Contribution of Abdominal Visceral Obesity and Insulin Resistance to the Cardiovascular Risk Profile of Postmenopausal Women

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The aim of this study was to determine the respective contribution of abdominal visceral adipose tissue (AT) accumulation and insulin resistance (IR) to the determination of a comprehensive cardiovascular metabolic risk profile in 108 postmenopausal women not receiving hormone therapy. Insulin sensitivity (*M/I*) was determined by a hyperinsulinemic-euglycemic clamp, and visceral AT area was measured by computed tomography. Median values of visceral AT (133.9 cm^2) and insulin sensitivity (0.010189 $mg \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$) were used to form four subgroups: *1*) low visceral AT–low IR ($n = 35$), *2*) low visceral AT–high IR ($n = 19$), *3*) high visceral AT–low IR ($n = 19$), and *4*) high visceral AT–high IR ($n = 35$). Women with isolated IR (low visceral AT and high IR) were characterized by significantly higher fasting and 2-h glycemia and higher fibrinogen, triglyceride, and VLDL-apolipoprotein (apo)B concentrations than women with low visceral AT and low IR ($P < 0.05$). The plasma lipid–lipoprotein profile and inflammatory markers were not significantly different between women with high visceral AT and low IR and women with low visceral AT and low IR. Women with high visceral AT and high IR had higher fasting and 2-h glycemia, triglyceride, and VLDL-apoB levels; lower apoAI and HDL_2 cholesterol levels; as well as higher C-reactive protein and interleukin-6 concentrations than women with low visceral AT and low IR ($P < 0.05$). In addition, 15 of the 35 women (42.9%) in the high visceral AT and high IR group were newly diagnosed with type 2 diabetes, whereas no women were diagnosed with type 2 diabetes in the group of women with low visceral AT and low IR. These results show that although the presence of high IR in its isolated form is associated with some metabolic alterations, it is the combination of both high visceral AT and high IR that is the most detrimental for the metabolic health in postmenopausal women. *Diabetes* 54:770–777, 2005

Postmenopausal women are at higher risk of cardiovascular disease (CVD) than premenopausal women. This increased CVD risk after menopause has been partly attributed to the increment in visceral adipose tissue (AT) deposition and worsening insulin-stimulated glucose disposal observed during the menopause transition (1,2). There is also evidence indicating that there is an increase in insulin resistance (IR) with aging (3). Insulin resistance has been suggested as an important risk factor in the development of the metabolic syndrome, a cluster of abnormalities comprising glucose intolerance, dyslipidemia, high blood pressure, and impaired fibrinolysis activity that is associated with increased risk of developing type 2 diabetes and CVD (4). It is well demonstrated that obesity is a risk factor for type 2 diabetes and CVD (5). In addition, body fat distribution is also related to the risk of type 2 diabetes and CVD, and studies have shown that individuals with increased accumulation of visceral AT appear to develop the metabolic syndrome more frequently than those with an increase in peripheral body fat distribution (i.e., subcutaneous AT) (6).

Postmenopausal women are more likely to be characterized by visceral obesity and related metabolic disturbances, such as type 2 diabetes, than premenopausal women. In fact, Hernandez-Ono et al. (7) found that postmenopausal women with more visceral AT accumulation were characterized by a less favorable metabolic profile. A recent study on postmenopausal Chinese women showed that postmenopausal women with abdominal obesity (as evaluated by waist circumference) carry a higher CVD risk and are more insulin resistant than those without abdominal obesity (8). Brochu et al. (9) also found that obese postmenopausal women with higher levels of visceral AT had lower insulin-mediated glucose disposal than those with less visceral AT.

Many studies have documented that abdominal visceral AT is closely associated with IR in obese nondiabetic and type 2 diabetic subjects (9–11). This close association between IR and obesity has made it difficult to establish whether IR per se (i.e., independent of obesity) is associated with various components of the metabolic syndrome. Previous studies suggested that IR might independently be

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2hPG, 2-h plasma glucose; apo, apolipoprotein; AT, adipose tissue; CRP, C-reactive protein; CT, computed tomography; CVD, cardiovascular disease; EE, energy expenditure; FFA, free fatty acid; FPG, fasting plasma glucose; FSH, follicule-stimulating hormone; hs-CRP, highly sensitive CRP; HT, hormone therapy; IL, interleukin; IR, insulin resistance; OGTT, oral glucose tolerance test.

associated with clustering of CVD risk factors in nondiabetic subjects as well as in subjects with type 2 diabetes (12). The respective contribution of visceral AT accumulation and IR to the determination of the CVD risk profile in postmenopausal women not receiving hormone therapy (HT) needs to be elucidated.

The aim of the present study was to determine the respective contribution of abdominal visceral AT accumulation and IR to the determination of a comprehensive metabolic risk profile in postmenopausal women not receiving HT. For that purpose, regional body fat distribution was determined by computed tomography (CT), and a hyperinsulinemic-euglycemic clamp was used to measure insulin-stimulated glucose disposal in a group of 108 postmenopausal women. Furthermore, a complete plasma lipid-lipoprotein profile and inflammatory markers were measured. We hypothesized that both visceral AT and IR would be significant correlates of metabolic parameters measured, with a more important contribution for IR.

RESEARCH DESIGN AND METHODS

This study was conducted in a sample of 108 postmenopausal women (aged between 46 and 68 years) recruited through the local newspapers of the Quebec City metropolitan area. Each woman was individually interviewed to evaluate if she corresponds to the study's criteria for age, menopausal status, HT, and other medication. Women were asked about their menstrual cycle. Those reporting that they did not had their menses for at least 1 year were considered postmenopausal and were included in the study. A measure of the follicule-stimulating hormone (FSH) was used to confirm the menopausal status (FSH value between 28 and 127 IU/l). All women included in our study were free from metabolic disorders, were not using any type of HT, and were not under treatment for coronary heart disease, diabetes, dyslipidemias, or endocrine disorders (except stable thyroid disease). Five women included in our study were smokers. One woman started HT during testing period because of severe menopausal symptoms. Analyses were therefore conducted with and without this woman for comparison purposes. None of the participants had received a diagnosis of type 2 diabetes before the study. All participants signed an informed consent document before entering the study, which was approved by the Laval University Hospital and Laval University Research Ethics Committees.

Anthropometric measurements. Body density was estimated by the hydrostatic weighing technique (13). The mean of six valid measurements was used to calculate the percentage of body fat from body density with the equation of Siri (14). Height, body weight, BMI, and waist circumference were determined following the procedures recommended at the Airlie Conference (15). Height was measured to the nearest millimeter with a stadiometer, and body weight was measured to the nearest 0.1 kg on a calibrated balance. Waist circumference was measured in duplicate at the mid-distance between iliac crest and last rib margin while the woman was in a standing position, and the measurement was recorded to the nearest millimeter. Participants were wearing swimming suits and were asked to remove their shoes for these last measurements.

CT. Measurements of abdominal AT areas were performed by CT scan with a GE High Speed Advantage CT scanner (General Electric Medical Systems, Milwaukee, WI) with the procedures of Sjöström et al. (16), as previously described (17). Briefly, women were examined in the supine position with both arms stretched above the head. The CT scan was performed at the abdominal level between L4 and L5 vertebrae. A radiograph of the skeleton was used as a reference to establish the position of the scan to the nearest millimeter. Total abdominal AT area was calculated by delineating the abdominal scan with a graph pen and then by computing the AT surface using an attenuation range of −190 to −30 Hounsfields units (18). Abdominal visceral AT area was measured by drawing a line within the muscle wall surrounding the abdominal cavity. The abdominal subcutaneous AT area was calculated by subtracting the visceral AT area from the total abdominal AT area.

Oral glucose tolerance test. A 75-g oral glucose tolerance test (OGTT) was performed in the morning after an overnight fast. Blood samples were collected in EDTA-containing tubes (Becton Dickinson, Franklin Lakes, NJ) through a venous catheter from an antecubital vein at −15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 min for the determination of plasma glucose, insulin, and C-peptide concentrations. Plasma glucose was measured enzymatically, whereas plasma insulin was measured by radioimmunoassay with polyethylene glycol separation (19,20). Plasma C-peptide levels were measured by a modification of the method of Heding (21) with polyclonal antibody A-4741 from Ventrex (Portland, ME) and polyethylene glycol precipitation (19). The interassay coefficient of variation was 1.0% for a basal glucose value set at 5.0 mmol/l. Type 2 diabetes was defined as a fasting plasma glucose (FPG) concentrations ≥7.0 mmol/l or 2-h plasma glucose (2hPG) concentrations ≥11.1mmol/l (22).

Hyperinsulinemic-euglycemic clamp. Insulin sensitivity was determined with a hyperinsulinemic-euglycemic clamp previously described by DeFronzo et al. (23). The hyperinsulinemic-euglycemic clamp was performed after a 12-h overnight fast. An antecubital arm vein was cannulated with a catheter for infusion of insulin and glucose (20% dextrose). A hand vein from the contralateral arm was cannulated to permit sampling of blood for the determination of plasma insulin and glucose concentrations. Fasting blood sample was drawn for baseline measurements. A primed continuous infusion of insulin (Humulin R) (40 $mU \cdot m^{-2} \cdot min^{-1}$) was then started. Adjustments in glucose infusion rate were performed to reach the FPG values and a steady state of ~5.5 mmol/l for women with FPG above the normal range (FPG ≥6.1 mmol/l). Once the steady state of glucose concentration was reached, the insulin infusion was continued for the next 2 h. The duration of the insulin infusion was such that the rate of infused glucose reached a constant value during the last hour of the clamp. Blood samples were collected in EDTA-containing tubes from time −15 min and then every 5 min during the test to measure blood glucose concentrations by using a glucometer-Elite Bayer (number 3903-E). Measurement of plasma glucose concentrations was then validated by enzymatic method (20). Plasma insulin concentrations were monitored from blood samples collected every 10 min and stored at −20°C for later analyses using radioimmunoassay with polyethylene glycol separation (19). The insulin-stimulated glucose disposal rate or M value was then calculated from the glucose infusion rate divided by kilograms of body weight during the last 30 min of the clamp. Insulin sensitivity (M/I) was determined as the M value divided by the mean insulin concentration during the last 30 min of the clamp, as defined previously (23). Insulin resistance was defined as $(M/I)^{-1}$.

Plasma lipoprotein–lipid profile. On the morning of the hyperinsulinemic-euglycemic clamp, blood samples were collected to measure a complete plasma lipid–lipoprotein profile by standard methods. Blood samples were collected after a 12-h overnight fast from an antecubital vein into vacutainer tubes containing EDTA. Cholesterol and triglyceride concentrations were determined enzymatically in plasma and lipoprotein fractions with a Technicon RA-500 analyzer (Bayer, Tarrytown, NY). Enzymatic reagents were obtained from Randox (Randox Laboratories, Crumlin, U.K). Plasma lipoprotein fractions (VLDL, LDL, and HDL) were isolated by ultracentrifugation as previously described (24). Plasma VLDL (density [d] < 1.006g/ml) were isolated by ultracentrifugation (25). The HDL fraction was obtained after precipitation of LDL in the infranatant (d >1.006 g/ml) with $MnCl_2$ and heparin (25). The cholesterol and triglyceride content of the infranatant were measured before and after the precipitation step. HDL_2 was precipitated from the HDL fraction with a 4% solution of low–molecular weight dextran sulfate (15–20 kDa) obtained from SOCHIBO (Boulogne, France). The cholesterol content of the supernatant fraction (HDL_3) was determined, and HDL_2 cholesterol levels were derived by subtracting HDL_3 from total HDL cholesterol concentrations (26). Apolipoprotein (apo)B was measured by nephelometry (BN ProSpec; Dade Behring, Newark, NJ) in plasma and lipoprotein fractions with reagents provided by this company (N antisera to Human Apolipoprotein B).

Inflammatory markers. Plasma C-reactive protein (CRP) levels were measured on plasma stored at −80°C using the Behring Latex-Enhanced highly sensitive CRP (hs-CRP) assay on a Behring Nephelometer BN-100 (Behring Diagnostic, Westwood, MA) and the calibrators (N Rheumatology Standards SL) provided by the manufacturer. Plasma interleukin (IL)-6 levels were measured on baseline samples using a commercially available enzyme-linked immunosorbent assay (ELISA), the Quantikinine HS Immunoassay kit (R&D Systems, Minneapolis, MN) and calibrators (Diluent HD6F), according to the manufacturers' procedures. Plasma fibrinogen was also measured by nephelometry (BN ProSpec).

Other measurements. Systolic and diastolic blood pressure were measured in the right arm of seated participants, as previously described (27). Women filled out a validated 3-day activity diary including 2 weekdays and 1 weekend day (28). The activities were categorized according to mean energy expenditure (EE) on a 1–9 intensity scale for each 15-min period during 24 h, and subjects used a list of categorized activities to fill out their diary. For example, category 1 indicated very low EE (such as sleeping), and category 9 indicated a very high EE (such as running). EE from moderate to vigorous physical activity corresponding to category 6–9 EE (EE6–9) was used in this study.

TABLE 1
Age and metabolic variables in the four groups of postmenopausal women separated on the basis of visceral AT and IR

Variables	Low VAT–low IR	Low VAT–high IR	High VAT–low IR	High VAT–high IR
n	35	19	19	35
Physical characteristics				
Age (years)	56.2 ± 4.2	57.2 ± 5.1	56.2 ± 4.1	57.9 ± 4.2
BMI (kg/m^2)	25.1 ± 3.4	26.5 ± 2.9	30.0 ± 5.3*†	31.8 ± 4.5*†
Body fat mass (kg)	22.9 ± 7.9	25.6 ± 6.1	33.1 ± 10.5*†	35.4 ± 8.8*†
Visceral AT (cm^2)	92 ± 26	100 ± 26	171 ± 33*†	190 ± 39*†‡
Energy from fat (%)	29.5 ± 4.9	33.4 ± 5.7*	32.6 ± 3.2*	34.0 ± 5.3*
EE6–9 (kcal · kg^{-1} · day^{-1})	4.13 ± 4.45	1.73 ± 2.34	3.76 ± 5.18	2.01 ± 3.23
Blood pressure				
Systolic (mmHg)	127 ± 13	129 ± 15	126 ± 13	138 ± 18*†‡
Diastolic (mmHg)	80 ± 6	82 ± 6	81 ± 10	86 ± 7*†‡
Glucose-insulin homeostasis				
Fasting glucose (mmol/l)	5.2 ± 0.5	5.7 ± 0.8*	5.4 ± 0.4	6.0 ± 1.0*‡
2-h glycemia (mmol/l)	6.1 ± 1.6	8.0 ± 2.5*	7.3 ± 2.5	10.4 ± 2.9*†‡
Insulin sensitivity (*M/I*)	0.0156 ± 0.0039	0.0077 ± 0.0019*	0.0127 ± 0.0024*†	0.0061 ± 0.0025*‡

Data are means ± SD. *Significantly different from the low VAT–low IR group; †significantly different from the low VAT–high IR group; ‡significantly different from the high VAT–low IR group, $P < 0.05$. Median values for VAT and *M/I* are 133.9 cm^2 and 0.010 mg · kg^{-1} · min^{-1} · pmol^{-1}, respectively.

Food intake was assessed by a 3-day dietary record, which was completed during 2 weekdays and 1 weekend day. The diary was explained and reviewed by the study nutritionist during an interview with the participant. Women were asked to weigh foods with a scale provided by the nutritionist. The evaluation of nutrient intakes derived from the food record was performed using Food Processor Nutrition Analysis software version 7.2 (ESHA Research, Salem, OR).

Statistical analyses. Statistical analyses were performed using software from the SAS Institute, Cary, NC (version 8.2). Pearson correlation coefficients were calculated to quantify the univariate associations between variables. Median values of visceral AT (133.9 cm^2) and insulin sensitivity (*M/I*) (0.010189 mg · kg^{-1} · min^{-1} · pmol^{-1}) were used to classify women into four subgroups: *1*) women with low visceral AT (<133.9 cm^2) and low IR (*M/I* >0.010189 mg · kg^{-1} · min^{-1} · pmol^{-1}); *2*) women with low visceral AT (<133.9 cm^2) and high IR (*M/I* ≤0.010189 mg · kg^{-1} · min^{-1} · pmol^{-1}); *3*) women with high visceral AT (≥133.9 cm^2) and low IR (*M/I* >0.010189 mg · kg^{-1} · min^{-1} · pmol^{-1}); and *4*) women with high visceral AT (≥133.9 cm^2) and high IR (*M/I* ≤0.010189 mg · kg^{-1} · min^{-1} · pmol^{-1}). Anthropometric and metabolic variables were compared between the four groups by using ANOVA with the general linear model procedure. The Duncan test was used in situations in which a significant group effect was observed. Multiple regression analyses were performed to determine the respective contribution of visceral AT and IR $(M/I)^{-1}$ to the variance of several metabolic variables using a general linear model procedure. The presence of possible interactions between visceral AT and IR was also evaluated. Confounding variables that are likely to affect metabolic profile were also included in multivariate models (age, EE from moderate to vigorous physical activity, and percentage of energy from carbohydrates and lipids). The source of variations in the metabolic variables was computed using the type III sum of squares. This sum of squares applies to unbalanced study designs and quantifies the effects of an independent variable after adjustment for all other variables included in the model. The critical *P* value for significance was set at 0.05. Some variables were not normally distributed (BMI, body fat mass, FPG, IR, triglycerides, VLDL cholesterol, hs-CRP, and IL-6 levels). For these variables, analyses were done on their log-transformed values.

RESULTS

Parameters measured in the four groups of postmenopausal women defined according to their levels of visceral AT and IR are presented in Tables 1 and 2. Women characterized by high visceral AT accumulation but low IR showed similar metabolic profile than control subjects (women with low visceral AT and low IR). Women with low visceral AT deposition but with high IR were characterized by increased FPG, 2hPG, triglyceride, VLDL-apoB, and fibrinogen and lower HDL_2 cholesterol concentrations than women with low visceral AT accumulation and low IR ($P < 0.05$). Except for IR, there were no significant differences in metabolic variables between women with low visceral AT accumulation and high IR and women with

TABLE 2
Metabolic variables in the four groups of postmenopausal women separated on the basis of visceral AT and IR

Variables	Low VAT–low IR	Low VAT–high IR	High VAT–low IR	High VAT–high IR
n	35	19	19	35
Lipoprotein-lipid profile				
Triglycerides (mmol/l)	0.95 ± 0.42	1.33 ± 0.69*	1.06 ± 0.30	1.68 ± 0.6*†‡
LDL cholesterol (mmol/l)	3.56 ± 0.74	3.63 ± 0.96	3.43 ± 0.82	3.61 ± 0.86
HDL cholesterol (mmol/l)	1.56 ± 0.38	1.43 ± 0.29	1.46 ± 0.27	1.21 ± 0.25*†‡
HDL_2 cholesterol (mmol/l)	0.76 ± 0.31	0.58 ± 0.22*	0.65 ± 0.22	0.43 ± 0.16*†‡
Total cholesterol–to–HDL cholesterol ratio	3.71 ± 0.98	4.07 ± 1.23	3.69 ± 0.83	4.68 ± 1.19*‡
apoB (g/l)	0.96 ± 0.15	1.02 ± 0.26	0.95 ± 0.21	1.07 ± 0.24
VLDL apoB (g/l)	0.09 ± 0.05	0.12 ± 0.06*	0.11 ± 0.04	0.14 ± 0.06*
Inflammatory markers				
hs-CRP (mg/l)	1.44 ± 2.34	1.81 ± 1.69	2.40 ± 3.62	4.80 ± 4.45*†‡
Fibrinogen (g/l)	2.61 ± 0.48	3.10 ± 0.75*	2.79 ± 0.58	3.22 ± 0.86
IL-6 (pg/l)	1.23 ± 0.50	1.25 ± 0.49	1.37 ± 0.47	2.10 ± 1.08*†‡

Data are means ± SD. *Significantly different from the low VAT–low IR group; †significantly different from the low VAT–high IR group; ‡significantly different from the high VAT–low IR group, $P < 0.05$.

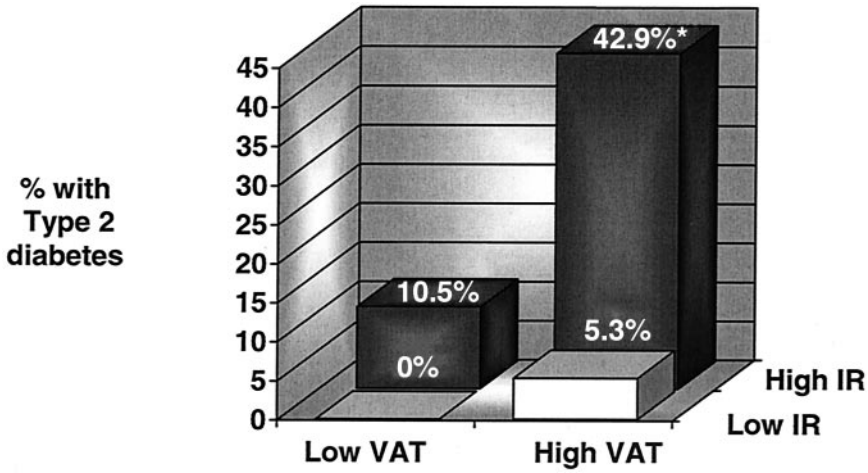

FIG. 1. Prevalence of type 2 diabetes in postmenopausal women separated on the basis of visceral AT and IR. Low VAT–low IR: women with low visceral AT (<133.9 cm^2) and low IR (*M/I* >0.010189 $mg \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$). Low VAT–high IR: women with low visceral AT (<133.9 cm^2) and high IR (*M/I* ≤0.010189 $mg \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$). High VAT–low IR: women with high visceral AT (≥133.9 cm^2) and low IR (*M/I* >0.010189 $mg \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$). High VAT–high IR: women with high visceral AT (≥133.9 cm^2) and high IR (*M/I* ≤0.010189 $mg \cdot kg^{-1} \cdot min^{-1} \cdot pmol^{-1}$). The determination of glucose tolerance status was based on results obtained by one OGTT.

high visceral AT and low IR. On the other hand, women with high visceral AT deposition and high IR were characterized by many alterations in their metabolic profile (hyperglycemia, increased diastolic and systolic blood pressure, higher triglyceride, and lower HDL cholesterol concentrations and increased inflammatory marker concentrations) compared with women with low visceral AT accumulation and low IR ($P < 0.05$). Women with high visceral AT accumulation and high IR also had higher FPG, 2hPG, triglyceride concentrations, total cholesterol–to–HDL cholesterol (cholesterol–to–HDL cholesterol) ratio, hs-CRP, and IL-6 and lower HDL cholesterol and HDL_2 cholesterol concentrations than women with high visceral AT accumulation but low IR. Because visceral AT area was significantly higher in women with high visceral AT and high IR than in women with high visceral AT accumulation and low IR, we paired women on the basis of visceral AT area (±10 cm^2) and then compared the two groups using a Student's *t* test. After this pairing procedure, differences between the two groups were no longer statistically significant for FPG concentrations ($P = 0.067$), hs-CRP ($P = 0.07$), and IL-6 ($P = 0.11$), whereas differences in the plasma lipid-lipoprotein profile and 2hPG concentrations remained significant. Finally, HDL cholesterol and HDL_2 cholesterol concentrations were significantly lower, whereas triglyceride, hs-CRP and IL-6 levels were significantly higher in women with high visceral AT and high IR compared with women with low visceral AT but high IR ($P < 0.05$). Analyses performed without the single woman taking HT provided similar results (results not shown).

Figure 1 shows that 42.9% of women in the group with high visceral AT and high IR and 10.5% of women with low visceral AT and high IR were diagnosed with type 2 diabetes. In women with high visceral AT and low IR, 5.3% were identified as having type 2 diabetes, while none of the women with low visceral AT and low IR had type 2 diabetes ($\chi^2 = 26.6$; $P < 0.0001$).

Table 3 shows that visceral AT and IR were significantly associated with several variables including FPG, 2hPG, systolic and diastolic blood pressure, triglyceride and HDL cholesterol concentrations, HDL_2 cholesterol concentrations and cholesterol–to–HDL cholesterol ratio. Visceral AT and IR were also correlated with hs-CRP, IL-6, and fibrinogen. BMI correlated with metabolic parameters in a similar manner as visceral AT, except that BMI was not significantly associated with cholesterol–to–HDL cholesterol ratio and VLDL-apoB. In contrast, subcutaneous AT correlated only significantly with systolic and diastolic blood pressure, triglycerides, FPG, 2hPG, hs-CRP, IL-6, and fibrinogen.

A multivariate regression analysis was used to determine the independent contribution of visceral AT, IR, and

TABLE 3
Coefficients of correlation (*r*) for the associations of visceral AT and insulin resistance $(M/I)^{-1}$ with selected metabolic variables

Variables	Visceral AT	Subcutaneous AT	$(M/I)^{-1}$	BMI
Fasting glucose	0.38‡	0.29†	0.52‡	0.35†
2-h glycemia	0.45‡	0.32†	0.66‡	0.38‡
Systolic blood pressure	0.31†	0.38‡	0.32†	0.41‡
Diastolic blood pressure	0.34†	0.42†	0.31†	0.43‡
Triglycerides	0.42‡	0.23*	0.54‡	0.32†
LDL cholesterol	0.004	−0.05	0.08	0.04
HDL cholesterol	−0.43‡	−0.18	−0.43‡	−0.27†
HDL_2 cholesterol	−0.43‡	−0.22	−0.48‡	−0.28†
Total cholesterol–to–HDL cholesterol	0.33†	0.07	0.37‡	0.18
apoB	0.17	0.01	0.25†	0.07
VLDL-apoB	0.29†	0.05	0.25†	0.18
hs-CRP	0.57‡	0.54‡	0.45‡	0.64‡
Fibrinogen	0.33†	0.40‡	0.38‡	0.43‡
IL-6	0.56‡	0.49‡	0.38‡	0.59‡

Significant correlation, *$P < 0.05$, †$P < 0.01$, ‡$P < 0.0001$.

TABLE 4
Multivariate regression analyses showing independent contributions of visceral AT and IR to the variance of metabolic variables in the total sample of postmenopausal women

Dependent variable	Independent variable	Partial ($r^2 \times 100$)	P
Triglycerides	IR $(M/I)^{-1}$	13.2	<0.0001
LDL cholesterol	Age	4.4	0.03
HDL cholesterol	Visceral AT	5.9	0.004
	IR $(M/I)^{-1}$	4.2	0.015
	Age	3.8	0.021
	EE6–9	3.2	0.035
HDL_2 cholesterol	Age	7.4	0.001
	IR $(M/I)^{-1}$	6.7	0.001
	Visceral AT	5.1	0.005
	EE6–9	3.9	0.01
Total cholesterol–to–HDL cholesterol ratio	IR $(M/I)^{-1}$	3.7	0.04
apoB	Age	6.8	0.006
hs-CRP	Visceral AT	14.6	<0.0001
	IR $(M/I)^{-1}$	2.8	0.04
IL-6*	VAT * M/I interaction	3.0	0.03
Fibrinogen	IR $(M/I)^{-1}$	4.3	0.03
	Visceral AT	2.7	0.08
Fasting glucose	Energy from carbohydrates	2.8	0.05
2-h glycemia*	Energy from carbohydrates	8.3	<0.0001
	Insulin resistance $(M/I)^{-1}$	3.1	0.02
	Energy from lipids	3.0	0.02
Systolic blood pressure	Age	3.9	0.03
Diastolic blood pressure	Visceral AT	4.0	0.03

Age, EE from moderate to vigorous physical activity (EE6–9), visceral AT, and IR were entered in the model. *For these variables, the interaction between visceral AT and IR was also entered in the multivariate model.

the possible interaction between visceral AT and IR to the variance of metabolic variables in the total sample of postmenopausal women (Table 4). Because EE from moderate to vigorous physical activity was significantly associated with HDL cholesterol, HDL_2 cholesterol, hs-CRP, FPG, 2hPG, and systolic and diastolic blood pressure, and because age was significantly associated with triglyceride, LDL cholesterol, apoB, IL-6, 2hPG, and systolic blood pressure in our study, these two variables were included in the regression models. Percentage of energy derived from carbohydrates and lipids was associated with FPG and 2hPG, and these two variables were also entered in the multivariate models. Interaction between visceral AT and IR was removed from the initial model for the variables of triglycerides, LDL cholesterol, HDL cholesterol, HDL_2 cholesterol, cholesterol–to–HDL cholesterol ratio, apoB, hs-CRP, fibrinogen, and diastolic and systolic blood pressure because they did not contribute significantly to the variance of these variables. Table 4 shows that visceral AT was an independent predictor of HDL cholesterol and HDL_2 cholesterol concentrations, hs-CRP, and diastolic blood pressure ($r^2 \times 100$ varied from 4.0 to 14.6). Insulin resistance was also found to be an independent predictor of many metabolic variables including triglyceride concentrations, HDL and HDL_2 cholesterol concentrations, cholesterol–to–HDL cholesterol ratio, hs-CRP, fibrinogen, and 2hPG ($r^2 \times 100$ between 2.8 and 13.2). EE from moderate to vigorous physical activity was found to be an independent predictor of HDL cholesterol ($r^2 \times 100 = 3.2$) and HDL_2 cholesterol concentrations ($r^2 \times 100 = 3.9$). The only predictor of LDL cholesterol and apoB concentrations in our study was age. A significant effect of interaction between visceral AT and IR was found for IL-6 concentrations ($r^2 \times 100 = 3.0$). Finally, the percentage of energy derived from carbohydrates was found to significantly explain the variance in FPG concentrations ($r^2 \times 100 = 2.8$), whereas a significant proportion of the variance of 2hPG was explained by the percentage of energy derived from carbohydrates ($r^2 \times 100 = 8.3$) and from lipids ($r^2 \times 100 = 3.0$).

DISCUSSION

In this study, we found that women with a combination of high visceral AT and high IR were characterized by a more deteriorated metabolic risk profile than women with increased visceral AT or increased IR as isolated conditions. These results support data from a recent study in which it was observed that subjects with both increased abdominal AT levels and IR were characterized by a more deteriorated lipid-lipoprotein profile compared with subjects with low abdominal AT levels and low IR (29). Women with low visceral AT and high IR were characterized by a deterioration of their metabolic risk profile (glucose–insulin homeostasis parameters, plasma lipid-lipoprotein profile, and inflammatory markers), whereas women with isolated high visceral AT were similar to women with low visceral AT and low IR, with respect to the metabolic risk profile. Therefore, it appears that an increased visceral AT accumulation is associated with a deteriorated metabolic profile only in the presence of IR. On the other hand, the deleterious effects of IR may be seen without visceral AT accumulation.

Many studies have reported the close relationship between IR and abdominal AT accumulation (30,31). It has been debated whether this association is determined pri-

marily by the accumulation of abdominal visceral or subcutaneous AT. Some studies have suggested that abdominal visceral AT is a more important determinant of IR and other features of the metabolic syndrome than abdominal subcutaneous AT (32–34), whereas others have shown the opposite (35). The mechanism by which visceral AT could cause IR has not been fully elucidated. Visceral AT, but not subcutaneous AT, is drained by the portal venous system. Mobilization of free fatty acids (FFAs) is more rapid from visceral than from subcutaneous fat cells because of the higher lipolytic activity in visceral adipocytes, which probably contributes significantly to increase FFA concentrations in the systemic circulation (36). The elevated FFA flux into the liver associated with increased visceral AT accumulation would decrease the hepatic insulin extraction by inhibiting insulin binding and degradation, leading to systemic hyperinsulinemia as well as inhibiting the suppression of hepatic glucose production by insulin (37–39).

Results from this study point out the synergic effect of high visceral AT and high IR in the determination of the metabolic risk profile found in postmenopausal women, as the subgroup of women with high visceral AT and high IR was the one showing the more abnormalities in metabolic variables. Because it has been suggested that hyperglycemia per se could be associated with increased CVD risk (40), we decided to compare women with type 2 diabetes with those without type 2 diabetes among our subgroup characterized by high visceral AT and high IR. Besides differences in FPG, 2hPG, and IR, women with type 2 diabetes did not differ significantly from nondiabetic women (data not shown), suggesting that the presence of women with type 2 diabetes in the high visceral AT and high IR group (42.9%) was not contributing significantly to the deteriorations in the CVD risk profile observed in this group of women.

Women with high visceral AT and low IR did not show a deteriorated metabolic profile despite the presence of high levels of visceral AT. Genetic background, diet, or physical activity could potentially explain their relatively good metabolic profile. Indeed, regular physical activity and proper diet have been shown to improve insulin sensitivity and related conditions in women (41). Additional analyses showed that physical activity habits and diet were similar between this group of women and the one with low visceral AT accumulation and low IR. Thus, our results suggest that these women with an increased visceral AT accumulation will be protected from features of the metabolic syndrome as long as they maintain a good degree of insulin sensitivity. These results also suggest that women with high visceral AT and high IR could benefit from interventions based on lifestyle modifications (diet and physical activity) or pharmaceutical agents that improve insulin sensitivity independently of weight loss. These results are relevant from a clinical point of view since they emphasize the importance of intervention strategies aiming at improving insulin sensitivity rather than focusing solely on a reduction in body weight, which is often difficult to achieve and maintain (42). However, weight still remains a relevant clinical target when appropriate because it has been shown to be associated with improved insulin sensitivity (43).

Women with low visceral AT and high IR displayed deterioration in their metabolic risk profile compared with those with low visceral AT and low IR. Using a simple questionnaire about family history of diabetes, we were not able to establish that these women had increased genetic susceptibility for type 2 diabetes that could have explained their increased IR. However, we found that women characterized by low visceral AT accumulation and high IR tended to have lower EE from moderate to vigorous physical activity compared with women with low visceral AT and low IR ($P = 0.055$). The reduction in the frequency of physical activity in this group could explain, at least in part, their deteriorated metabolic risk profile since physical activity has been shown to be an independent predictor of some metabolic parameters (44). Thus, increasing regular physical activity and preventing weight gain could be essential in these women in order to slow down their transition toward the high visceral AT and high IR state shown to display the highest metabolic risk.

Our results are not perfectly in line with those of Nieves et al. (29), who concluded that the dyslipidemia typically found in nonobese IR subjects was mainly explained by increased visceral AT accumulation. In fact, they found that nonobese subjects (BMI <27.5 kg/m^2) with IR had alterations in their lipid-lipoprotein profile (higher levels of triglyceride, LDL cholesterol, and apoB and lower HDL cholesterol concentrations), as compared with nonobese insulin-sensitive subjects, but also had increased visceral AT accumulation, suggesting that IR independently of obesity status could contribute significantly to dyslipidemia (29). In our study, our groups of women with low visceral AT had similar visceral AT levels (92 vs. 100 cm^2) but differed in terms of IR. In addition, significant differences in the lipid-lipoprotein profile were observed. Therefore, we could not attribute these alterations in lipid-lipoprotein profile to an increase in visceral AT, and an independent contribution of IR can thus be suggested.

Results from multivariate regression analyses confirmed those obtained with group comparisons, which suggest that both visceral AT and IR contribute to the deteriorated metabolic risk profile, with their respective contribution varying according to the metabolic variable studied. In fact, IR seems to be more closely associated with some variables of the plasma lipid–lipoprotein profile (such as triglycerides, HDL_2 cholesterol, and cholesterol–to–HDL cholesterol ratio), glucose-tolerance homeostasis variables, and fibrinolysis parameters. IR seems to be particularly important in the determination of triglyceride concentrations. The independent contribution of IR to the variance in triglyceride concentrations may be explained through reduced antilipolytic action of insulin. This generates an increase in circulating FFAs and FFA flux to the liver that can stimulate triglyceride formation (45). A recent study has shown that IR in the skeletal muscle of healthy, young, lean, insulin-resistant offspring of subjects with type 2 diabetes was associated with dysregulation of intramyocellular fatty acid metabolism. These alterations in fatty acid metabolism may represent a mechanism by which IR could be linked to hypertriglyceridemia (46).

Our results showed that HDL cholesterol concentrations, hs-CRP, and diastolic blood pressure were more closely associated with visceral AT levels than with IR.

More specifically, visceral AT explained almost 15% of the variance in plasma hs-CRP levels. The association between visceral AT and hs-CRP has also been observed in another study on postmenopausal women and might be mediated by IL-6, which is expressed in AT and referred to as the main regulator of CRP production in the liver (47).

In conclusion, results from this study performed in postmenopausal women suggest that visceral fat and IR both mediate the metabolic risk profile and that the combination of high visceral AT and high IR in postmenopausal women appears to be the most detrimental combination of factors for the metabolic health of these women. Although our results clearly show the independent contribution of visceral AT accumulation and IR to the determination of many metabolic parameters, it should be kept in mind that these two conditions appear mostly in combination. In addition, the fact that some metabolic risk variables were more closely associated with visceral AT and that other parameters were rather more stronger related to IR needs to be considered in an optimal preventive strategy and in the selection of an adequate treatment. The underlying mechanisms involved explaining the effects of visceral AT, IR, and their interaction on the determination of the CVD risk profile in postmenopausal women will require further investigation.

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