Relationship of Visceral Adipose Tissue to Metabolic Risk Factors for Coronary Heart Disease: Is There a Contribution of Subcutaneous Fat Cell Hypertrophy?

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Visceral adipose tissue (VAT) accumulation is an important correlate of the metabolic complications found in obese patients. The aim of this study was to evaluate the respective contribution of VAT deposition versus subcutaneous abdominal or femoral fat cell hypertrophy as correlates of the metabolic risk profile in 69 men and 65 premenopausal women (aged 35 ± 5 years) with a wide range of fatness (body mass index, 18 to 57 kg/m^2). In both genders, VAT accumulation was positively correlated with fasting plasma insulin, triglyceride (TG), and low-density lipoprotein (LDL)-apolipoprotein B (apo B) levels and the cholesterol (CHOL)/high-density lipoprotein (HDL)-CHOL ratio ($.24 \leq r \leq .71$, $P < .05$). A similar pattern of positive relationships was found between subcutaneous abdominal fat cell weight and metabolic risk variables in men and women ($.33 \leq r \leq .60$, $P < .01$). Positive associations were also observed in women between femoral fat cell weight and fasting plasma insulin, TG, and CHOL levels and the CHOL/HDL-CHOL ratio ($.29 \leq r \leq .42$, $P < .05$). However, only plasma TG concentrations and the CHOL/HDL-CHOL ratio were positively correlated with femoral fat cell weight in men ($r = .30$, $P < .05$). To better investigate the relationships between the metabolic risk profile and hypertrophic subcutaneous obesity, individuals with small versus large subcutaneous abdominal adipocytes were matched according to VAT accumulation. Men with large abdominal fat cells displayed higher plasma TG and LDL-apo B levels compared with men characterized by small abdominal adipocytes ($P < .05$). Stepwise multiple regression analyses showed that subcutaneous abdominal fat cell weight was the best independent variable predicting plasma TG and LDL-apo B levels in men. No significant difference was found in the metabolic profile of subjects displaying small versus large femoral adipocytes. Taken together, these results suggest that for a given VAT deposition, the presence of hypertrophied subcutaneous abdominal adipocytes in men appears to be associated with further deterioration in the metabolic risk profile. On the other hand, the hypertrophy of femoral adipocytes does not further alter the metabolic complications generally related to obesity in both men and women.

SEVERAL EPIDEMIOLOGICAL and experimental studies have confirmed the pioneering observations of Vague[1] emphasizing the importance of body fat distribution. Indeed, it is now commonly accepted that a preferential accumulation of fat in the abdominal region is associated with an increased risk of non–insulin-dependent diabetes mellitus and coronary heart disease (CHD).[2-7] Moreover, the development of new imaging techniques has allowed several groups of investigators to propose that visceral adipose tissue (VAT) deposition is a critical correlate of the metabolic complications found among obese patients.[4,7,8] However, some recent studies have reported that a variation in subcutaneous abdominal fat is also an important determinant of individual differences in insulin sensitivity.[9-11]

The apparent heterogeneity of human obesity has led clinicians to propose several classifications of this condition.[7] One of these is based on the cellular characteristics of adipose tissue and identifies two main subtypes of obesity: "hypertrophic obesity," resulting from an enlargement of adipocytes, versus "hyperplastic obesity," which is related to an increased number of adipose cells. Krotkiewski et al[12] have examined this issue in a comprehensive study of adipose cellularity. They concluded that body fat accretion was mainly due to fat cell weight enlargement, the latter being subsequently followed by an increase in adipose cell number. In this regard, Björntorp[13] has already suggested that adipose tissue (AT) hyperplasia occurs when fat cell weight approaches 0.6 μg lipid per cell.

The impact of individual variation in adipose tissue cellularity on the metabolic abnormalities of obesity has also been examined. Björntorp et al[14] have reported that fasting plasma insulin is positively related to the fat cell size of adipocytes derived from subcutaneous abdominal and gluteal fat depots in middle-aged men. Similarly, subcutaneous epigastric fat cell weight has also been associated with fasting plasma insulin, glucose, and triglyceride (TG) levels in women, whereas epigastric fat cell weight has been related to plasma insulin and TG levels in men.[12] Kissebah et al[15] have also shown that upper-body obese premenopausal women also display enlarged subcutaneous abdominal fat cells and elevated plasma glucose and insulin levels following an oral glucose load, whereas lower-body obese women have smaller subcutaneous abdominal adipocytes and lower glycemic and insulinemic responses. Taken together, these observations suggest that excessive abdominal fat deposition and the presence of enlarged subcutaneous adipose cells could synergistically act in the etiology of the metabolic complications associated with obesity. However, to the best of our knowledge, no study has attempted to investigate the independent contribution of VAT versus subcutaneous adipose cell hypertrophy to the metabolic risk profile of men and women.

The aim of the present investigation was therefore to evaluate the respective contribution of VAT and subcutaneous abdominal and femoral fat cell hypertrophy to the variation in the metabolic profile predictive of CHD risk in a sample of 69 men and 65 premenopausal women.

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SUBJECTS AND METHODS

Subjects

One hundred thirty-four healthy subjects (69 men and 65 women) aged 35 ± 6 years (mean ± SD) were recruited through the media and provided written informed consent to participate in a study to examine the potential relationship of obesity and body fat distribution indices to metabolic risk variables.[16,17] This study has been approved by the Laval University Medical Ethics Committee. All individuals were subjected to a medical evaluation by a physician, which included a medical history. Subjects with cardiovascular disease, diabetes mellitus, or endocrine disorders or those on medication that could influence carbohydrate or lipid metabolism (β-blockers, antihypertensive, etc.) were excluded from the study. All subjects were sedentary nonsmokers and moderate alcohol consumers. None had recently been on a diet or involved in a weight loss program, and their body weight was stable for at least 6 months prior to the experiment. The women had regular menstrual cycles, and none were using oral contraceptives or lactating at the time of study. All measurements were performed while women were in the early follicular phase of the menstrual cycle.

Total Body Fatness and Regional Fat Distribution

Body density was determined by the underwater weighing technique,[18] and percent body fat was derived from body density.[19] Pulmonary residual volume was measured using the helium dilution method.[20] Fat mass was calculated as total body weight minus fat-free mass. Waist girth was measured according to the procedures recommended at the Airlie Conference.[21] Computed tomography (CT) was performed on a Siemens Somaton DRH scanner (Siemens, Erlangen, Germany) according to the methodology of Sjöström et al.[22] Briefly, the subjects were examined in the supine position with both arms stretched above their head. CT scans were performed at both the abdominal (between L4 and L5 vertebrae) and femoral (midthigh) levels, using a radiograph of the skeleton as a reference to establish the position of the scans to the nearest millimeter as previously described.[23] Total adipose tissue (AT) areas were calculated by delineating these areas with a graph pen and then computing the AT surfaces with an attenuation range of −190 to −30 Hounsfield units[22] as previously described.[23] The abdominal VAT 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 VAT area from the total abdominal AT area.

AT Biopsy Procedure

After an overnight fast, participants were subjected to biopsies of subcutaneous fat, one in the periumbilical region (abdominal site) and the other at the midthigh level (femoral site). A small cutaneous incision (1 cm) was made at both sites, and about 100 mg subcutaneous AT was surgically removed from the two depots. Adipocytes were isolated according to the method of Rodbell[24] in a Krebs-Ringer bicarbonate buffer (pH 7.4) containing 4% bovine serum albumin (KRBA) and 5 mmol/L glucose plus 1 mg/mL collagenase, as previously described.[25] Digestion was performed in a shaking water bath under an air gas phase of 95% O_2 and 5% CO_2 for 40 minutes at 37°C. The suspension was then filtered, and the cellular filtrate obtained was rinsed three times with 5 mL KRBA. Isolated adipocytes were finally resuspended in KRBA to obtain a final concentration of approximately 500 cells/50 μL. Fat cell diameter was determined using a Leitz microscope equipped with a graduated ocular (Leitz, Rockleigh, NJ). The mean adipose cell diameter was assessed from the measurement of at least 500 cells, and the density of triolein was used to transform adipose cell volume to fat cell weight as previously described.[25]

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 tubes containing EDTA and Trasylol (Miles Pharmaceutics, Rexdale, Ontario, Canada) through a venous catheter from an antecubital vein at −15, 0, 15, 30, 45, 60, 90, 120, 150, and 180 minutes. Plasma glucose levels were measured enzymatically,[26] and plasma insulin concentrations were determined by radioimmunoassay with polyethylene glycol separation.[27] Plasma free fatty acid (FFA) levels were determined at −15, 0, 30, 60, 120, and 180 minutes with a colorimetric method.[28] The total glucose and insulin areas under the curve measured during the OGTT were calculated using the trapezoid method.

Plasma Lipids and Lipoproteins

Blood samples were obtained in the morning after a 12-hour fast from an antecubital vein into vacutainer tubes containing EDTA. Plasma cholesterol (CHOL) and TG levels were measured enzymatically in plasma and lipoprotein fractions on a RA-1000 Autoanalyzer (Technicon Instruments, Tarrytown, NY) referenced to the Centers for Disease Control (Atlanta, GA). Plasma very–low-density lipoproteins ($d < 1.006$ g/mL) were isolated by ultracentrifugation,[29] and the high-density lipoprotein (HDL) fraction was obtained after precipitation of low-density lipoprotein (LDL) in the infranatant ($d > 1.006$ g/mL) with heparin and $MnCl_2$.[30] Plasma LDL-apolipoprotein B (apo B) levels were measured by the rocket immunoelectrophoretic method of Laurell as previously described.[31]

Reagents and Chemicals

Collagenase and bovine serum albumin were obtained from Boehringer Mannheim (Montreal, Canada). All other chemicals and organic solvents were of the highest purity commercially available. The same batches of collagenase and albumin were used in all experiments.

Statistical Methods

Data reported in the tables are expressed as the mean ± SD, whereas values shown in the figures are the mean ± SE. Associations between two variables were quantified using Pearson's product-moment correlation coefficients. Partial correlation analyses were also performed to estimate the independent contribution of VAT deposition and abdominal and femoral fat cell weight to the variance of several metabolic variables. Since the results obtained from partial correlation analyses were essentially similar to data obtained from the matching procedure, only results on matched subjects are reported. The normality of the distribution for all variables was studied with a Shapiro-Wilk W test. For variables that were not normally distributed, the Mann-Whitney test for nonparametric variables was used instead of Student's t test. All analyses were performed using JMP Version 3.1.5 for Macintosh (SAS Institute, Cary, NC).

RESULTS

Subject Characteristics

The subjects' physical and metabolic characteristics are presented in Tables 1 and 2, respectively. The wide range of percent body fat (10% to 39% and 16% to 59% for men and women, respectively) indicated that our sample included lean to obese individuals. Gender comparisons showed that men displayed both higher body weight and waist girth compared with women ($P = .0001$ to $.001$). However, women were characterized by a greater fat mass and a higher percent body fat than men ($P = .0001$ to $.05$). Both subcutaneous abdominal and midthigh AT areas determined by CT were larger in women than

Table 1. Physical Characteristics of the Subjects

Characteristic	Women (n = 65)	Men (n = 69)
Age (yr)	35 ± 5	36 ± 4
Anthropometric variables		
Body weight (kg)‖	73 ± 21	81 ± 12†
BMI (kg/m²)‖	28 ± 9	27 ± 4
Fat mass (kg)‖	29 ± 16	22 ± 8*
% Fat	37 ± 11	26 ± 6‡
Waist girth (cm)‖	84 ± 17	94 ± 11‡
AT areas measured by CT (cm²)‖		
Abdomen (L4-L5)		
Subcutaneous	361 ± 202	242 ± 101†
Visceral	90 ± 50	121 ± 48†
Midthigh subcutaneous	176 ± 68	97 ± 35‡
Regional fat cell weight (μg lipid/cell)		
Abdominal	0.57 ± 0.25	0.51 ± 0.13
Femoral	0.67 ± 0.22§	0.55 ± 0.12†

NOTE. Values are the mean ± SD.
Abbreviation: BMI, body mass index.
Significant gender difference: *$P < .05$, †$P < .001$, ‡$P < .0001$.
§Regional variation in adipose cell size of women, $P < .05$.
‖Mann-Whitney test for nonparametric variables was performed because variables were not normally distributed.

in men ($P = .0001$ to $.001$). On the other hand, men were characterized by a greater VAT accumulation compared with women ($P < .001$). Femoral fat cells were also significantly larger than subcutaneous abdominal adipocytes in women ($P < .05$), although no regional variation was observed in men. Finally, women displayed larger subcutaneous femoral adipose cells than men ($P < .001$), whereas no gender difference was found for subcutaneous abdominal fat cell weight. On the other hand, men had higher fasting plasma glucose and insulin levels, as well as lower HDL-CHOL levels and a larger CHOL/HDL-CHOL ratio, than women ($P = .0001$ to $.01$). Moreover, the insulin response to an oral glucose load was also higher in men versus women ($P < .0001$).

In an attempt to investigate the contribution of VAT accumulation and regional subcutaneous fat cell size to the metabolic complications associated with obesity, relationships between

Table 2. Metabolic Profile of the Subjects

Parameter	Women (n = 65)	Men (n = 69)
Glucose (mmol/L)‖	4.8 ± 0.5	5.2 ± 0.6‡
Insulin (pmol/L)‖	66.5 ± 47.9	78.7 ± 35.1*
FFA (mmol/L)	0.5 ± 0.2	0.6 ± 0.2
TG (mmol/L)‖	1.3 ± 0.6	1.6 ± 0.1
CHOL (mmol/L)	4.9 ± 0.9	4.9 ± 0.7
LDL-CHOL (mmol/L)	3.3 ± 0.9	3.3 ± 0.7
HDL-CHOL (mmol/L)	1.2 ± 0.3	1.0 ± 0.2†
LDL-apo B (mg/dL)	79.3 ± 21.4	81.7 ± 18.2
CHOL/HDL-CHOL ratio	4.3 ± 1.2	5.0 ± 1.3*
Glucose area	1.12 ± 0.23	1.17 ± 0.28
Insulin area‖	46.0 ± 34.8	71.9 ± 34.2‡

NOTE. Values are the mean ± SD. Glucose and insulin areas represent integrated plasma concentrations measured for 3 hours after an oral glucose load (75-g OGTT). Glucose area is expressed in mmol/L/min × 10^{-3} and insulin area is expressed in pmol/L/min × 10^{-3}.
Significant gender difference: *$P < .01$, †$P < .001$, ‡$P < .0001$.
‖Mann-Whitney test for nonparametric variables was performed because variables were not normally distributed.

Table 3. Pearson Correlation Coefficients for the Associations Between Subcutaneous Abdominal and Femoral Fat Cell Weight and VAT Accumulation Versus Metabolic Variables in the Sample of 69 Men

Variable	VAT	Fat Cell Weight	
		Abdominal	Femoral
Glucose	.43§	.26*	.02
Insulin	.55§	.35†	.06
FFA	.28*	.33†	.13
TG	.28*	.34†	.30†
CHOL	.16	.36†	.12
LDL-CHOL	.13	.27*	.09
HDL-CHOL	−.22	−.21	−.32†
LDL-apo B	.28*	.40‡	.14
CHOL/HDL-CHOL ratio	.24*	.33†	.29*
Glucose area	.56§	.35†	.08
Insulin area	.65§	.56§	.20

*$P < .05$.
†$P < .01$.
‡$P < .001$.
§$P < .0001$.

VAT and between subcutaneous abdominal and femoral fat cell weight and the metabolic risk profile were examined in both men and women (Tables 3 and 4, respectively).

Correlation Analyses

In both genders, fasting plasma glucose, insulin, FFA, TG, and LDL-apo B levels and the CHOL/HDL-CHOL ratio were positively related to VAT accumulation ($.24 \leq r \leq .71$, $P = .0001$ to $.05$). In addition, VAT deposition was positively associated with both the glucose and insulin areas measured during the oral glucose load ($.42 \leq r \leq .65$, $P < .001$). A similar pattern of significant associations was found between subcutaneous abdominal fat cell weight and both the plasma lipid-lipoprotein profile and indices of glucose-insulin homeostasis in both genders ($.26 \leq r \leq .60$, $P = .0001$ to $.05$). On the other hand, positive associations were observed between femo-

Table 4. Pearson Correlation Coefficients for the Associations Between Subcutaneous Abdominal and Femoral Fat Cell Weight and VAT Accumulation Versus Metabolic Variables in the Sample of 65 Women

Variable	VAT	Fat Cell Weight	
		Abdominal	Femoral
Glucose	.57§	.52§	.38‡
Insulin	.62§	.60§	.52§
FFA	.52§	.42‡	−.13
TG	.71§	.56§	.45‡
CHOL	.27*	.31†	.29*
LDL-CHOL	.24*	.32†	.29*
HDL-CHOL	−.51§	−.46§	−.33†
LDL-apo B	.33†	.36†	.20
CHOL/HDL-CHOL ratio	.54§	.52§	.42‡
Glucose area	.42‡	.27*	.06
Insulin area	.44‡	.42‡	.31*

*$P < .05$.
†$P < .01$.
‡$P < .001$.
§$P < .0001$.

ral fat cell weight and fasting plasma glucose, insulin, TG, and CHOL levels and the CHOL/HDL-CHOL ratio in women ($.29 \leq r \leq .52$, $P = .0001$ to .05), whereas plasma TG levels and the CHOL/HDL-CHOL ratio were the only variables correlated with femoral fat cell weight in men ($r = .30$, $P = .01$ to .05). However, correlation coefficients between the latter metabolic variables and femoral fat cell weight were generally lower than those found when subcutaneous abdominal fat cell weight was plotted against these metabolic indices. Finally, the metabolic profile was correlated with the subcutaneous abdominal fat deposition measured by CT ($.25 \leq r \leq .48$ and $.28 \leq r \leq .68$ in men and women, respectively, $P = .0001$ to .05; not shown), and these correlation coefficients were of similar magnitude to those previously observed with subcutaneous fat cell weight.

To further quantify the metabolic complications associated with hypertrophic subcutaneous obesity, two subgroups of subjects matched for similar VAT accumulation but with either small or large subcutaneous abdominal or femoral fat cells were compared (Figs 1 and 2). In both genders, subgroups with either small or large subcutaneous abdominal fat cells did not differ by age, total body fatness, and subcutaneous abdominal fat deposition. However, men characterized by large subcutaneous abdominal fat cells had higher plasma TG and LDL-apo B levels ($P < .05$). In contrast, there was no difference in the metabolic profile of women with either small or large subcutaneous abdominal fat cells (Fig 1). Moreover, no significant difference was found for the metabolic risk profile of subgroups displaying small or large subcutaneous femoral adipocytes in both genders (Fig 2).

Stepwise Multiple Regression Analyses

To estimate the respective contributions of regional and total adiposity to the variance in TG, LDL-apo B, HDL-CHOL, and insulin levels, a stepwise multiple regression analysis was performed. Our model included fat mass, femoral fat, VAT and subcutaneous abdominal AT accumulation measured by CT, as well as subcutaneous abdominal and femoral fat cell weight. In men, 35% of the variance in fasting insulin was best predicted by VAT (Table 5). Moreover, 31% of the variance in TG was predicted by subcutaneous abdominal fat cell weight and femoral and abdominal subcutaneous AT accumulation. On the other hand, subcutaneous abdominal fat cell weight was the only variable retained as a significant predictor of LDL-apo B levels (15% of variance), while femoral fat cell weight was the best predictor of the variance in HDL-CHOL concentrations (10%). With the exception of fat mass and femoral fat cell weight, all variables included in our stepwise regression model accounted for 55% of the variance in the insulin area. However, VAT was the best predictor of the variance in the insulin area (43%) determined during the oral glucose load, in men.

In women, 65% of the variance in fasting insulin was accounted for by fat mass, femoral and subcutaneous abdominal AT accumulation, and femoral fat cell weight (Table 6). In addition, VAT was the only variable that accounted for 51% and 25% of the variance in TG and HDL-CHOL levels, respectively. Moreover, 23% of the variance in LDL-apo B levels was best predicted by subcutaneous abdominal AT accumulation and fat mass. Finally, subcutaneous abdominal fat accounted for 21% of the variance in the insulin area.

DISCUSSION

The present study was designed as an attempt to verify whether variations in either subcutaneous abdominal or femoral adipose cell size could influence the metabolic risk profile of both men and women after controlling for individual differences in VAT accumulation.

It is now well recognized that there are gender differences in body fat distribution, with women being characterized by a greater accumulation of subcutaneous fat than men.[1,7,13,32] Furthermore, women display a preferential accumulation of gluteal-femoral AT, a finding concordant with the fact that they show enlarged gluteal-femoral adipocytes as compared with subcutaneous abdominal adipose cells.[33-37] On the other hand, men have a larger proportion of intraabdominal fat and do not show a marked regional variation in subcutaneous adipose cell size.[37,38]

The significant correlations observed in the present study between VAT measured by CT and fasting plasma glucose, insulin, and FFA levels in both genders (Tables 3 and 4) are in good accordance with the positive associations previously reported between intraabdominal fat deposition and alterations in both insulin-glucose homeostasis and plasma lipoprotein-lipid levels.[6,7,16,17,39] Although the mechanism(s) responsible for the deleterious metabolic impact of excess intraabdominal fat is not fully understood, it has been hypothesized that AT lipolysis could play a nonnegligible role in the metabolic complications related to abdominal obesity.[3,7,40,41] Indeed, it is now well established that due to its important lipolytic rate, VAT, which is drained by the portal vein, could expose the liver to an enhanced FFA flux, which in turn could alter glucose-insulin homeostasis by promoting a reduction of hepatic insulin degradation and an inhibition of peripheral glucose utilization,[4,6,7] which could lead to systemic hyperinsulinemia and in vivo insulin resistance.

In the present study, significant relationships were also observed between subcutaneous abdominal fat cell weight and most of the metabolic indices measured in both genders (Tables 3 and 4). These results are in good accordance with the previous associations reported between large subcutaneous adipocytes and metabolic aberrations such as hyperinsulinemia,[42] hypertriglyceridemia,[14] and non–insulin-dependent diabetes mellitus.[43] Thus, the present results reemphasize the notion that the hypertrophy of subcutaneous abdominal adipose cells is a significant correlate of the metabolic disturbances associated with abdominal obesity in both genders.[38,44] However, although these previous studies have provided interesting results, they did not take into account the concomitant variation in VAT accumulation, which may have affected the conclusions relating hypertrophic obesity to the metabolic deteriorations that generally accompany abdominal fat deposition.[14,38,42-44]

To the best of our knowledge, our study is the first to investigate the independent contribution of hypertrophic subcutaneous obesity versus VAT accumulation to the variation observed in the metabolic risk profile of both men and women. Our results showed that for similar levels of VAT, men with enlarged subcutaneous abdominal adipocytes displayed further deterioration of the metabolic risk profile versus individuals

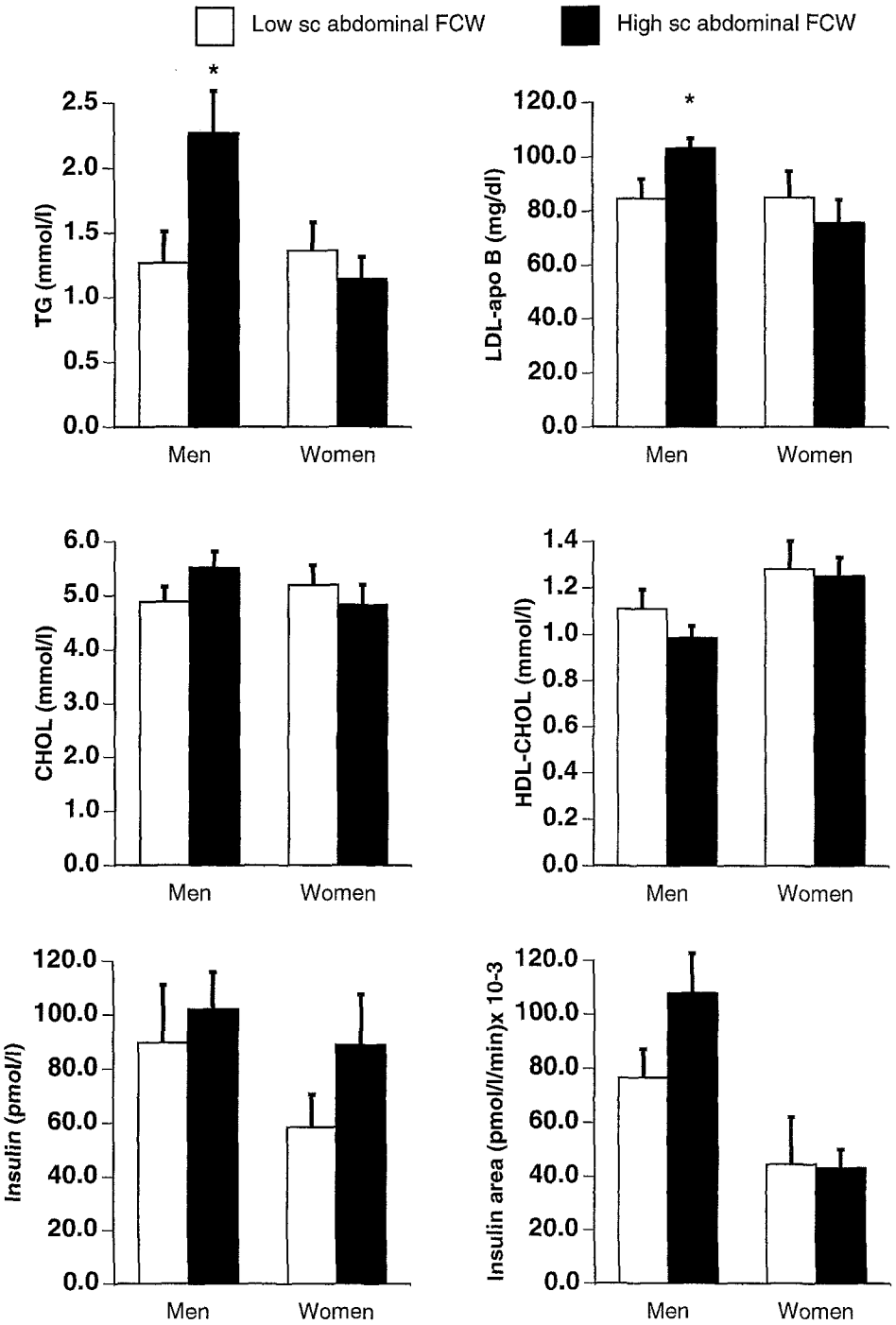

Fig 1. Comparison of selected metabolic indices for 8 pairs of men with low (0.44 ± 0.05 μg/lipid) or high (0.71 ± 0.04 μg/lipid) subcutaneous (sc) abdominal fat cell weight (FCW) versus 9 pairs of women with low (0.43 ± 0.07 μg/lipid) or high (0.85 ± 0.09 μg/lipid) sc abdominal FCW. Subjects were matched for similar VAT accumulation (137 ± 21 *v* 141 ± 22 and 102 ± 17 *v* 104 ± 18 cm² in men and women, respectively). *$P <$ .05.

with small subcutaneous abdominal adipose cells (Fig 1). Considering the fact that large adipocytes are generally characterized by a high lipolytic rate,[15,25,35,45] the hypertrophy of subcutaneous abdominal adipocytes could lead to an increased adipocyte-hepatocyte fatty acid flux, which in turn may partly explain metabolic disturbances such as hypertriglyceridemia[46] or hyperapobetalipoproteinemia.[47] This hypothesis does not appear to be justified in women, since no significant difference was found in the metabolic profile of subjects displaying small versus large subcutaneous abdominal adipose cells. The latter observation is confirmed by the stepwise regression analysis, which showed that the metabolic profile in women seems to be influenced more by both VAT and subcutaneous abdominal AT deposition, as well as fat mass, than by hypertrophied subcutaneous abdominal fat cells.

However, the fact that a high lipolytic rate of enlarged subcutaneous abdominal fat cells could contribute to the observed hypertriglyceridemia and hyperapobetalipoproteinemia is based exclusively on in vitro measurements. Some discrepancies exist between in vitro and in vivo findings regarding adipose cell lipolysis. There is now increasing evidence that such discordant observations are probably due, in

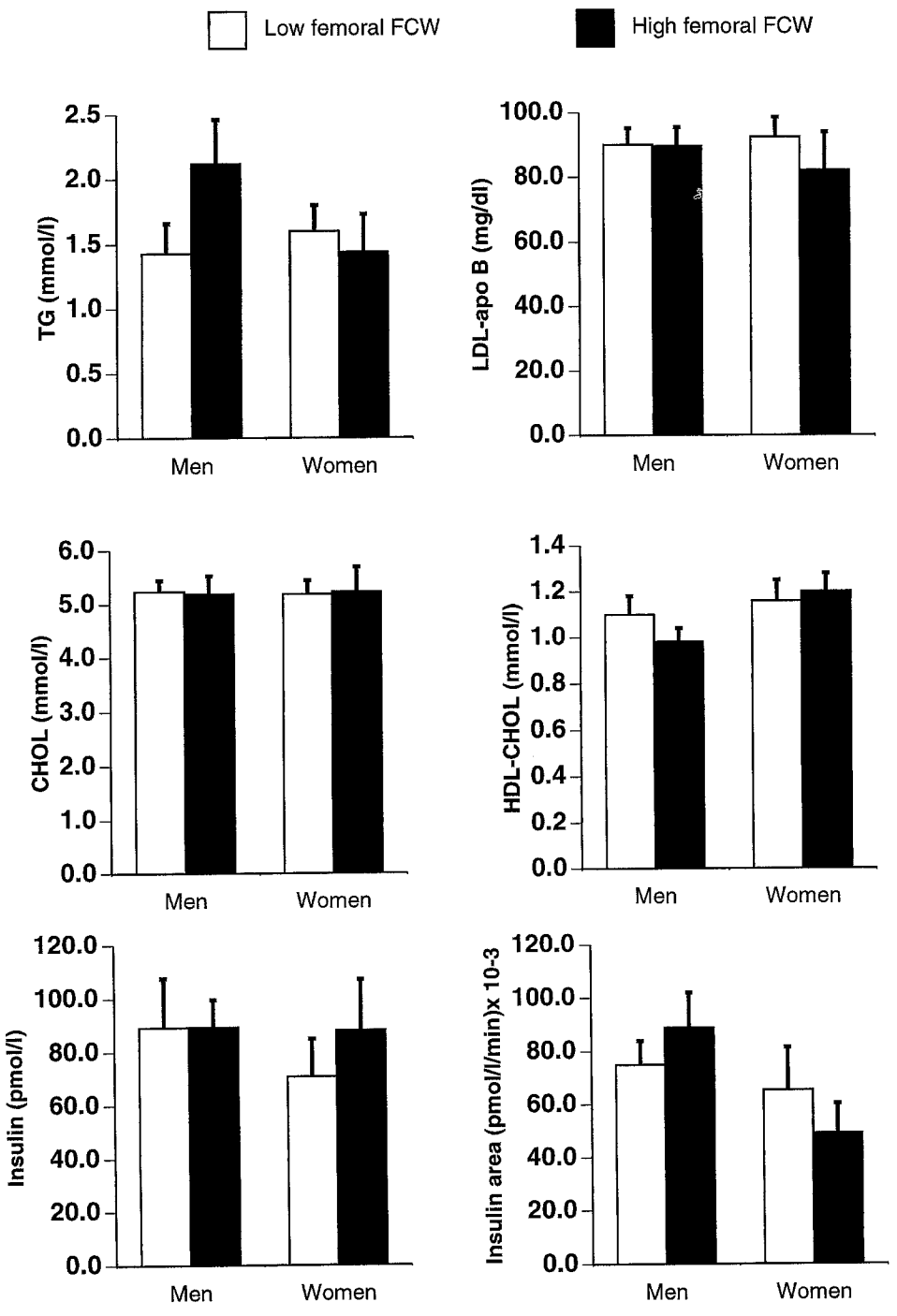

Fig 2. Comparison of selected metabolic indices for 9 pairs of men with low (0.44 ± 0.02 μg/lipid) or high (0.74 ± 0.04 μg/lipid) femoral fat cell weight (FCW) versus 9 pairs of women with low (0.56 ± 0.04 μg/lipid) or high (0.99 ± 0.05 μg/lipid) femoral FCW. Subjects were matched for similar VAT accumulation (136 ± 18 *v* 139 ± 19 and 108 ± 16 *v* 111 ± 17 cm^2 in men and women, respectively).

part, to blood flow effects.[48] In this regard, it has already been shown that when expressed per kilogram of fat mass, whole-body lipolytic rates determined during infusions of stable isotopically labeled glycerol are lower in obese versus lean individuals.[49] However, the main problem concerning the assessment of in vivo lipolysis in obesity relates to the expression of FFA and glycerol release (R_a) in terms of lean body mass or AT mass. These different modes of expression may have a considerable influence on the data interpretation. Indeed, it has previously been reported that obese subjects display increased basal rates of lipolysis when FFA R_a is expressed per unit of lean body mass.[50] On the other hand, an in vivo study using microdialysis reported higher glycerol concentrations in obese versus lean men,[51] a difference that was also ascribed to an increased lipolytic rate in larger fat cells. In addition, interstitial glycerol levels were higher in abdominal versus femoral subcutaneous AT,[52] which attests for an increased lipolytic rate from the abdominal depot. Based on these observations, although in vivo lipolysis measurements may explain the associations reported between the metabolic profile and fat cell hypertrophy, in vitro fat cell metabolic data should also be considered.

On the other hand, results of the present study also suggest that plasma insulin levels are best accounted for by VAT

Table 5. Stepwise Multiple Regression Analysis Showing the Independent Contributions of Anthropometric Variables to the Variation in the Metabolic Risk Profile of 69 Men

Dependent Variable	Independent Variable	Partial R^2 (×100)	Total R^2 (×100)	P
Model 1				
TG	Abdominal fat cell weight (+)	16.5	30.7	.0009
	Femoral fat (+)	5.0		.05
	Subcutaneous abdominal fat (+)	9.2		.007
Model 1				
LDL-apo B	Abdominal fat cell weight (+)	15.1	15.1	.002
Model 1				
HDL-CHOL	Femoral fat cell weight (−)	9.5	9.5	.01
Model 1				
Insulin	Visceral fat (+)	35.4	35.4	.0001
Model 1				
Insulin area	Visceral fat (+)	43.0	54.4	.0001
	Abdominal fat cell weight (+)	5.4		.01
	Femoral fat (+)	2.7		.05
	Subcutaneous abdominal fat (+)	3.3		.04

NOTE. Model 1 includes fat mass, femoral fat, VAT and subcutaneous abdominal AT measured by CT, and subcutaneous abdominal and femoral fat cell weight. Positive (+) or negative (−) relationships are indicated between independent and dependent variables.

Table 6. Stepwise Multiple Regression Analysis Showing the Independent Contributions of Anthropometric Variables to the Variation in the Metabolic Risk Profile of 65 Women

Dependent Variable	Independent Variable	Partial R^2 (×100)	Total R^2 (×100)	P
Model 1				
TG	Visceral fat (+)	50.7	50.7	.0001
Model 1				
LDL-apo B	Subcutaneous abdominal fat (+)	18.0	23.2	.01
	Fat mass (+)	5.2		.05
Model 1				
HDL-CHOL	Visceral fat (−)	25.5	25.5	.0001
Model 1				
Insulin	Fat mass (+)	46.4	65.0	.0001
	Femoral fat (+)	6.8		.005
	Subcutaneous abdominal fat (+)	7.6		.002
	Femoral fat cell weight (+)	4.2		.01
Model 1				
Insulin area	Subcutaneous abdomimal fat (+)	21.0	21.0	.0002

NOTE. Model 1: includes fat mass, femoral fat, VAT and subcutaneous abdominal AT measured by CT, and subcutaneous abdominal and femoral fat cell weight. Positive (+) or negative (−) relationships are indicated between independent and dependent variables.

accumulation in men, a finding that reemphasizes the important role of "portal" AT as a correlate of insulin sensitivity.[4,6,8] However, subcutaneous abdominal fat has recently been shown also to be an important correlate of insulin resistance.[10,11] One potential explanation for this finding could be the method used to study in vivo insulin action. Indeed, insulin sensitivity was measured using a euglycemic clamp in those studies,[10,11] whereas we used an OGTT. During the euglycemic clamp, the glucose-insulin infusion bypasses the gut, and it is possible that under such conditions subcutaneous fat may be more critical than VAT in modulating in vivo insulin action. However, during the oral glucose load, it is possible that hormonal or metabolic stimuli originating from the gut may play a more important role in explaining the greater contribution of VAT when the body is challenged by oral glucose. This hypothesis requires further studies.

Finally, previous studies have already proposed that a preferential femoral fat accumulation could even be associated with a "protective" metabolic risk profile in obese men[17] and women.[53] Results of the present study indicate that femoral fat cell hypertrophy per se did not have deleterious consequences for the metabolic risk profile in both genders (Fig 2), a finding already observed in men.[38] On the other hand, fat cell hypertrophy has been shown to be related to increased lipolysis.[45] However, femoral adipocytes of obese subjects display similar basal lipolytic rates compared with those of lean individuals, probably due to the strong α_2-adrenoceptor antilipolytic component found in these cells.[33,34] Such observations suggest that large femoral adipocytes do not significantly contribute to increase FFA flux in the circulation and are unlikely deleterious to the metabolic risk profile of both men and women.

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