CHAPTER 3

RESULTS

*Independent and opposite associations of hip and waist circumference with metabolic syndrome components, inflammatory and atherothrombotic risk factors in overweight and obese women

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ABSTRACT

Recent studies have shown independent and opposite associations of hip circumference (HC) and waist circumference (WC) with glucose intolerance, insulin resistance (IR), and type 2 diabetes mellitus. However, no studies have simultaneously considered the independent contributions of both markers to metabolic proinflammatory and atherosclerotic risk factors. In this study we investigated the independent associations of WC and HC with metabolic syndrome, pro-inflammatory and atherothrombotic features. Independent associations of thigh adipose and muscle tissue compartments with metabolic features were also examined. Abdominal and thigh adipose and muscle tissue distribution were assessed by computed tomography in 140 overweight and obese women. Blood lipids, inflammatory and atherothrombotic markers were also measured. For a given, a larger HC was inversely associated with fasting insulin, plasminogen activator inhibitor-1 (PA1-1), and hemoglobin A1c (Hb A1c) concentrations. Contrarily, WC was associated with an unfavourable metabolic profile. For a given WC, higher TTAT and TTSAT mass were associated with lower Hb A1c concentrations and LDL-C/HDL-C ratio. Additionally, TTAT was inversely related with leptin, revealing TTSAT opposite associations with insulin and HDL-C concentrations. TTMT was related with lower PAI-1 and fibrinogen concentrations. In conclusion, HC revealed independent and opposite associations with IR markers and atherothrombotic disturbances. Contrarily, WC was related with a higher metabolic risk. These contrasting effects in diabetogenic and atherothrombotic risk factors were, respectively, mediated by gluteofemoral adipose tissue and thigh muscle tissue. In addition to BMI and WC screening relevance, HC can contribute to additionally predict health risk in overweight and obese women.

Keywords: Waist circumference, hip circumference, metabolic syndrome, proinflammatory and atherothrombotic risk factors, obesity, body fat distribution

INTRODUCTION

Central and peripheral fat accumulation defines two different phenotypes of body composition. When trunk fatness is taken into account, fat accumulation within the hips and legs independently protect against impaired glucose metabolism (1-5), and development of type 2 diabetes mellitus (DM) (1,6,7) and cardiovascular disease (CVD) related mortality (7-9). Additionally, it was recently observed, both in a cross-sectional and prospective follow-up studies, that a greater fat deposition in the legs protects against insulin resistance (IR) and aortic calcification (10,11). Most population studies employ waist circumference (WC) to determine health risk because it is a well-established marker of several metabolic syndrome outcomes and CVD such as coronary artery disease (CAD) (12,13).

On the other hand, for a given WC, age and body mass index (BMI), a larger hip circumference (HC) is associated with enhanced glucose tolerance (2,4), better blood lipid profile, lower incidence of some CVD endpoints (8,9) as well as type 2 DM risk (1,2). These associations seem to be present not only in Caucasian individuals but are also present across different ethnic groups (14,15). Regarding the independent and opposite associations and the potential protective role of a larger HC, if WC is taking into account, it has been suggested that both thigh adipose and muscle tissue seem to contribute to the decreased risk observed (1,14).

Additionally, it has been proposed that both anthropometric markers may play different metabolic roles (4). Previous studies that have investigated the independent associations of central and peripheral fat mass or WC and HC with glucose intolerance (1-5), type 2 DM (1,2,14), CVD, and atherogenic risk profile (10-12) and mortality (9,11), have employed measuring techniques that do not allow separate quantification of trunk visceral and subcutaneous fat as well as thigh subcutaneous and intermuscular adipose tissue (AT). Indeed, current knowledge regarding separate contributions of central and peripheral fatness

to health-related effects are based in large measure on fat mass determination using dual-energy X-ray absorptiometry (DXA), which is unable to distinguish different abdominal and thigh AT and muscle compartments. However, evidence using advanced imagiologic methods, such as computed tomography and magnetic resonance imaging has been suggesting that these body composition compartments are differentially related with metabolic disturbances and thus, with health risk (16).

In this context, we investigated the independent associations of WC and HC to metabolic syndrome features, and proinflammatory and atherothrombotic disturbances, in a large sample of overweight and obese premenopausal women. Furthermore, we also examined the relevance of each thigh adipose and muscle tissue compartments to the selected metabolic syndrome risk factors.

SUBJECTS AND METHODS

Subjects

The participants in this investigation were recruited from the community for a 2-year weight loss program as previously described (17). The study population included 140 Caucasian sedentary women. Inclusion criteria required that the subjects were older than 24 years, had a BMI greater than 24.9 kg/m^2, were pre-menopausal, not currently pregnant, not under medication that affected weight or body composition, no history of cancer in the last five years, and no clinical evidence of liver disease. Diabetes mellitus was also an exclusion criterion as well as hormonal dysfunction, Cushing syndrome, hypertension, CVD, stroke, CHD, and resting and exercise electrocardiograms abnormalities. Subjects taking oral medication to treat hyperglycemia, hypercholesterolemia, or hypertriacylglycerolemia were also excluded. All volunteers were informed about the research design and gave written consent to participate. The study protocol was design in accordance to the Helsinki

Declaration and was approved by the Human Subjects Institutional Review Board of the Faculty of Human Movement, Technical University of Lisbon.

Body composition assessments

Anthropometric variables.

Body mass was measured to the nearest 0.01 kg on a calibrated scale after removing shoes and heavy clothing. Height was measured to the nearest 0.1 cm with a stadiometer (Seca, Hamburg, Germany). WC and HC measurements were taken using standard procedures (18). BMI was calculated as weight divided by height squared (kg/m^2).

Measurement of thigh adipose tissue distribution.

Cross-sectional computed tomography (CT) (Siemens, Somaton Plus) thigh images were obtained using standard procedures described elsewhere (19). All images were obtained using 120kV, 480 mA, 512 × 512 matrix with a 48-cm field of view. With the subjects supine and arms extended above their head, contiguous 7-mm-thick cross-sectional images of both legs were obtained between the inferior ischial tuberosity and the superior border of the patella. Several compartments of thigh adipose tissue (total, subcutaneous, and subfascia lata) and muscle tissue cross-sectional areas were measured.

The tissue volume (cm^3) identified in each image was calculated by multiplying the tissue area (cm^2) by the image thickness (7 mm). Thigh adipose tissue volume (litters) were converted to mass units (kilograms) by multiplying the volume by the assumed constant fat density (0.92 kg/L) (20). Total thigh muscle mass was also obtained multiplying volume (L) by the constant density assumed for adipose tissue-free skeletal muscle (1.04 kg/L) (20).

A 7-mm cross-sectional image of both mid-thighs, located at the mid-point distance between the anthropometric markers previously described, was selected from the thigh scans performed.

Abdominal adipose tissue distribution.

Abdominal adipose tissue was determined by acquisition of a single axial image at the L4-L5 inter-vertebral space (19,21). Total abdominal adipose tissue (TAAT), visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (Ab SAT) and superficial and deep subcutaneous adipose tissue areas were measured. The boundary between visceral and subcutaneous AT was defined using the abdominal and oblique wall muscles in continuity with the deep fascia of the paraspinal muscles and the anterior aspect of the vertebral body (22).

Measurement reliability.

The reliability for both thigh and abdominal body composition compartments was calculated in 30 women, with intra-observer analyses performed on same images separated by 3 months. The same technician made all segmentation measurements, thus only intra-observer error was calculated. Regarding mid-thigh adipose tissue, subfascial adipose tissue (SFAT) and mid-thigh muscular tissue, the intra-observer coefficient of variation (CV) were, 0.4%, 2.5% and 0.1%, respectively.

The intra-observer CV for TAAT was 0.7% and 0.9% for VAT. For Ab SAT, superficial and deep Ab SAT the intra-observer CV were, 0.8%, 3.1% and 2.8%, respectively.

Image analysis.

Once obtained CT data was analyzed (Slice-O-matic, Version 4.2, Tomovision, Montreal, Canada) based on image morphology. A combination of edge detection filters and watershed techniques was employed (23). Tissue segmentation was computed using standard Hounsfield units (HU) ranges: -190 to –30 HU for adipose tissue and -29 to +150 HU for skeletal muscle (23). Thigh fascia lata was used to subdivide the subcutaneous adipose tissue from subfascial AT as described elsewhere (19).

Blood analysis

Venous blood samples were collected at the antecubital vein, after a 12-hour overnight fast. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and uric acid were measured by enzymatic colorimetric methods. Insulin was determined by electrochemiluminescence immunoassay (ECLIA), interleukin-6 (IL-6) by chemiluminescence immunoassay and glycemia was assessed by a hexoquinase method.

Plasma apolipoprotein A1 (apo A1), apolipoprotein B100 (apo B100) and C-reactive protein (CRP) concentrations were measured by a high-sensitivity particle-enhanced turbidimetric assay. Tumor necrosis factor-alpha (TNF-α) was measured using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) principle. Plasminogen activator inhibitor-1 (PAI-1) was measured in iced citrated plasma using the Coatest PAI method (enzyme immuno assay - EIA) and fibrinogen was measured by clotting time. Hemoglobin A(1c) (Hb A1c) was determined by high-pressure liquid chromatography (HPLC). Adiponectin, leptin, and urinary cortisol were measured by radioimmunoassay (RIA).

Blood pressure

Diastolic and systolic blood pressures were measured in seated position after at least 5-minute rest with a Dinamap semiautomatic oscillometric recorder (Dinamap, Critikon, Tampa, FL). A suitable cuff size was applied to the participant's upper arm, at the heart level. The mean of three measurements in each arm was calculated.

Statistical analysis

Data are presented as mean ± SD, unless otherwise is indicated. Normality and homocedasticity of all variables were analysed. Based on skewed distributions, log

transformation was used to normalize distributions when necessary. Pearson correlation coefficients between both waist and hip circumference and metabolic syndrome features studied were calculated. Multiple linear regressions, adjusted for age and BMI, were performed to study the independent associations of continuous HC and WC with major metabolic syndrome components, proinflammatory and atherothrombotic metabolic disturbances (all entered as continuous variables). Independent contributions of WC and HC to abdominal and thigh body composition compartments, adjusted for age and BMI, were also studied. Further multiple linear regressions were developed to examine the associations of each thigh body composition compartment with metabolic syndrome features, independently of age, BMI, and WC.

Standardized beta values are presented for direct comparisons of the multiple linear regression models results. Multicolinearity was studied by statistic tolerance, which determines how much the independent variables are linearly related to each another. The tolerance is calculated as $1-R^2$ for an independent variable when it is predicted by the other independent variables already included in the model. If tolerance is inferior to 0.1, the stability of the regression model is disturbed by multicolinearity. Statistical significance was set as $p<0.05$. All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA).

RESULTS

The body composition and metabolic syndrome characteristics of the study population are presented in **Table 1** and **Table 2**. Despite some variation in the degree of obesity ($25<BMI\leq45$ kg/m^2), the majority of the sample was obese, with an average BMI of ~30 kg/m^2.

Table 1. Characteristics of the study population (n=140)

	mean±SD	Range
Anthropometric data		
Age, y	38.3±0.5	25.0-49.0
Weight, kg	78.1±1.0	59.1-107.8
BMI, kg/m^2	30.4±0.3	25.1-45.2
WC, cm	87.2±0.8	71.1-123.4
HC, cm	111.4±0.7	94.7-134.6
Abdominal adipose tissue		
TAAT, cm^2	470.9±12.1	211.9-910.8
VAT, cm^2	111.3±4.3	24.9-266.8
Ab SAT, cm^2	353.6±9.1	145.0-633.4
Thigh Compartments		
Thigh AT, cm^2	270.7±6.9	132.9-509.1
Thigh SAT, cm^2	261.6±6.8	129.4-501.6
Thigh SFAT, cm^2	3.5±0.2	1.0-11.9
Muscle area, cm^2	234.3±2.6	176.3-324.7
TTAT mass, kg	8.4±2.1	4.0-14.8
TTSAT mass, kg	7.9±2.1	3.8-14.0
TTSFAT mass, kg	0.6±0.2	0.3-1.5
TTMT mass, kg	6.1±0.9	4.4-10.3

Values are means ± SD. BMI, body mass index; WC, waist circumference; HC, hip circumference; TAAT, total abdominal adipose tissue; VAT, visceral adipose tissue; Ab SAT, abdominal subcutaneous adipose tissue; Thigh AT, mid-thigh adipose tissue; Thigh SAT, mid-thigh subcutaneous adipose tissue; Thigh SFAT, mid-thigh subfascial adipose tissue; TTAT, total thigh adipose tissue; TTSAT, total thigh subcutaneous adipose tissue; TTSFAT, total thigh subfascial adipose tissue; TTMT, total thigh muscular tissue.

Table 2. Metabolic syndrome characteristics of the study population (n=140)

	mean±SD	Range
Triacylglycerols, mg/dL	101.48±4.86	32.00-329.0
Fasting insulin, μIU/mL	8.22±0.32	2.40-17.9
Fasting glycemia, mg/dL	89.48±0.65	73.00-113.0
Total cholesterol, mg/dL	194.74±3.86	101.00-307.0
HDL cholesterol, mg/dL	54.09±1.05	29.00-91.0
LDL cholesterol, mg/dL	123.50±3.54	45.00-255.0
TC/HDL-C ratio	3.74±1.11	2.04-9.55
LDL-C/HDL-C ratio	2.38±0.08	0.94-6.13
Apo A1, mg/dL	139.05±2.33	77.00-195.0
Apo B100, mg/dL	86.68±2.43	38.00-156.0
Apo A1/Apo B100 ratio	1.74±0.05	0.78-3.31
Systolic BP, mmHg	1 20.65±1.43	90.00-175.0
Diastolic BP, mmHg	75.83±0.93	50.00-101.0
CRP, mg/dL	0.45±0.03	0.03-1.14
IL-6, pg/mL	10.32±0.56	0.80-31.5
TNF-α, pg/mL	3.87±0.23	0.90-14.1
PAI-1, ng/mL	21.18±2.01	1.00-100.0
Fibrinogen, mg/dL	369.38±6.48	201.00-552.0
Adiponectin, ng/mL	9.18±6.44	2.93-41.0
Hb A1c, %	4.87±0.04	4.00-7.0
Cortisol, ug/day	41.04±1.69	6.00-105.0
Uric acid, mg/dL	4.39±0.97	2.40-8.5
Leptin, ng/mL	32.92±43.33	0.90-167.4

Values are means ± SD. HDL, high-density lipoprotein; LDL-C, low-density lipoprotein; TC, total cholesterol; Apo A1, apolipoprotein A1; Apo B100, apolipoprotein B100; BP, blood pressure; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; PAI-1, plasminogen activator inhibitor-1; Hb A1c, hemoglobin A(1c).

Subcutaneous adipose tissue was the major constituent of both abdominal and thigh adipose tissue area (75.1% and 93.9%, respectively) while visceral adipose tissue represented 23.6% of abdominal adipose tissue area. On the other hand, total thigh adipose tissue mass represented ~57.9% of the total thigh mass. In our sample, 9.3% of the women met the ATP III criteria for metabolic syndrome (24).

In bivariate models, WC showed positive associations with several metabolic syndrome features, while HC was positively correlated with PAI-1 and systolic BP (data not shown). However, after adjustment for each other, WC and HC revealed opposite associations with risk factors, being elevations in HC associated with improvements metabolic profile, whereas elevations in WC were related to metabolic deterioration.

The results of simultaneously adding waist and hip circumference, adjusting for age and BMI, to predict metabolic syndrome components, and proinflammatory and atherothrombotic risk factors for CVD are shown in **Table 3.** A large WC was associated with increased TG, fasting glycemia, apo A1 and PAI-1 concentrations, as well as with a higher TC/HDL-C ratio. Additionally, a larger WC was inversely related with and lower concentrations of HDL-C, IL-6, and leptin.

On the contrary, for a given WC, a large HC was inversely associated with fasting insulin, Hb A1c and PAI-1concentrations. The explained variance for each metabolic risk factor by HC and WC, independently of age and BMI, varied between 3.8% and 26.7%.

Table 3. Independent contributions (standardized beta coefficients) of waist and hip circumference to metabolic syndrome components, adjusted for age and BMI.

	Waist Circumference	Hip Circumference	Percentage of variance explained[**](%)
Triacylglycerols, mg/dL	0.337[*]	-0.062	17.3[#]
Fasting insulin, μIU/mL	0.125	-0.288[†]	24.5[#]
Fasting glycemia, mg/dL	0.490[‡]	-0.067	16.3[#]
TC/HDL-C ratio	0.347[*]	-0.149	19.0[#]
LDL-C/HDL-C ratio	0.267	-0.215	15.4[#]
Apo A1/Apo B100 ratio	-0.137	0.197	7.8[#]
Systolic BP, mmHg	0.170	0.227	8.6[#]
CRP, mg/dL	-0.063	0.030	9.7[#]
IL-6, pg/mL	-0.396[*]	-0.163	5.2
TNF-α, pg/mL	0.123	-0.049	9.4[#]
PAI-1, ng/mL	0.349[*]	-0.241[*]	26.7[#]
Fibrinogen, mg/dL	-0.037	-0.183	8.4[#]
Adiponectin, ng/mL	-0.186	-0.025	3.6
Hb A1c, %	0.202	-0.246[*]	13.0[#]
Cortisol, ug/day	0.272	0.004	3.8
Uric acid, mg/dL	-0.273	-0.111	15.9[#]
Leptin, ng/mL	-0.327[*]	-0.094	12.3[#]

All variables were entered in the regression models as continuous variables. TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Apo A1, apolipoprotein A1; Apo B100, apolipoprotein B100; BP, blood pressure; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; PAI-1, plasminogen activator inhibitor-1; Hb A1c, hemoglobin A(1c); Age had only independent significant contribution in PAI-1 and uric acid.

[**] Variance explained by age, BMI, and waist and hip circumference.

[#] Independent significant contribution of BMI, $P < 0.001$.

[*] $P < 0.05$.

[†] $P < 0.01$.

[‡] $P < 0.001$.

Table 4. Independent contributions (standardized beta coefficients) of waist circumference and thigh adipose and muscle tissue compartments to metabolic syndrome components, adjusted for age and BMI.

	WC	TTAT	WC	TTSAT	WC	TTMT
Triacylglycerols, mg/dL	0.297	-0.124	0.280	-0.142	0.321*	0.029
Fasting insulin, μIU/mL	0.172	-0.227	0.150	-0.239*	0.255	-0.023
Fasting glycemia, mg/dL	0.502†	-0.100	0.503†	-0.088	0.468†	0.147
TC/HDL-C ratio	0.308*	-0.188	0.308*	-0.172	0.374*	0.001
LDL-C/HDL-C ratio	0.228	-0.252*	0.221	-0.245*	0.334*	-0.034
Apo A1-Apo B100 ratio	-0.133	0.218	-0.120	0.228	-0.234	0.050
Systolic BP, mmHg	0.169	0.104	0.156	0.066	0.069	0.126
CRP, mg/dL	-0.015	0.233	-0.016	0.192	-0.058	-0.072
IL-6, pg/mL	-0.314	0.146	-0.318	0.122	-0.314	-0.122
TNF-α, pg/mL	0.223	0.232	0.212	0.168	0.158	-0.028
PAI-1, ng/mL	0.362*	-0.129	0.338*	-0.157	0.478‡	-0.164*
Fibrinogen, mg/dL	-0.018	-0.020	-0.029	-0.042	0.092	-0.222*
Adiponectin, ng/mL	-0.245	-0.085	-0.250	-0.084	-0.247	0.062
Hb A1c, %	0.221	-0.244*	0.210	-0.233*	0.266	0.071
Cortisol, ug/day	0.258	0.118	0.237	0.132	0.168	0.101
Uric acid, mg/dL	-0.275	-0.028	-0.287	-0.051	-0.244	-0.048
Leptin, ng/mL	-0.211	0.310*	-0.225	0.225	-0.297	-0.035

All variables were entered in the regression models as continuous variables. WC, waist circumference; TTAT, total thigh adipose tissue; TTSAT, total thigh subcutaneous adipose tissue; TTSFAT, total thigh subfascial adipose tissue; TTMT, total thigh muscular tissue; TC, total cholesterol; HDL, high-density lipoprotein; LDL-C, low-density lipoprotein; Apo A1, apolipoprotein A1; Apo B100, apolipoprotein B100; BP, blood pressure; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha; PAI-1, plasminogen activator inhibitor-1; Hb A1c, hemoglobin A(1c).

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.001$.

The associations of each thigh adipose and muscle tissue compartments with metabolic risk factors, independently of age, BMI and WC are presented in **Table 4**. For a given WC, a higher TTAT and TTSAT mass were both associated with lower Hb A1c concentrations, as well as with a lower LDL-C/HDL-C ratio. Additionally, while TTAT mass was inversely related with leptin, TTSAT mass revealed independent and opposite associations with fasting insulin and HDL-C concentrations. On the contrary, TTSFAT did not revealed associations with any of the metabolic syndrome features studied. Furthermore, for a given WC, a higher TTMT mass was associated with lower PAI-1 and fibrinogen concentrations.

DISCUSSION

This study demonstrates the opposite associations between both WC and HC and atherogenic and prothrombotic features of metabolic syndrome and CVD, extending the previous knowledge about the separate contributions of each anthropometric marker to health risk (**Figure 1**). To our knowledge, it is the first study that has observed independent and opposite effects of WC and HC not only with major metabolic syndrome components, but also with specific metabolic risk factors, which are relevant for diabetogenic and atherogenic risk assumption (2,3,8). Furthermore, in this context of the opposite contributions of WC and HC to metabolic risk, as a unique feature of this paper, it is the first time that it is addressed the contribution of abdominal and thigh adipose tissue compartments, and muscle tissue distribution, measured by CT, to the observed associations. It was already reported that, for a given WC, a larger HC was related with a lower risk for metabolic syndrome disturbances (1,2,14), type 2 DM (1,3,6,9), and CVD morbidity and mortality (8,9,25).

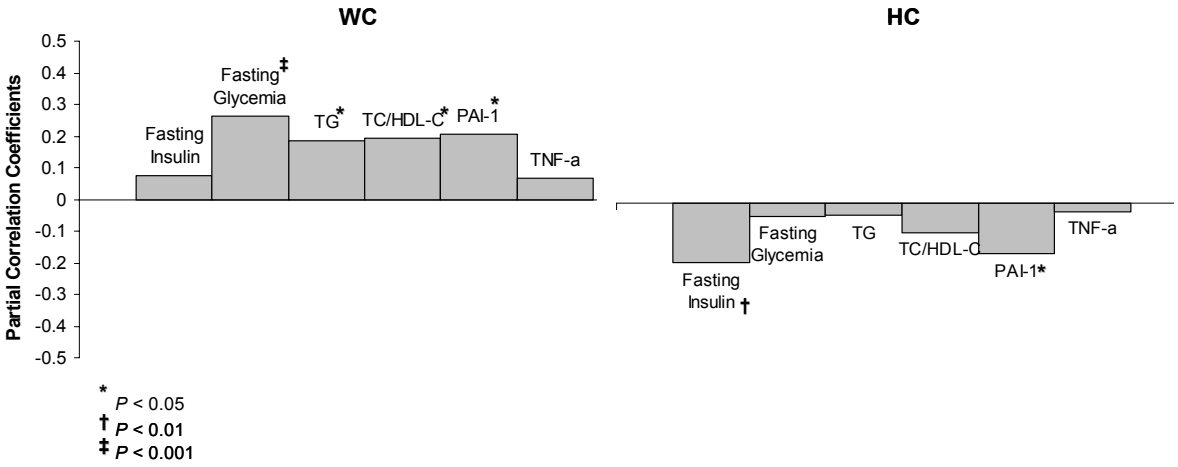

Figure 1. Partial correlations, controlling for age and BMI, between both WC and HC and the metabolic syndrome features studied.

However, this independent and relative HC protective contribution to disease risk disappeared when WC was not taken into account. Indeed, in our study, bivariate models revealed that larger WC and HC were both associated with an unfavourable metabolic profile and a higher CVD risk.

On the other hand, consistently with previous observations (26), we observed that WC was related with increased VAT and Ab SAT areas, reflecting a morbidogenic body composition phenotype and metabolic profile, while a larger HC was inversely associated with both abdominal AT compartments (data not shown). After adjustment for age and BMI, multiple regression analyses between both anthropometric markers and the metabolic risk factors revealed positive associations between WC and glucose metabolism markers, hypercholesterolemia, hypertriacylglycerolemia, and PAI-1, a specific indicator of impaired fibrinolysis and atherothrombotic state (27).

In addition, WC was also inversely associated with leptin and apo A1 concentrations (data not shown). In this context, it was recently proposed a synergistic effect promoted by a combination of hyperinsulinemia, hyperglycemia and hypercholesterolemia in obese

individuals to enhance plasma PAI-1 concentrations, which could explain the verified association between this atherothrombotic marker and WC (28). However, previous evidence have shown that hyperinsulinemia alone is also related with increased PAI-1 concentrations in obese and type 2 DM subjects, suggesting a direct link between IR and PAI-1 (27). However, it is still unclear whether insulin acts directly or via IR to enhance PAI-1 concentrations. PAI-1 has been associated with dyslipidemia, abdominal adiposity and hypertension (28). Moreover, it is known that AT is also an important source of angiotensin II, which might link the PAI-1 increase to renin-angiotensin system, and hence, to hypertension (29). Despite the controversial opinions about regional AT differences in PAI-1 secretion, it was recently suggested that VAT, rather than Ab SAT, could be responsible for the raised PAI-1 values and IR observed in metabolic syndrome patients (30).

On the contrary, in our study, for a given WC, a large HC was associated with lower fasting insulin, Hb A1c and PAI-1 concentrations. These results reinforce the protective effect of relatively larger hips to glucose metabolism and IR markers, and further extend this notion to specific atherogenic and prothrombotic disturbances. Similarly, other studies have also reported that a larger HC (or a higher peripheral fat mass) was independently associated with a more favourable plasma glucose and lipid profile (1,7,10), and a lower type 2 DM (3,4,14,31), and CVD risk (1,9,11). These protective contributions of a larger HC to morbidity seem also to be present with risk of premature mortality, after adjustment for BMI (9,25).

A larger WC, reflecting central obesity, has been associated with a chronic inflammatory state, promoted by a low-grade plasma elevation of some adipokines and acute-phase reactants, such as TNF-α, IL-6 and CRP (32). These inflammatory markers have been associated with type 2 DM (33), atherogenesis and CVD (34). Although not significant, WC and HC revealed an opposite association tendency not only with dyslipidemia markers,

but also with some inflammatory risk factors, such as TNF-α, which seem to play an important mechanistic role in IR, down-regulating GLUT-4, and inhibiting insulin receptor activity (35). Since TNF-α can induce IL-6 release, it has been suggested that TNF-α may be the "driver" behind metabolic syndrome (36). In abdominal obese women, the concentrations of these adipokines are increased, while adiponectin, produced by both visceral and peripheral adipocytes, is commonly decreased (37). Adiponectin present anti-atherogenic, anti-inflammatory, and insulin sensitising effects (38), which seem to be relevant to counteract the diabetogenic and atherogenic risk associated with obesity. In previous studies which have examined the contrasting contributions of both central fat mass (CFM) and peripheral fat mass (PFM) to atherogenic glucose and lipid markers, as well to aortic calcification (7,10,11,14), the verified PFM protection against type 2 DM and atherosclerosis seemed to be mediated by insulin sensitization effects associated with adiponectin physiological metabolism (39).

It is well recognized that HC variations can be explained by skeletal frame size, gluteofemoral muscle mass or AT accumulation (3,4). Moreover, it has been postulated that relatively narrow hips due to lack of thigh muscle mass are associated with a lower muscle insulin clearance (40) and an impaired muscle fatty acid oxidation capacity (6,8). However, authors have been suggesting that HC seems to be more closely associated with leg fat mass in women (4). In our study, contrarily to WC, a larger HC was not only independent and inversely related with both VAT and Ab SAT areas, but revealed also additional associations with gluteofemoral AT and thigh muscle tissue compartments (data not shown).

Therefore, further analyses were developed to highlight the relevance of each thigh AT and muscle tissue compartment to the observed relative HC protective role to metabolic risk. For a given WC, higher TTAT and TTSAT mass were both associated with lower Hb A1c concentrations and a lower LDL-C/HDL-C ratio. A higher TTSAT mass was also

inversely related with both fasting insulin and HDL-C concentrations, being a higher TTAT mass associated with lower leptin concentrations. In contrast, TTSFAT did not reveal any association with the metabolic syndrome features studied. Furthermore, for a given WC, a higher TTMT mass was a significant predictor of lower PAI-1 and fibrinogen concentrations.

These observations suggest that, in overweight or obese women, the verified protective HC role in dyslipidemia and IR, when WC is taken into account, could be mediated by subcutaneous gluteofemoral AT. Indeed, it was already observed that, for a given amount of abdominal fat, low subcutaneous fat in the legs was associated with an unfavourable lipid profile (16). In this context, it has been proposed that underlying hormonal factors, such as estrogens concentrations may regulate preferential thigh AT accumulation (41). In addition, gluteofemoral adipocytes are relatively less sensitive to catecholamine-stimulated lipolysis, being more sensitive to anti-lipolytic stimuli, when compared to VAT adipocytes (42). These metabolic differences combined with a relatively higher activity of lipoprotein lipase (LPL) in these thigh adipocytes promote the uptake of free fatty acids from circulation, providing a "buffer" which may carry out an anti-diabetogenic and anti-atherogenic effect, as well as a protection against liver, pancreas and muscle ectopic fat storage (43).

Conversely, our results also suggest that thigh muscle tissue seem to be relevant for the observed protection against prothrombotic and atherosclerotic abnormalities. Despite evidence has been highlighting the contribution of muscle tissue to a better metabolic profile (44), and lower insulin metabolism (40) and fatty acid oxidation capacity disturbances (6,8), these are novel observations that need further research. Furthermore, it is noteworthy that disturbances in glucocorticoid and growth hormone metabolism, age, gender and behavioral

factors, such as physical activity and diet may underlie and confound these associations, needing therefore to be taken in consideration in future studies (45).

In summary, we found that, for a given WC, HC was inversely associated with IR markers and atherothrombotic disturbances. On the contrary, a larger WC was associated with a higher metabolic risk. The protective effect of relatively larger HC, when WC is taken into account, was extended to novel and specific metabolic syndrome features, being these contrasting effects in diabetogenic markers mediated by gluteofemoral AT, while thigh muscle tissue seemed to mediate the protection against atherothrombotic risk factors. Therefore, in addition to BMI and WC screening relevance, HC can also contribute to additionally predict cardiovascular disease risk in overweight and obese women.

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