatic glucose output, and inhibits monocyte adhesion and macrophage transformation to foam cells within the vascular wall [78–80].

I.R. and visceral obesity determine BP elevation by activating SNS and renin-angiotensin-aldosterone system (RAAS) [40], resulting in sodium retention and volume expansion as well as and endothelial and renal dysfunction [41, 44, 81]. Hyperinsulinemia activates RAAS in both heart and blood vessels, with production of angiotensin II which has proatherogenic effects. Angiotensin II inhibits vasodilator effects of insulin on blood vessels and glucose uptake into the skeletal muscle [41, 44, 82] resulting in decreased NO production, vasoconstriction, and GLUT 4 inhibition [40].

The presence of endothelial dysfunction in patients with obesity and I.R. was first reported by Steinberg and coworkers. Adipokines dysregulation and inflammatory state disrupt vascular homeostasis by causing an imbalance between the NO pathway and the endothelin 1 system, with impaired insulin-stimulated endothelium-dependent vasodilation [83].

It is noteworthy that in obesity vascular dysfunction also involves the other layers of the vessel wall. Obesity-induced changes in medial smooth muscle cells disrupt the physiological facilitatory action of insulin on the responsiveness to vasodilator stimuli, whereas the adventitia and perivascular fat appear to be a source of proinflammatory and vasoactive factors possibly contributing to the endothelial and smooth muscle cell dysfunction and to the pathogenesis of vascular disease as pointed out by Tesauro and coworkers [84, 85].

At the present time, body weight control has not yet proved to prevent metabolic and cardiovascular complications of obesity on a large scale. Recent data from the same investigators show that in the forearm circulation of hyperinsulinemic MetS patients, Rho-Kinase inhibition by fasudil improves both endothelium-dependent and independent vasodilator responsiveness, possibly by increased oxidative stress [86].

7. Effects of Adipokines on Sympathetic Overactivity

7.1. Adipokines. Adipose tissue produces bioactive substances, known as adipokines, and releases them into its direct surroundings and into the bloodstream [87]. Among the different actions of the adipokines is the regulation of arterial tone [80, 88]. Therefore, adipose tissue affects not only metabolism but also many functions of organs and tissues, such as brain, muscle, liver, and blood vessels. The presence of a normal amount of adipose tissue is essential. An imbalance can cause dysregulation in the release of adipokines that may result in vascular disturbances and inflammation [89]. Adipokines may alter SNS activity and impair insulin signaling. Adipokines mainly involved in obesity and MetS are leptin, nonesterified free fatty acids (NEFAs), reactive oxygen species (ROS), adipocytic angiotensinogen and resistin. A reduction of adiponectin may be involved as well. An unbalanced interplay between these adipokines may lead to an impaired insulin signaling as well as to a state of inflammation and/or alter sympathetic regulation [29]. The aim of this section is to focus on the main SNS-activating adipokine, leptin, and on the main SNS-inhibiting one, adiponectin.

7.2. Leptin and Leptin Resistance. Leptin is a 167 aminoacid-16 kDa protein. It is secreted from adipocytes proportionally to the adipose tissue mass [90–92]. Physiologically, leptin represents the inhibitory signal from fat that informs the brain about the body's stocks of stored energy [93]. Its ability to produce anorexigenic effects has been extensively studied and is beyond the scope of this review. Leptin decreases food intake, and this is particularly dependent on the depolarization and hyperpolarization of neurons in the AN of the hypothalamus [94, 95]. Leptin resistance develops in obesity because the ARH neurons expressing leptin receptors do not become further activated from baseline in response to exogenous leptin; consequently, increased leptin levels do not increase energy expenditure or decrease food intake [96, 97]. How leptin resistance develops and how it could be treated in obesity is now under investigation.

Whether SNA increase in obesity [31, 98, 99] leads to innervation of all organs or it is organ-specific is still matter of debate. An interesting finding, as it is now readily hypothesized, is that a chronic increase of SNA to the kidney contributes to the development of HT. HT is part of

the MetS and is well known to contribute significantly to the development of CVD. Leptin acutely increases SNA [100, 101], although, at the present time, no conclusive data demonstrate that leptin increases SNA chronically, leading to HT. The increase of SNA observed in obesity also appears to cause organ damage, which exacerbates the risk of MetS and CVD [102]. When acutely injected into the dorsomedial hypothalamus of anesthetized rats leptin determines an increase in HR and BP [103]. Very recent animal studies show that leptin exerts its action at the level of the nucleus of the solitary tract where it alters the activity of neurons that mediate the cardiovascular responses to the activation of the aortic baroreceptor reflex [104] and in the forebrain to influence the baroreflex control of lumbar, renal, and splanchnic SNA and finally the HR [105]. Also, leptin activates brain centers that regulate SNS activity through a melanocortin-system-dependent pathway [106].

The interactions between the brain melanocortin system and leptin represent an important area of research to further understand the mechanisms leading to SNS activation in obesity.

Determining whether hyperleptinemia may be the cause of chronically elevated SNA in obesity, via activation of leptin receptors in higher brain regions, will hopefully lead to new treatment options for obesity. In a recent work, Curry and coworkers showed that a low MSNA and a lack of SNS-mediated support of resting energy expenditure 3 years after gastric by-pass should possibly be multifactorial in origin and involve changes in insulin sensitivity, body composition, and leptin [107].

Physiologically, leptin contributes to BP by its vasorelaxing and vasocontractile effects [108, 109]. While the contractile effect of leptin is attributed to SNS activation [110], various mechanisms seem to be responsible for leptin-induced vasorelaxation. This latter effect can be endothelium-dependent, either through the release of NO [110] or by other mechanisms [109, 111]. Recent findings from Schinzari and coworkers suggest that, under physiologic conditions, leptin stimulates both endothelin-1 and NO activity in the human circulation. This effect is absent in hyperleptinemic patients with MetS who are unresponsive to additional leptin [112].

7.3. Nonesterified Fatty Acids. Nonesterified fatty acids (NEFAs) act on all the aspects of glucose homeostasis, from uptake at peripheral tissue to hepatic production and disposal. NEFAs are increased in obesity and are inversely correlated with insulin sensitivity [113, 114]. Increased NEFAs may inhibit glucose uptake into peripheral tissues by impairing PI3-kinase activation [115]. PI3-kinase activation is lost indeed as a result of a high fat diet in mice [116]. Impaired PI3-kinase activation may also be due to excess of protein kinase C [117]. NEFAs can cross the blood-brain barrier, cause a central activation of MSNA in lean subjects [118, 119], and reduce cardiovagal baroreflex in lean and obese subjects [120]. NEFAs also stimulate plasminogen activator inhibitor-1, which may contribute to the association between increased plasma NEFA in obesity and the augmentation of MSNA [121]. However, other investigations demonstrated no significant effect of NEFAs on MSNA or sympathetic baroreflex sensitivity [122]. Furthermore, whole body and renal NE spillover did not change during infusion of NEFAs [123].

7.4. Adiponectin. Adiponectin is an anti-inflammatory, insulin-sensitizing, and antiatherogenic protein exclusively secreted by adipocytes. Adiponectin is an adipose tissue hormone, also known as gelatin-binding protein-28 (GBP28), AdipoQ, adipocyte complement-related protein (ACRP30), or apM1. Adiponectin circulates in trimeric, hexameric, and high-molecular-mass species. Different forms of adiponectin may play distinct roles in the balance of energy homoeostasis. Adiponectin is an insulin sensitizing hormone that exerts its action through its receptors Adipo R1, Adipo R2, and T-cadherin. Adipo R1 is expressed mainly in muscle, whereas Adipo R2 is predominantly expressed in the liver.

Adiponectin is inversely related to obesity, DM, and other I.R. states that cause metabolic dysfunction; adiponectin deficiency may also contribute to coronary heart disease, steatohepatitis, I.R., nonalcoholic fatty liver disease, and a wide array of cancers.

The human adiponectin gene was cloned through systematic sequencing of an adipose-tissue library [124, 125]. The apM1 gene encodes a 244 amino acid open reading frame containing a putative signal sequence repeat (66 amino acids) followed by a cluster of aromatic residues near the C terminus that shows a high local resemblance to collagens X and VIII and complement factor C1q [126].

The regulation of adiponectin receptors Adipo R1 and Adipo R2 is important to facilitate essential physiological functions. Adipo R1 is expressed ubiquitously and exhibits high affinity to the ligand, whereas Adipo R2 exhibits intermediate affinity. The expression of adiponectin and its receptors has been investigated in streptozotocin (STZ)-induced diabetic rat heart and in mouse skeletal muscle. STZ-induced DM upregulates adiponectin receptors in the heart [127].

Some evidence suggests that T-cadherin can bind to the hexameric and HMW forms of adiponectin but not to monomer globular and trimeric forms. T-cadherin is expressed ubiquitously, with the highest expression found in the heart and the aortic, carotid, iliac, and kidney arteries [128]. T-cadherin is bound to adiponectin and is critical for the association of adiponectin protection with cardiac stress in mice.

The pleiotropic roles of adiponectin have been studied in multiple *in vitro* and *in vivo* models. The multiple molecular targets of adiponectin mediate multiple pharmacological actions.

A steep rise in the prevalence of obesity has occurred over the past few decades. Obesity is inversely related to adiponectin, making adiponectin a negative marker of MetS. Furthermore, the expression of the receptors Adipo R1 and Adipo R2 declines by 30% in the subcutaneous fat of obese individuals, while they normalize following weight loss [129]. Adiponectin may play an important role in type II DM, HT, multiple sclerosis, and the dyslipidemias. The most significant role played by adiponectin is that of its insulin-sensitizing

effect. Adiponectin in the diabetic's blood is lower than normal, whereas higher adiponectin in plasma minimizes the risk of type II DM [130]. Adiponectin relates negatively to blood glucose and insulin. Total adiponectin, HMW adiponectin, and the total/HMW ratio are all inversely related to homeostasis model assessment I.R. index. The total/HMW ratio is considered a better indicator of I.R. than total plasma adiponectin [131]. The role of adiponectin in I.R. was determined using knockout mice. These mice had normal plasma insulin, but its capability of lowering blood glucose was severely impaired, this clearly pointing to the role of adiponectin in glucose tolerance [132]. The absence of serum adiponectin in lipoatrophic mice causes hyperglycemia and hyperinsulinemia, which can be normalized by adiponectin injections. All studies on the putative role of adiponectin in IR and type II DM suggest that low plasma adiponectin causes susceptibility to these disorders.

In conclusion, adiponectin exerts an insulin-sensitizing action with profound effects on fatty acid oxidation and inflammation. Drugs affecting serum adiponectin may have a role in the treatment of type II DM and possibly the Mets. In obese adiponectin-knockout mice with HT adiponectin replenishment lowers elevated BP [133]. Existing drugs such as peroxisome proliferator-activated receptor agonists (thiazolidinediones), some angiotensin Adipo R1 receptor blockers (telmisartan), angiotensin-converting enzyme inhibitors, and cannabinoid Adipo R1 receptor blockers (rimonabant and taranabant) may increase circulating adiponectin [134]. However, future strategies should focus on upregulation of adiponectin/adiponectin receptors expression or on targeting adiponectin receptors with specific agonists [132]. Modulation of adiponectin actions through the expression of adiponectin receptors may thus be a novel and promising therapeutic option.

7.5. Ghrelin. Ghrelin is a 28-amino-acid growth-hormone-releasing peptide secreted by the stomach [135]. It causes increased food intake and weight gain in rodents [136, 137]. But in obese subjects circulating levels are decreased. This observation is against a central role of ghrelin in the determination of common obesity [138, 139]. It is noteworthy that ghrelin infusion decreases BP by 5–10 mmHg, although it also increases SNS activity, perhaps through compensatory baroreflex activation [140]. The long-term central nervous system mediated cardiovascular actions of ghrelin are still unknown. A recent work of Freeman et al. demonstrated that chronic central ghrelin infusion reduces BP and HR despite increasing appetite and promoting weight gain in normotensive and hypertensive rats [141].

Ghrelin may also improve endothelial function mimicking phosphoinositol 3-kinase-dependent actions of insulin to stimulate production of NO by endothelial cells and restoring the endothelin 1/nitric oxide balance in patients with obesity-related MetS, as observed by Tesauro and coworkers [142–144].

7.6. Further Perspectives. The prevalence of the MetS is escalating worldwide. Sympathetic overdrive may be the common thread of visceral adiposity, HT, dyslipidemia, and glucose intolerance in the clinical diagnosis of the MetS [29]. MetS is thus characterized by sympathetic overdrive with outflow activation to both kidneys and adipose tissue among others. Although the mechanisms responsible for the initial activation of SNS are still to be fully elucidated, hyperinsulinemia, derangement of circulating adipokines, and beta receptor polimorphisms are all implicated and may cause the development of HT, I.R., diastolic dysfunction, and finally renal disease. Although lifestyle correction and hypotensive medications are the first-line therapy for obesity and HT in the MetS, interventions that target the SNS directly may be of further benefit. Such benefits may even be weight-unrelated and associated with a significant reduction in end-organ damage [37]. Afferent adrenergic signaling from the kidneys was recently identified as an important contributor to central SNS overdrive and SNS outflow to the kidneys is involved in cardiovascular, renal, and metabolic control [145, 146]. In recent clinical investigations, functional renal denervation obtained by means of catheter-based radiofrequency or ultrasound technologies achieved BP control in patients with resistant HT [147, 148] and polycystic ovary syndrome [149], further emphasizing the link between SNS overdrive and I.R., HT, and other comorbidities in the MetS. Finally, catheter-based renal denervation was investigated in end-stage renal disease (ESRD) patients with resistant intradialytic HT, who are supposed to compel a significant SNS overdrive [150, 151]. In preliminary investigations, a reduction of SNS overdrive with good control of blood pressure was obtained in small series of ESRD disease patients on maintenance hemodialysis by Di Daniele and coworkers [152], Ott et al. [153], and Schlaich and Coworkers [154].

Further investigations addressing the still open questions in the treatment of resistant HT and evaluating potential new indications such as the MetS or heart failure are still necessary to prove the safety and effectiveness of renal denervation in these patients. By modulating sympathetic activity, renal denervation may have the potential to provide significant benefits in a variety of diseases.

Abbreviations

MetS: Metabolic syndrome
CVD: Cardiovascular disease
BP: Blood pressure
HT: Hypertension
CKD: Chronic kidney disease
SNS: Sympathetic nervous system
RAAS: Renin-angiotensin system
I.R.: Insulin resistance
SNA: Sympathetic nerve activity
MSNA: Muscle sympathetic nerve activity
NE: Norepinephrine
AN: Arcuate nucleus
DM: Diabetes
HR: Heart rate
NO: Nitric oxide
PI3K: Phosphatidylinositol-3′-kinase
MAPK: Mitogenic-activated protein-kinase

NEFAs: Nonesterified fatty acids
STZ: Streptozotocin
HMW: High molecular weight
ESRD: End-stage renal disease
ROS: Reactive oxygen species.

Conflict of Interests

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

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Research Article

Cross-Sectional and Longitudinal Associations between Body Mass Index and Cardiometabolic Risk Factors in Adolescents in a Country of the African Region

Tanica Lyngdoh,[1] Bharathi Viswanathan,[2] Edwin van Wijngaarden,[3] Gary J. Myers,[4] and Pascal Bovet[1,2]

[1] *Institute of Social and Preventive Medicine, Lausanne University Hospital, CH-1010 Lausanne, Switzerland*
[2] *Section of Noncommunicable Diseases, Ministry of Health, Victoria, Seychelles*
[3] *Department of Community and Preventive Medicine, University of Rochester Medical Center, Rochester, NY, USA*
[4] *Departments of Neurology, Pediatrics, and Environmental Medicine, University of Rochester Medical Center, Rochester, NY, USA*

Correspondence should be addressed to Pascal Bovet; bovet.pascal@gmail.com

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We assessed the association between several cardiometabolic risk factors (CRFs) (blood pressure, LDL-cholesterol, HDL-cholesterol, triglycerides, uric acid, and glucose) in 390 young adults aged 19-20 years in Seychelles (Indian Ocean, Africa) and body mass index (BMI) measured either at the same time (cross-sectional analysis) or at the age of 12–15 years (longitudinal analysis). BMI tracked markedly between age of 12–15 and age of 19-20. BMI was strongly associated with all considered CRFs in both cross-sectional and longitudinal analyses, with some exceptions. Comparing overweight participants with those having a BMI below the age-specific median, the odds ratios for high blood pressure were 5.4/4.7 (male/female) cross-sectionally and 2.5/3.9 longitudinally ($P < 0.05$). Significant associations were also found for most other CRFs, with some exceptions. In linear regression analysis including both BMI at age of 12–15 and BMI at age of 19-20, only BMI at age of 19-20 remained significantly associated with most CRFs. We conclude that CRFs are predicted strongly by either current or past BMI levels in adolescents and young adults in this population. The observation that only current BMI remained associated with CRFs when including past and current levels together suggests that weight control at a later age may be effective in reducing CRFs in overweight children irrespective of past weight status.

1. Introduction

Obesity is known to be associated with cardiovascular outcomes [1–3] and with elevated levels of several cardiometabolic risk factors (CRFs), including blood pressure and several metabolic factors [4]. The association between obesity and several CRFs has already been observed in children and adolescents both cross-sectionally [5–7] and longitudinally [8–10], but the data are limited. Furthermore, intervention studies through lifestyle interventions [11], and bariatric surgery [12] in children show that a reduction of body weight among obese children results in reduced levels of several CRFs, such as blood pressure [13–15], lipids [16–18], and insulin [19]. There are several methods to measure adiposity in children, including BMI, waist circumference, or the waist-to-hip ratio. There is continued debate on which indicator best predicts cardiovascular risk [20] but several studies suggest that body mass index (BMI) can adequately predict cardiovascular risk in adults, adolescents, and young adults [9, 21, 22].

There is, however, a relative scarcity of studies that have examined the relation between obesity and CRFs in population-based samples of children and adolescents (i.e., outside of the clinical setting). Furthermore, most such

studies in children and adolescents have relied on a cross-sectional design, although a few did have a prospective design, but most such studies were conducted in high-income countries [8, 9]. Furthermore, only a few studies have simultaneously compared the relative strength of the associations between obesity and a broad set of CRFs including both physiological risk factors (e.g., blood pressure) and biological markers (e.g., blood lipids, glucose, and uric acid) in youths so that the associations between obesity and different CRFs can be directly compared.

In this study, we examined both the cross-sectional and longitudinal relationships between overweight and several CRFs in a population-based sample of adolescents and young adults. Data on CRFs were derived from a cohort study of children followed since their birth to examine the association between maternal fish consumption and the children's neurological development [23, 24]. In this cohort study, BMI and CRFs were measured for the first time on the seventh follow-up visit at the age of 19-20 years. We then linked the CRF data with BMI values measured at the age of 12–15 years previously obtained during a routine screening program in all schools of the country [25, 26]. The main objectives of the present study were to directly compare the associations between obesity and several CRFs, to compare the associations based on cross-sectional or longitudinal designs, and to examine the contribution of current and past weight statuses in the prediction of current CRF levels.

2. Methods

2.1. Study Population. The Republic of Seychelles is a rapidly developing small island state in the Indian Ocean located east of Kenya. The population is mostly of African descent. Between February 1989 and February 1990, a cohort of 779 children was enrolled at the age of 6 months as part of the Seychelles Child Development Study (SCDS). Details of this cohort study have been previously described [24]. The SCDS was undertaken to assess the association between prenatal exposure to methyl mercury from fish consumption and neurocognitive development in a sample of children representative of the general population. BMI and a broad set of cardiometabolic risk factors were measured for the first time in the seventh follow-up visit in 2008-2009 when the participants were aged 19-20 years (age range: 18.7–20.5; mean age: 19.5; SD: 0.5).

2.2. Linkage of Data. BMI at the age of 12–15 years was derived from a routine school-based surveillance program. BMI was measured when children were in the 7th and 10th grades and were aged approximately 12.7 years for children in the 7th grade (SD: 0.4; range: 11.6–13.4) and 13.9 years for children in the 10th grade (SD: 0.6; range: 12.1–15.9). The school screening program was conducted in all public and private schools in Seychelles under the auspices of the Ministries of Health and Education. The methods and results of this screening program have been published previously [25–27]. Linkage of BMI in the school-based screening program with CRFs measured at the age of 19-20 years within the SCDS study was based on the national identification number that is available for all Seychelles citizens. We had previously linked data from the SCDS with data from the school screening program in order to examine the relationship between exposure to prenatal mercury (SCDS) and blood pressure at the age of 12–15 years (school screening program) [28]. Informed consent was obtained from the subject and caregiver of every participant for both the SCDS and the school screening program. The school screening program and the SCDS were approved by the Research and Ethical Committee of the Ministry of Health, Victoria, Seychelles. In addition, the SCDS was approved by the Human Subjects Review Board at the University of Rochester, Rochester, NY, USA.

2.3. Clinical Measurements. Measurement of anthropometrics at age of 12–15 years has been detailed previously [27]. Briefly, weight was measured without shoes and in light garments by trained school nurses in the schools, during regular school hours (8 am–2:30 pm), using electronic scales (Seca 870, Hamburg, Germany). Height was measured with a fixed stadiometer (Seca 208). The same weight scales and stadiometers were available in all schools and the instruments were regularly checked for accuracy by the screening nurses and further checks were preformed at least once per year by the manager of the screening program. BMI was calculated as weight (kg) divided by height (meters) squared. We used the average of the two values of BMI measured at ages 12 and 15 years, to reflect BMI in early adolescence.

At the age of 19-20 years, all measurements took place between 8 a.m. and 10 a.m. at the SCDS study center. Weight was measured with a precision electronic scale (Seca 870) and height was measured with a fixed stadiometer (Seca 208). The instruments were regularly checked for accuracy. BP was measured using a validated oscillometric automated device (Omron M5). Three readings were measured by a nurse, on the left arm, and using an appropriately sized cuff. Readings were taken at intervals of at least 1 minute between the BP readings after the participant had been sitting for at least 5 minutes. Systolic BP (SBP) and diastolic BP (DBP) were based on the average of the three readings.

2.4. Biological Samples. The methods used to measure venous blood taken at the age of 19-20 years have been described previously [21]. Briefly, venous blood samples were collected after an overnight fast. Glucose, total cholesterol, HDL-cholesterol, triglycerides, and uric acid were measured at the central laboratory of the main hospital in Seychelles (Seychelles Hospital), using standard enzymatic methods (Konelab T series reagents, Thermo Scientific) with a Thermo Konelab 30 automatic analyzer (Konelab Corp., Espoo, Finland). LDL-cholesterol was calculated using the Friedewald formula.

2.5. Statistical Analyses. Among the 423 adolescents who had complete data on CRFs and BMI at the age of 19-20 years in the SDCS (77% of 549 children seen at the age of 19-20 years), 390 (92%) also had data on BMI at the age of 12–15 years; hence, this study includes 390 participants.

Analyses were conducted separately in males and females because of substantial sex differences in the distribution of several variables, including BMI and several CRFs, and the possibility that the relations between BMI and CRFs may differ according to sex. In order to examine the associations between BMI and CRFs, we considered three categories of body weight: (1) "low" BMI was defined as a BMI below the median values for age and sex (both at age of 19-20 years and at age of 12–15 years); (2) "elevated" BMI was defined as BMI above the age- and sex-specific median values of BMI and below the age- and sex-specific BMI cut-off values for "overweight"; and (3) "overweight." Overweight at age of 12–15 years was defined according to the age- and sex-specific IOTF criteria of overweight [29]. Overweight in persons aged 18 years and above follows guidelines in adults and are defined as a BMI of 25 or above [30]. In both adolescents and young adults our cut-off values for "overweight" encompass both "overweight" and "obesity" categories. We did not distinguish between "overweight" and "obesity" because of an insufficient number of participants. We also conducted analyses examining the relationship between CRFs and joint categories of body weight at both age of 12–15 years and at age of 19-20 years (e.g., high-high, low-low, high-low, low-high) but the sample sizes in some categories (high-low, and low-high) were too small for meaningful statistical inference (results are available from the authors). We tabulated the mean values and 95% confidence intervals of CRFs according to these three categories of BMI separately in males and females. In order to directly compare the magnitude of the differences of the mean values of CRFs between these three BMI categories, we plotted the percent changes between values in the "elevated weight" and "overweight" categories compared to participants with low body weight.

We also provide results in terms of odds ratios of elevated levels of CRFs according to the three defined categories of BMI, at age of 12–15 years or at age of 19-20 years, as determined by logistic regression. Regression models were not adjusted for other covariates, since the aim of the study was to examine the performance of BMI to predict CRFs. We defined dichotomized categories of CRFs in youths aged 19-20 years based on recent guidelines for subjects aged 18–21 years [31]. However, because only 4% of subjects had triglycerides ≥1.3 mmol/L, we used a lower cutoff (≥1.0 mmol/L) for this parameter which resulted in a prevalence of 12%. There is no widely used cutoff for serum uric acid in adolescents or young adults so we defined elevated levels as levels above the sex-specific 80th percentile. The cut-off values for the dichotomized categories of CRFs appear in Table 3.

We also examined the linear relationship between CRFs and BMI (used as a continuous variable) for both the crosssectional (BMI and CRFs measured at the age of 19-20 years) and longitudinal (BMI measured at the age of 12–15 years and CRFs measured at the age of 19-20 years) associations using linear regression, separately in males and females. Analyses were done separately for males and females. We also examined regression analysis models that included both BMI at the age of 12–15 years and BMI at the age of 19-20 years to determine how well the BMI predicted the defined outcomes at each age (i.e., to examine the association between CRFs and BMI at age of 19-20 years given a certain BMI at age of 12–15 years and, inversely, the association between CRFs and BMI at age of 12–15 years given a certain BMI at age of 19-20 years). To permit a direct comparison of the results based on cross-sectional and longitudinal analyses, we used standardized regression coefficients, which express change in the considered outcomes associated with a 1 standard deviation change in the exposure (BMI). Analyses were performed using Stata 11.2. All analyses were done among the 390 children who had complete data at age of 12–15 years and at age of 19-20 years. We used two-sided tests and considered statistical significance as $P < 0.05$.

3. Results

Table 1 presents the mean values and standard deviations of BMI and CRFs as well as the prevalence of the defined categories of BMI in participants aged 12–15 years and aged 19-20 years, separately in males and females. The proportion of participants who were overweight in the school evaluations was 18.9% among males and 19.5% among females aged 12–15 years. At age of 19-20 years, 12.6% of males and 27.9% of females were overweight (this also includes obesity). BMI and several CRFs differed substantially between males and females at both the age of 12–15 years and at the age of 19-20 years. The Spearman correlation coefficients between BMI at age of 12–15 years and BMI at age 19-20 years were 0.69 ($P < 0.001$) in males and 0.83 ($P < 0.001$) in females, which suggests a high degree of tracking of BMI over age.

The distribution of the CRFs at the age of 19-20 years according to our three defined BMI categories at the age of 12–15 years and at the age of 19-20 years is presented in Table 2. A significant positive linear trend was found between categories of BMI and most CRFs at both ages. Associations did not reach statistical significance for triglycerides and glucose in males and LDL-cholesterol, HDL-cholesterol and triglycerides in females at age of 12–15 years and glucose in males at age 19-20 years.

Figure 1 shows the percent changes in mean CRFs levels in males and females comparing the categories "elevated BMI" and "overweight" with "BMI below the median." Overall, these changes were quite similar whether based on the longitudinal or cross-sectional analyses. However, the cross-sectional associations tended to be slightly larger than the longitudinal ones. The magnitude of the percent changes tended to be larger for several metabolic markers than for BP. However, the associations for the metabolic CRFs were not always statistically significant, which is consistent with larger variability (e.g., larger SD) for metabolic CRFs than for BP (Table 1).

Table 3 shows the odds ratios (ORs) for the presence of elevated CRFs in participants with elevated BMI and with overweight compared to participants with low BMI. Comparing participants with overweight versus participants with low BMI, these ORs were as high as 4.0 (95% CI: 1.5–10.4) for LDL-cholesterol among males aged 12–15 years and 8.0 (95% CI: 2.3–28.6) for triglycerides among males aged 19-20 years. Similar to the results from the stratified (Table 2)

Table 1: Characteristics of the participants at the age of 12–15 years and at the age of 19-20 years.

	Males (n = 175)	Females (n = 215)	P value
Age 12–15 years			
Mean age	13.88 (0.62)	13.89 (0.53)	
Age range	12.01–15.98	12.01–14.57	
BMI (kg/m^2)	19.35 (3.31)	20.29 (3.95)	0.013
Categories of BMI			
Overweight	33 (18.86)	42 (19.53)	
Elevated weight	43 (24.57)	77 (35.81)	
Low weight	99 (56.57)	96 (44.65)	0.035
Age 19 years			
Mean age	19.51 (0.33)	19.51 (0.29)	
Age range	18.8–20.4	18.8–20.5	
BMI (kg/m^2)	21.57 (3.47)	22.71 (5.45)	0.017
Categories of BMI			
Overweight	22 (12.57)	60 (27.91)	
Elevated weight	62 (35.43)	51 (23.72)	
Low weight	91 (52.00)	104 (48.37)	<0.001
Cardiovascular risk factors			
SBP (mmHg)	121.23 (10.74)	110.49 (9.75)	<0.001
DBP (mmHg)	67.36 (7.80)	69.06 (7.90)	0.034
LDL-cholesterol (mmol/L)	2.44 (0.77)	2.74 (0.84)	<0.001
HDL-cholesterol (mmol/L)	1.43 (0.41)	1.35 (0.39)	0.038
Triglycerides (mmol/L)	0.69 (0.28)	0.69 (0.35)	0.963
Glucose (mmol/L)	5.22 (0.58)	4.96 (0.54)	<0.001
Uric acid (mmol/L)	0.35 (0.08)	0.24 (0.07)	<0.001

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-cholesterol: low-density lipoprotein cholesterol; HDL-cholesterol: high-density lipoprotein.
Results are presented as mean (standard deviation) or as number (percentage).

and linear regression (Table 3) analyses, the magnitude of the associations based on dichotomous categories of CRFs (i.e., ORs for the presence of elevated CRFs versus non elevated CRFs) in relation to BMI categories was generally not different whether the results were derived from longitudinal analysis (BMI measured at the age of 12–15 years and CRFs measured at the age of 19-20 years) or cross-sectional analysis (BMI and CRFs both measured at the age of 19-20 years), with some exceptions.

Table 4 shows the standardized linear coefficients derived from linear regression between CRFs measured at the age of 19-20 years and BMI measured either at the age of 12–15 years or at the age of 19-20 years, in males and females separately. Most of the associations were highly significant for most CRFs at both the earlier and later ages. This is in line with what was found for stratified analysis (Table 2, Figure 1). The associations tended to be slightly stronger for the cross-sectional associations between BMI and CRFs measured at 19-20 years than for the longitudinal associations (BMI measured at the age of 12–15 years and CRFs measured at the age of 19-20 years). Consistent with results of stratified analysis (Table 2), no associations were found for glucose in males (with BMI measured at any age) as well as for LDL-cholesterol and HDL-cholesterol in females when BMI was measured at the age of 12–15 years.

In analyses including BMI measured at both ages, only BMI measured at the age of 19-20 years was significantly associated with CRFs, but not BMI measured at the age of 12–15 years. The results may be sensitive to the variability in the measurements of the exposure variable and reflect that BMI was measured more accurately during the research evaluation at 19-20 years (highly standardized protocol with few observers and scales). However, the standard deviations of BMI were not substantially different whether BMI was measured when participants were aged 12–15 years or 19-20 years. These results suggest that current BMI matters more than past BMI when predicting current levels of CRFs.

4. Discussion

We found that elevated levels of BP and several metabolic risk factors measured at the age of 19-20 years are predicted by either current BMI or BMI levels measured several years earlier (age of 12–15 years). Furthermore, when relating CRFs at the age of 19-20 years toboth current and past BMI levels, only current levels were significantly associated with CRFs. This suggests that a life course approach to weight control is needed but that current weight status is particularly important in order to limit impact of adiposity on CRFs. This is the first study to examine both the cross-sectional and longitudinal associations between obesity and a broad panel of CRFs in a population-based sample of adolescents and young adults in the African region.

Our findings of strong associations between obesity and several CRFs in adolescents and young adults are consistent with earlier studies and holds true whether analyses are cross-sectional [5–7] or longitudinal [8–10]. The CRFs most consistently associated with obesity in youth include high blood pressure, dyslipidemia, hyperinsulinemia, and insulin resistance [9, 10, 32]. What our study adds, is that a large impact of obesity on several CRFs can be observed both cross-sectionally and longitudinally in adolescents and young adults in a country in the Africa region. These data provide further useful evidence for the need to address obesity and its impact on CRFs in youth in the African region.

The increase in mean levels of CRFs in a dose-response manner across categories of BMI is consistent with previous studies showing a strong association and clustering of obesity with CRFs in young people. It is interesting to note, from our stratified analysis (Table 2), that the relation between BMI and mean levels CRFs seemed to be linear (i.e., an effect was also seen with the intermediate "elevated" category) in the case of BP, while the impact of BMI on several metabolic CRFs was most apparent only in the highest BMI category (i.e., overweight, which also includes obesity). An implication of these findings is that interventions targeting the entire population are useful for blood pressure control at

Table 2: Distribution of mean levels (and 95% confidence intervals) of cardiometabolic risk factors at the age of 19 years according to categories of body mass index measured at the age of 12–15 years or at the age of 19-20 years.

		Age 12–15 years			Age 19-20 years		
		Overall	M	F	Overall	M	F
SBP (mmHg)	Low weight	113.7 (112.2–115.4)	119.4 (117.3–121.5)	107.9 (106.1–109.8)	112.3 (110.7–113.9)	118.3 (116.0–120.5)	107.01 (105.3–108.8)
	Elevated weight	115.4 (113.4–117.4)	122.2 (119.1–125.4)	111.6 (109.5–113.7)	118.9 (116.9–120.9)	123.8 (121.3–126.2)	113.0 (110.48–115.6)
	Overweight	119.2 (116.4–122.0)	125.5 (121.5–129.5)	114.3 (111.1–117.5)	117.5 (115.2–119.9)	126.4 (121.9–130.8)	114.3 (111.9–116.7)
P-trend		*0.002*	*0.015*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
DBP (mmHg)	Low weight	66.5 (65.5–67.6)	66.1 (64.6–67.7)	66.9 (65.5–68.4)	66.0 (65.0–67.1)	65.3 (63.8–66.9)	66.6 (65.1–68.1)
	Elevated weight	68.7 (67.3–70.0)	67.5 (65.3–69.8)	69.3 (67.6–71.0)	68.9 (67.5–70.3)	68.3 (66.3–70.3)	69.6 (67.6–71.7)
	Overweight	72.3 (70.5–74.1)	70.9 (68.2–73.5)	73.4 (70.9–76.0)	72.9 (71.3–74.6)	73.2 (70.2–76.2)	72.8 (70.9–74.8)
P-trend		<0.001	0.003	<0.001	<0.001	<0.001	<0.001
LDL-cholesterol (mmol/L)	Low weight	2.50 (2.39–2.61)	2.33 (2.20–2.46)	2.67 (2.51–2.84)	2.44 (2.34–2.55)	2.26 (2.12–2.39)	2.60 (2.46–2.75)
	Elevated weight	2.71 (2.54–2.87)	2.46 (2.20–2.71)	2.85 (2.64–3.05)	2.68 (2.51–2.84)	2.57 (2.36–2.78)	2.81 (2.54–3.07)
	Overweight	2.73 (2.54–2.92)	2.74 (2.42–3.07)	2.72 (2.48–2.96)	2.90 (2.71–3.09)	2.82 (2.43–3.20)	2.93 (2.71–3.16)
P-trend		*0.009*	*0.010*	*0.428*	*<0.001*	*0.001*	*0.020*
HDL-cholesterol (mmol/L)	Low weight	1.42 (1.36–1.48)	1.47 (1.40–1.55)	1.36 (1.27–1.45)	1.46 (1.40–1.51)	1.51 (1.42–1.59)	1.41 (1.33–1.49)
	Elevated weight	1.37 (1.30–1.44)	1.41 (1.28–1.55)	1.35 (1.26–1.44)	1.34 (1.26–1.42)	1.33 (1.23–1.43)	1.35 (1.23–1.47)
	Overweight	1.33 (1.25–1.42)	1.34 (1.19–1.50)	1.32 (1.22–1.42)	1.29 (1.21–1.37)	1.43 (1.24–1.63)	1.24 (1.15–1.32)
P-trend		*0.062*	*0.051*	*0.596*	*0.001*	*0.058*	*0.008*
Triglycerides (mmol/L)	Low weight	0.66 (0.62–0.70)	0.67 (0.62–0.72)	0.65 (0.60–0.71)	0.63 (0.60–0.66)	0.63 (0.58–0.67)	0.63 (0.58–0.67)
	Elevated weight	0.67 (0.61–0.73)	0.66 (0.59–0.73)	0.68 (0.59–0.77)	0.64 (0.60–0.69)	0.69 (0.63–0.74)	0.59 (0.53–0.66)
	Overweight	0.78 (0.68–0.88)	0.78 (0.63–0.93)	0.78 (0.65–0.92)	0.89 (0.78–1.00)	0.94 (0.72–1.15)	0.87 (0.74–1.01)
P-trend		*0.165*	*0.405*	*0.211*	*<0.001*	*<0.001*	*0.007*
Fasting glucose (mmol/L)	Low weight	5.03 (4.96–5.11)	5.21 (5.09–5.33)	4.86 (4.77–4.95)	5.00 (4.92–5.08)	5.18 (5.05–5.32)	4.84 (4.76–4.93)
	Elevated weight	5.04 (4.94–5.13)	5.20 (5.01–5.39)	4.95 (4.84–5.05)	5.13 (5.03–5.22)	5.30 (5.17–5.43)	4.92 (4.81–5.04)
	Overweight	5.26 (5.11–5.42)	5.31 (5.12–5.50)	5.23 (4.99–5.47)	5.20 (5.05–5.34)	5.19 (4.93–5.43)	5.20 (5.02–5.38)
P-trend		*0.034*	*0.569*	*0.004*	*0.008*	*0.509*	*<0.001*
Uric acid (mmol/L)	Low weight	0.28 (0.27–0.29)	0.32 (0.31–0.34)	0.24 (0.23–0.26)	0.27 (0.26–0.29)	0.33 (0.31–0.34)	0.23 (0.22–0.24)
	Elevated weight	0.27 (0.26–0.29)	0.33 (0.31–0.35)	0.24 (0.22–0.25)	0.29 (0.27–0.30)	0.33 (0.32–0.35)	0.24 (0.22–0.25)
	Overweight	0.31 (0.29–0.34)	0.38 (0.35–0.41)	0.26 (0.24–0.29)	0.31 (0.29–0.33)	0.39 (0.34–0.44)	0.28 (0.26–0.29)
P-trend		*0.080*	*0.002*	*0.078*	*0.011*	*0.008*	*<0.001*

SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-cholesterol: low-density lipoprotein cholesterol; HDL-cholesterol: high-density lipoprotein cholesterol.
Elevated weight is defined for weight above sex- and age-specific medians and below cut off for overweight.

Table 3: Odds ratios for the association between cardiometabolic risk factors at the age of 19-20 years and body mass index categories measured at the age of 12–15 years or at the age of 19 years.

	12–15 years		19-20 years	
	Males (n = 175)	Females (n = 215)	Males (n = 175)	Females (n = 215)
Blood pressure ≥120/80 mmHg (M: 53.1%; F: 21.9%)				
Low BMI	1	1	1	1
Elevated BMI	1.27 (0.62–2.61)	3.89 (1.73–8.78)	3.12 (1.59–6.13)	4.70 (1.96–11.26)
Overweight	2.54 (1.10–5.90)	3.86 (1.53–9.73)	5.44 (1.84–16.06)	4.70 (2.02–10.94)
LDL-cholesterol ≥3.0 mmol/L (M: 17.7%; F: 36.3%)				
Low BMI	1	1	1	1
Elevated BMI	2.11 (0.81–5.56)	1.73 (0.93–3.24)	4.17 (1.60–10.88)	1.08 (0.52–2.22)
Overweight	4.00 (1.53–10.42)	1.42 (0.66–3.04)	6.86 (2.15–21.91)	2.52 (1.30–4.86)
HDL-cholesterol <1.03 mmol/L (M: 15.4%; F: 20.0%)				
Low BMI	1	1	1	1
Elevated BMI	0.38 (0.14–1.03)	0.98 (0.48–2.04)	0.33 (0.13–0.84)	0.68 (0.30–1.55)
Overweight	0.27 (0.10–0.75)	2.07 (0.72–5.93)	0.33 (0.10–1.12)	0.76 (0.34–1.68)
Triglycerides ≥1.00 mmol/L (M: 10.9%; F: 13.0%)				
Low BMI	1	1	1	1
Elevated BMI	0.67 (0.17–2.56)	0.86 (0.31–2.38)	2.19 (0.66–7.24)	1.39 (0.37–5.16)
Overweight	1.98 (0.66–5.94)	3.05 (1.18–7.89)	8.03 (2.25–28.64)	7.00 (2.60–18.88)
Glycemia ≥5.6 mmol/L (M: 24.0%; F: 8.8%)				
Low BMI	1	1	1	1
Elevated BMI	0.64 (0.26–1.56)	1.27 (0.39–4.10)	0.86 (0.40–1.84)	1.02 (0.24–4.26)
Overweight	0.90 (0.36–2.24)	3.00 (0.94–9.55)	0.87 (0.29–2.62)	3.27 (1.12–9.50)
Uric acid >0.39 (M) or 0.31 (F) mmol/L (M: 18.9%; F: 20.9%)				
Low BMI	1	1	1	1
Elevated BMI	1.70 (0.69–4.16)	0.90 (0.42–1.94)	1.06 (0.44–2.56)	1.18 (0.48–2.89)
Overweight	1.79 (0.68–4.71)	1.62 (0.70–3.74)	3.81 (1.37–10.59)	2.75 (1.29–5.86)

LDL-cholesterol: low-density lipoprotein cholesterol; HDL-cholesterol: high-density lipoprotein. M: males; F: females.
The prevalence of elevated CRFs in males (M) and females (F) is indicated between brackets.
Low levels of BMI refer to BMI values below the age- and sex-specific medians; overweight (which also includes obesity) is defined according to standard BMI cut-off values; and elevated BMI refers to intermediate BMI values.

Table 4: Standardized linear regression coefficients for the associations between cardiometabolic risk factors at the age of 19-20 years and body mass index measured at the age of 12–15 years or at the age of 19-20 years.

	SBP	DBP	LDL-cholesterol	HDL-cholesterol	Triglyceride	Fasting glucose	Uric acid
Males							
BMI at 12–15 years	0.24^{**}	0.27^{***}	0.20^{**}	−0.12	0.17^{*}	−0.01	0.27^{***}
BMI at 19 years	0.27^{***}	0.34^{***}	0.26^{***}	-0.16^{*}	0.38^{***}	0.05	0.33^{***}
$BMI_{12–15}$ adj for BMI_{19}	0.11	0.07	0.03	−0.03	−0.18	−0.09	0.08
BMI_{19} adj for $BMI_{12–15}$	0.19^{*}	0.28^{**}	0.24^{*}	−0.14	0.51^{***}	0.11	0.28^{**}
Females							
BMI at 14 years	0.23^{**}	0.31^{***}	0.04	−0.05	0.14^{*}	0.29^{***}	0.17^{*}
BMI at 19 years	0.31^{***}	0.36^{***}	0.09	-0.17^{*}	0.26^{***}	0.39^{***}	0.27^{***}
$BMI_{12–15}$ adj for BMI_{19}	−0.1	0.01	−0.15	0.35^{**}	-0.29^{*}	−0.15	−0.22
BMI_{19} adj for $BMI_{12–15}$	0.39^{**}	0.35^{**}	0.22	-0.47^{***}	0.51^{***}	0.52^{***}	0.46^{***}

adj: adjusted.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-cholesterol: low-density lipoprotein cholesterol; HDL-cholesterol: high-density lipoprotein.

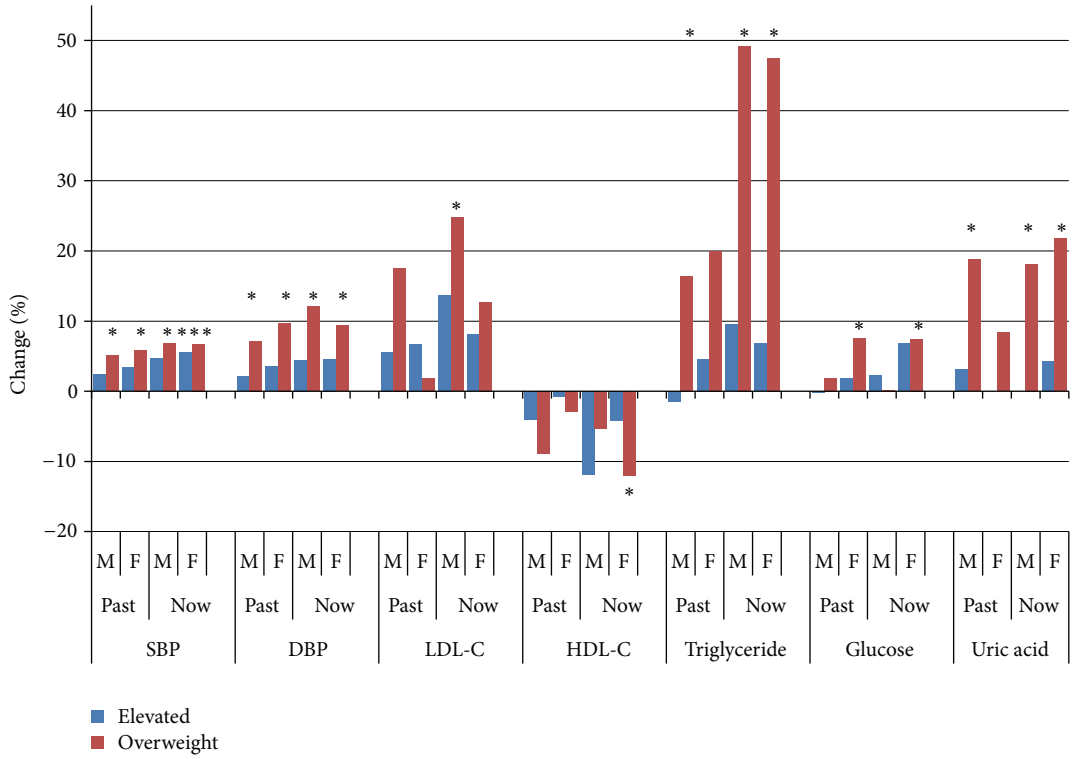

Figure 1: Relative difference (in percent) in the mean levels of cardiometabolic risk factors at the age of 19-20 years according to BMI categories measured at the age of 12–15 years (past) or at the age of 19-20 years (now). The reference BMI category ("low") includes children whose BMI is below the age- and sex-specific median; "overweight" (which also includes obesity) is defined according to standard guidelines and "elevated" BMI refers to BMI values between the "low" and "overweight" categories. SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-C: LDL cholesterol; HDL-C: HDL cholesterol; M: male; F: female; past: BMI measured at the age of 12–15 years; now: BMI measured at age of 19-20 years. The stars indicate association with statistical significance of $P < 0.05$.

the population level while abnormal levels of biological CRFs might be better addressed by targeting obese adolescents and young adults.

The question of whether BMI or different obesity indicators would perform differently in their ability to predict CRFs is still ongoing. Based on data of participants aged 19-20 years (SCDS), we previously showed that BMI was at least as adequate as several other adiposity markers to predict mean levels of CRFs [21]. We found in this study that the magnitude of the associations between overweight and CRFs was important, with odds ratios of elevated levels of CRFs associated with overweight being larger than 2-3 for most CRFs. These results are consistent with findings in other studies among young people, for example, the Bogalusa Heart Study in the USA, in which a BMI larger than the 95th percentile was associated with odds ratios of 2.4, 4.5, 3.0, 7.1, and 12.1 for raised diastolic BP, systolic BP, LDL-cholesterol, triglycerides, and insulin concentration, respectively, and 3.4 for low HDL cholesterol [8]. Overweight was also related strongly to lipid markers among children aged 6–11 years in Qatar [7]. Of note, we found strong associations between overweight in adolescents and young adults and virtually all components of the metabolic syndrome. The magnitude of the observed associations and the broad scope in terms of the many factors involved stress the importance of weight control programs in youth.

It is interesting to note that the association between BMI and CRFs was almost as strong in our study when BMI was measured 4–6 years before measuring the CRF outcomes (longitudinal design) as when they were measured at the same time (cross-sectional design). Analyses based on the longitudinal design strengthen the possibility that elevated body weight is causally associated with elevated levels of CRFs. Confounding or reverse causation is unlikely to account for these findings (e.g., high BP or dyslipidemia is unlikely to be the cause of obesity). It is remarkable, however, that the longitudinal associations between BMI and CRFs were quite strong in comparison to the cross-sectional associations. This reinforces the conclusion that overweight is very likely an important issue for cardiovascular health and that weight control needs be addressed early in life.

The finding that only BMI at age of 19-20 years remained a strong predictor of CRFs in a regression model including both BMI at age of 12–15 years and BMI at age of 19-20 years suggests that current BMI is particularly important in relation to the prediction of blood pressure and metabolic risk factors. Conclusions must, however, be carefully drawn on these results in view of possible methodological issues, for example,

the possibility that that BMI was measured with less precision at age of 12–15 years in the context of a routine screening than at age of 19-20 years in a study with highly standardized methods. Nonetheless, this finding (i.e. that CRFs are strongly associated with current BMI but not with BMI measured several years earlier when considering both weight statuses together) implies the important role of current versus past BMI in relation to current CRFs levels. This is welcome news since current weight, not past weight, can possibly be addressed through interventions. However, overweight tends to track over age, and children who were overweight when they were younger are largely the same children who are overweight when they are older. This is confirmed by the Spearman correlation coefficient of BMI at age of 12–15 years and at age of 19-20 years. The larger importance of current versus past weight has been emphasized in previous research. Several longitudinal studies of both past and current weights have shown that obesity in childhood has only limited impact when assessing cardiovascular health in adulthood [33, 34], but other studies have found an impact of obesity in young people on both risk factors [9, 10] and actual CVD outcomes [35–37] in adulthood. Overall, our findings that CRFs are associated with current and past BMI, and that BMI tracks over time, support a life course approach to obesity including both population-based interventions aimed at preventing the occurrence of overweight in the population at all ages and individual-based interventions targeting overweight among adolescents and young adults.

The distribution of several CRFs was markedly different between sexes. The observation of higher uric acid blood levels in males compared to females was expected [38] and consistent with the uricosuric effect of estrogens in females, which confers a protective effect on several metabolic CRFs [39]. A higher SBP in males compared to females may be related to males being generally taller since height is an important determinant of BP in adolescents and young adults [40]. We found a significant association between BMI and glucose only in females even though males had higher blood glucose levels than females. A possible explanation for the observed sex differences could be the greater adiposity, in particular fat mass, in females than in males [41]. The influence of abdominal fat, which is particularly active metabolically [42], may not be fully captured by the measurement of BMI [43] and might be a key determinant of some of the observed sex differences.

Our study has several strengths, including the population-based sample; a fairly large sample size for a study including blood markers among healthy adolescents and young adults; a broad panel of CRF markers considering the study included healthy participants; analyses based on both longitudinal and cross-sectional designs; and the presence of a minimal number of potential confounders, comorbid conditions, and related treatments at this early age. There are also some potential limitations. Accuracy of BMI measurements at the age of 12–15 years may be less than optimal in the context of routine school screening programs. In addition, although participants were asked to fast, they may not have been all fasting when blood was collected at the age of 19-20 years. A larger sample size would also have been useful to assess the associations separately among overweight and obese children and in order to generate different cohorts with sufficient numbers of children who either gained or lost weight between age of 12–15 and age of 19-20 and the subsequent effects on CRFs at the age of 19-20 years.

In conclusion, we found strong associations between adolescents and young adults who were overweight and several CRFs in both cross-sectional and longitudinal analyses. These findings indicate that a life course approach to weight control with interventions as early as possible is warranted but that weight control even at a later age is equally or perhaps more efficacious in reducing cardiovascular risk. These findings in a country of the African region extend previous similar findings in high-income countries and highlight the adverse consequences of obesity on CRFs in adolescents and young adults in all regions.

Conflict of Interests

The authors declare that they have no conflict of interests.

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