Liver Fat Is Not a Marker of Metabolic Risk in Lean Premenopausal Women

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We examined the independent associations among abdominal adipose tissue (AT) depots, liver fat, cardiorespiratory fitness (CRF), and metabolic risk factors in 86 lean premenopausal women. We measured abdominal AT and liver fat by computed tomography (CT), and CRF by a maximal treadmill exercise test. Liver fat was not related to any abdominal AT depot, metabolic risk factor, or CRF ($P > .10$). Visceral AT mass (kilograms) remained a significant ($P < .05$) predictor of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), TC/high-density lipoprotein cholesterol (HDL-C), and LDL-C/HDL-C after statistical adjustment for CRF. Abdominal subcutaneous AT mass was also a significant ($P < .05$) correlate of TC/HDL-C and LDL-C/HDL-C after control for CRF. Visceral AT remained a significant predictor ($P < .05$) of TC and LDL-C after control for abdominal subcutaneous AT. Conversely, subcutaneous AT did not remain a significant correlate after control for visceral AT. However, the deep subcutaneous AT depot remained significantly associated with LDL-C, TC/HDL-C, and LDL-C/HDL-C after control for visceral AT. In contrast, visceral AT remained correlated with triglycerides (TG) alone, after control for the deep subcutaneous AT. These observations suggest that liver fat is not a determinant of metabolic risk in lean women. Conversely, both visceral and the deep subcutaneous depot are determinants of metabolic risk in premenopausal woman despite the absence of obesity.

SEVERAL RECENT studies suggest that fat accumulation in the liver is a component of fat distribution that explains variation in metabolic risk independent of abdominal and visceral adiposity.[1-3] Indeed, Seppala-Lindroos et al[1] reported that despite similar amounts of visceral adipose tissue (AT), men with high liver fat have a greater metabolic risk than those with low liver fat. These findings are consistent with Nguyen-Duy et al,[3] who recently observed that liver fat was a significant correlate of fasting glucose and plasma triglycerides (TG) independent of visceral AT. Similarly, Tiikkainen et al[2] reported that liver fat is a significant predictor of fasting insulin and TG levels independent of visceral and abdominal subcutaneous AT in obese women with previous gestational diabetes. Together, these studies suggest that liver fat is indeed a predictor of metabolic risk independent of abdominal adiposity. However, the studies reporting liver fat as an independent predictor of metabolic risk have been conducted in populations with a wide range of abdominal adiposity, or homogeneous groups of obese individuals.

Based on these observations, we reasoned that if liver fat was in fact related to metabolic risk independent of obesity, then variation in liver fat might well predict metabolic risk in a sample of lean women characterized by low levels of adiposity, in particular, visceral fat. If this were true, it would reinforce the notion that liver fat is an independent component of abdominal fat distribution and an antecedent for the development metabolic risk. Furthermore, no study has considered whether liver fat remained a significant predictor of metabolic risk after segmentation of abdominal subcutaneous AT into its superficial and deep AT compartments. This is relevant as it has been reported that the more metabolically active deep subcutaneous AT depot predicts metabolic risk independent of visceral AT.[4]

To consider this hypothesis we investigated the independent relationships among all abdominal AT depots, liver fat, cardiorespiratory fitness, and features of the metabolic syndrome in a group of lean, premenopausal women characterized by low levels of total and abdominal obesity.

MATERIALS AND METHODS

Subjects

Subjects consisted of a subset of 86 lean premenopausal women selected from a larger cohort who received a medical examination at the Cooper Clinic in Dallas, TX, between 1995 and 2002. Inclusion criteria required that the subjects were nonsmokers, had received a computed tomography (CT) scan of the abdominal region, self-reported regular menstrual cycles, and were not using oral contraceptives. Further, lean individuals in this study were defined as having a body mass index (BMI) less than 25 kg/m^2 and a waist circumference of less than 88 cm. Subjects were from a middle to upper socioeconomic background. Exclusion criteria included persons with history of diabetes mellitus, cardiovascular disease, stroke, cancer, abnormal resting or exercise electrocardiograms (ECG), or failure to reach at least 85% of their age-predicted maximal heart rate during the treadmill test. Subjects taking medication to treat hypertension and those with high cholesterol were also excluded. All subjects gave their fully informed written consent prior to participation in the examination according to the ethical guidelines of The Cooper Institute Institutional Review Board, and the study was reviewed and approved annually.

Clinical Examination

In the morning following an overnight fast of at least 12 hours, study participants completed a comprehensive medical examination. This evaluation included a physical examination, a questionnaire on demographic factors and health habits (alcohol consumption, cigarette smoking, physical activity, family history, and medication), anthropometric and blood pressure measurements, blood chemistry analyses, resting ECG, a standardized maximal treadmill test, and a CT scan of the abdominal region. Body weight and height were measured using a standard physician's scale and stadiometer, and were used to calculate

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Submitted October 16, 2003; accepted February 20, 2004.

Supported in part by research grants from the National Institutes of Health to S.N.B. (AG06945) and M.Z.N. (HL62508), and from the Canadian Institutes of Health Research to R.R. (MT13448).

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0026-0495/04/5308-0004$30.00/0

doi:10.1016/j.metabol.2004.02.016

the BMI (weight in kilograms/height in meters squared). Waist circumference was measured at the level of the umbilicus using a plastic tape measure.

Maximal Treadmill Test

Cardiorespiratory fitness was evaluated using a modified Balke maximal exercise test protocol performed on a treadmill.[5] The initial treadmill speed was 88 m/min. The grade was 0% for the first minute, and was raised to 2% the second minute. Each subsequent minute the grade was further increased by 1%. After 25 minutes, the incline remained at 25% while the speed was increased 5.4 m/min every minute until fatigue ensued, and the test was terminated. Total treadmill endurance time was converted to a maximal oxygen consumption value (Vo_{2max}) using a standard prediction equation,[6] as it has been shown to correlate very well with Vo_{2max} ($r = 0.94$).[6]

Biochemistry Analyses

Venous blood samples were taken from the antecubital vein, and were analyzed using automated methods in a laboratory that participates in and meets quality control standards of the Centers for Disease Control and Prevention Lipid Standardization Program. Measures included total serum TG, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and fasting blood glucose levels. Low-density lipoprotein cholesterol (LDL-C) levels were estimated using the Friedewald equation.[7]

Measurement of Abdominal AT Distribution

Axial images of the abdominal region were obtained using an electron-beam CT (Imatron, General Electric, Milwaukee, WI) standard protocol used to quantify coronary calcium.[8] Subjects were examined while in a supine position with their arms extended above their head. Approximately 40 contiguous images (6 mm thickness) were acquired from the distal iliac crest to the caudal region of the heart. Images were obtained using 130 kV and 630 mA with a 48-cm field of view and a 512×512 matrix. The CT data collected in Dallas was electronically transferred to the laboratory in Kingston for analysis using specialized image analysis software (Tomovision, Montreal, Canada).

A continuous series of 5 to 7 CT images corresponding to the L4-L5 to L3-L4 vertebral disc spaces for each subject were selected for analysis. The AT volumes were calculated using a truncated pyramid method as described previously.[9] AT volumes (liters) were converted to mass units (kilograms) by multiplying the volumes by the assumed constant density for fat (0.92 kg/L). AT areas (centimeters squared) were computed using an attenuation range of -190 to -30 Hounsfield units (HU). Visceral AT was determined by delineating the intra-abdominal cavity at the innermost aspect of the abdominal and oblique wall musculature and the anterior aspect of the vertebral body. Abdominal subcutaneous AT area was defined as the area of adipose tissue between the skin and the outermost aspect of the abdominal muscle wall. The deep and superficial depots of the abdominal subcutaneous AT were identified for a single image at the level of L4-L5 using the subcutaneous fascia.

The interobserver (2 observers) error for separation of subcutaneous AT area measurements into the superficial and deep depots was determined from the analyses of a single image (L4-L5) in a subset of 50 subjects. Intra-observer error for the same depots was determined by duplicate analysis of the L4-L5 image separated by 3 months. The inter- and intra-observer coefficient of variations for the division of the abdominal subcutaneous AT were: deep 7.5% and 6.7%, respectively, and superficial subcutaneous AT 5.2% and 4.4%, respectively.

Measurement of Liver Fat

CT is capable of differentiating tissues on the basis of their attenuation characteristics, which are a function of tissue density and chemical composition. A normal liver is usually denser and consequently has a higher attenuation value than the spleen. Therefore, a lower mean liver attenuation value relative to that of the spleen is an indication of fatty infiltration of the liver.[10] Liver fat was represented as a ratio of mean liver to spleen attenuation values (CTL/CTS).[10,11] CTL and CTS were calculated using the average attenuation values of 2 regions of interest within each organ obtained by from a single CT image that clearly displayed both the liver and spleen. The regions of interest were consistently placed in the parenchyma of the right lobe of the liver and in a similar region within the spleen, being careful to avoid blood vessels, artifacts, and other areas of inhomogeneity.

In a subset of 30 women, no significant differences were observed among the 3 images for determination of CTL, CTS, and corresponding CTL/CTS values (data not shown), and the calculated standard error was less than 1.6% for all comparisons. Therefore, one image was arbitrarily chosen to serve for the analysis of liver fat content.

We examined the reliability for liver fat measurements in 50 women. Intra-observer analyses were performed on the same image separated by 3 months. The coefficients of variation for repeated CTL, CTS, and CTL/CTS measurements by the same observer were 3.9%, 3.6%, and 5.6%, respectively. The interobserver coefficients of variation for CTL, CTS, and CTL/CTS measurements of the same image were 3.8%, 4.6%, and 5.9%, respectively.

Statistical Analyses

Pearson correlation coefficients were computed to determine univariate associations between variables. Partial correlation analyses were performed to determine the independent relationships among measures of abdominal obesity, CRF, liver fat, and lipid variables. Log transformations were used to normalize the distribution for TG, TC/HDL-C, LDL-C/HDL-C, deep abdominal subcutaneous AT, and visceral mass. All other variables were normally distributed. Intra- and interobserver reliability data for duplicate measurements of subcutaneous AT areas and liver fat were compared using a paired *t* test. A 1-way analysis of variance (ANOVA) was used to compare CTL, CTS, and CTL/CTS values among the 3 selected liver images. All statistical analyses were performed using SPSS software (Chicago, IL).

RESULTS

Subject Characteristics

Subject characteristics are presented in Table 1. Despite a low BMI (21.4 ± 1.7) and visceral adiposity (42 ± 23 cm at L4-L5), the sample was characterized by a wide range in abdominal adiposity, CRF, and all metabolic variables. Absolute liver density values (47.0 to 76.4 HU) were within a normal range. The deep abdominal subcutaneous AT was significantly correlated with the superficial depot ($r = 0.84$, $P < .01$) and was consistently smaller than the superficial depot, comprising on average 38.4% of the total abdominal subcutaneous AT area at the level of L4-L5.

Self-reported alcohol consumption averaged 7.5 g/d (range, 0 to 48.2 g/d). Eight subjects consumed greater than 20 g alcohol per day (>1 to 2 drinks per day), which has been suggested to have hepatotoxic effects in women[12]; however, none of these individuals had fatty liver. "Fatty liver," defined as a ratio of liver to spleen CT attenuation values less than one,[10] was observed in only 3 individuals.

Relationships Between Visceral AT, Abdominal Subcutaneous AT, CRF, Liver Fat, and Metabolic Variables

CRF was negatively correlated with visceral AT ($r = -0.29$, $P < .01$) and abdominal subcutaneous AT ($r = -0.40$, $P <$

Table 1. Subject Characteristics

	Mean ± SD	Range
Anthropometric data*		
Age (yr)	40.8 ± 3.9	30-46
BMI (kg/m^2)	21.4 ± 1.7	18.5-24.8
Waist circumference (cm)	69.2 ± 5.2	59-87
AT mass (kg)†		
Total abdominal	0.57 ± 0.26	0.16-1.36
Visceral§	0.14 ± 0.08	0.04-0.41
Abdominal subcutaneous	0.43 ± 0.21	0.12-1.03
Adipose tissue area (L4-L5, cm^2)†		
Visceral	42 ± 23	8-115
Abdominal subcutaneous	155 ± 75	32-394
Superficial subcutaneous	95 ± 42	26-226
Deep subcutaneous§	59 ± 35	6-176
Liver/spleen variables (HU)‡		
CTL	62.8 ± 5.2	47.0-76.4
CTL/CTS	1.24 ± 0.13	0.85-1.78
Metabolic variables (mol/L)*		
TG§	0.8 ± 0.6	0.4-4.8
TC	4.8 ± 1.0	2.5-8.9
LDL-C	2.6 ± 0.8	0.9-6.7
HDL-C	1.8 ± 0.4	1.0-3.1
TC/HDL-C§	2.8 ± 0.8	1.7-5.9
LDL-C/HDL-C§	1.6 ± 0.7	0.4-3.7
Fasting glucose	5.1 ± 0.4	4.2-6.9
Systolic blood pressure (mm Hg)	107 ± 12	80-150
Estimated Vo_{2max} (mL/kg/min)	38.6 ± 5.2	28.0-51.8

*n = 86,
†n = 84,
‡n = 85.
§Log transformations were used for analyses.

.001). Visceral AT, abdominal subcutaneous AT, and CRF were significantly related ($P < .05$) to the metabolic variables by a similar magnitude (Fig 1 and Table 2). Visceral AT remained a significant correlate of TC and LDL-C after statistical control for abdominal subcutaneous AT. However, abdominal subcutaneous AT did not remain a significant predictor of the metabolic profile after adjusting for visceral AT. When isolated, the deep subcutaneous AT depot remained a significant correlate of LDL-C, TC/LDL-C, and LDL-C/HDL-C after statistical adjustment for visceral AT. Conversely, the superficial subcutaneous AT depot was not a significant correlate of any of the metabolic variables after control for visceral AT (Table 3). Visceral AT remained a significant predictor ($P < .05$) of TC and LDL-C after control for total abdominal subcutaneous AT. After statistical adjusted for deep subcutaneous AT alone, visceral AT remained associated with TG ($r = 0.24$, $P < .05$) alone. Conversely, subcutaneous AT did not remain a significant correlate after control for visceral AT ($P > .05$). CRF was also not significantly correlated with any of the metabolic variables after control for visceral AT or abdominal subcutaneous AT.

Liver fat content (CTL or CTL/CTS) was not related to any AT depot, CRF, or any of the metabolic variables ($P > .05$) with or without control for alcohol consumption (Fig 1).

DISCUSSION

The findings of this study suggest that contrary to earlier findings in obese men and women, liver fat is not a significant correlate of metabolic risk in lean premenopausal women. In contrast, both visceral and subcutaneous AT depots were significant markers of metabolic risk independent of liver fat and cardiorespiratory fitness. Moreover, the more metabolically active deep subcutaneous AT depot was a predictor of metabolic risk independent of visceral adiposity. Thus, it would appear that the accumulation of abdominal fat, and in particular the visceral and deep subcutaneous AT in lean women, is associated with a deterioration in the metabolic profile prior to the development of obesity as measured by BMI.

Emerging evidence suggests that the accumulation of liver fat may be a marker of metabolic profile independent of total and abdominal adiposity. Indeed, we[3] and others[1,2,13] report that liver fat measured by CT[3,13] or proton magnetic resonance spectroscopy[1,2] is a significant correlate of metabolic risk in obese men[1,3,13] and women.[2] Our failure to reproduce these earlier observations may partially be explained by the relatively low and narrow range of CT-measured liver fat values observed. For example, the liver fat scores in this study (CTL/CTS = 1.24 ± 0.13 HU) are characteristic of lean livers by comparison to our previous study in obese men (1.12 ± 0.17).[3] Further, the range of liver fat scores in this study varied 2-fold by comparison to the 3-fold variation observed in obese men.[3] It is unlikely that differences between the studies are due to methodological limitations as CT has been validated as a sensitive tool for hepatic lipid content measurement even in instances of low fatty infiltration.[11] Thus, the difference in liver fat values between the 2 studies may well be explained by concomitant variation in obesity as it is often reported that abdominal obesity,[13] in particular visceral AT, is strongly associated with liver fat[3,14] and provides indirect support that obesity-related fatty liver is preceded by an accumulation in visceral AT. Nevertheless, the relationship between abdominal AT and liver fat does not appear to be the conduit that links abdominal obesity and metabolic risk. We[3] and others[1,2] report that liver fat remains a marker of metabolic risk independent of total and abdominal obesity in obese men and women. The findings reported here provide indirect support for these observations as both visceral and abdominal subcutaneous AT were strong markers of metabolic risk, whereas liver fat was not. Whether a threshold of liver fat accumulation is required to influence metabolic risk is unknown.

In this study, both visceral and abdominal subcutaneous AT per se were significant correlates of metabolic risk. However, only visceral AT remained an independent predictor of select lipid variables. The singular importance of visceral AT to the development of metabolic risk has been clearly shown in men and women across a wide range of adiposity.[15] Accordingly, despite the extremely low levels of visceral AT (42 cm^2) in this study, we observed a positive association between metabolic risk and visceral adiposity. Although it has been suggested that accumulation of visceral AT in the order of 110 cm^2 (measured at the L4-L5 intervertebral space) is required prior to observing frank dyslipidemia,[16,17] the findings here suggest that visceral AT is associated with increased disturbances in lipid and car-

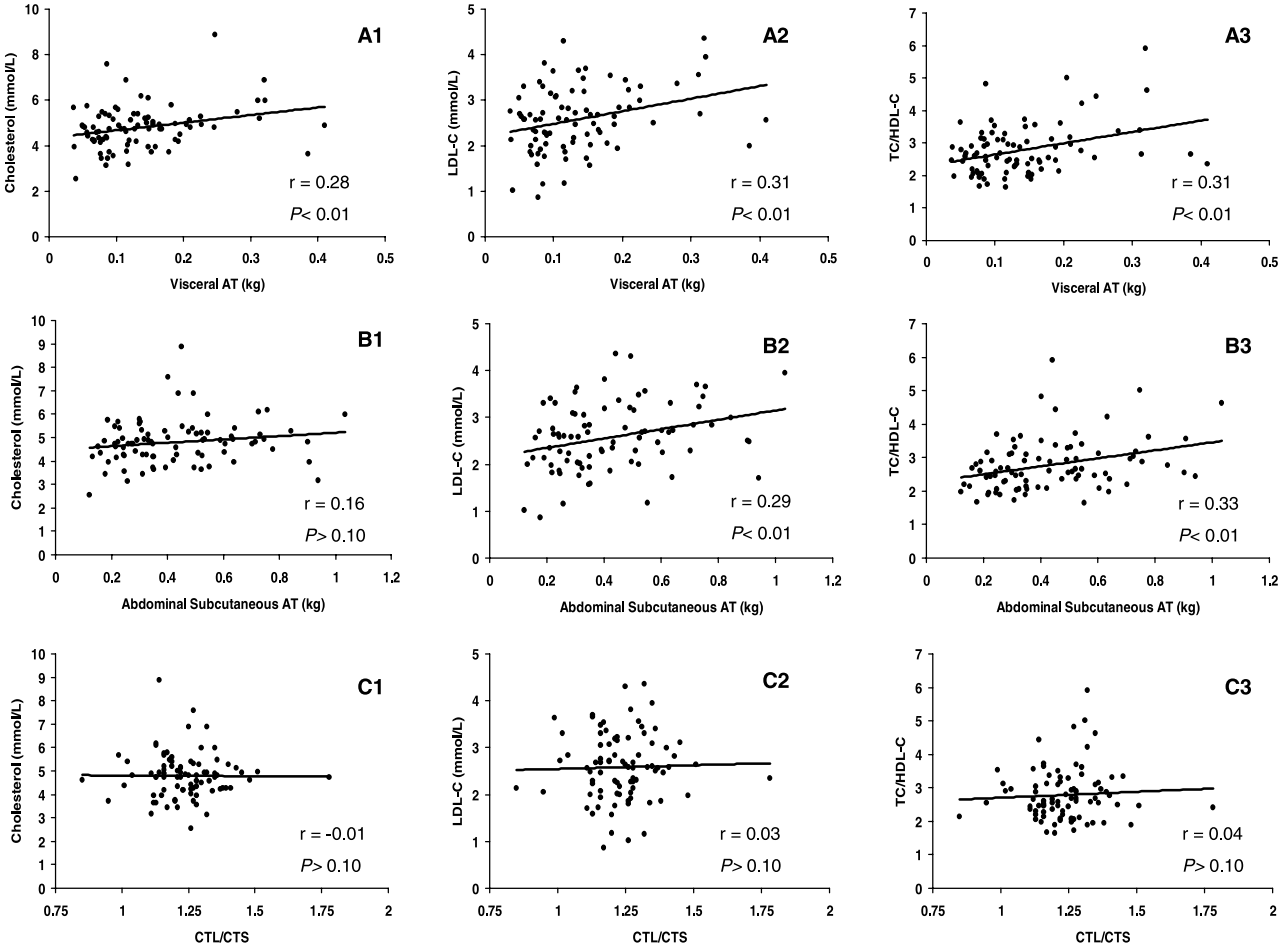

Fig 1. Associations between visceral AT mass and (A1) TC, (A2) LDL-C, and (A3) TC/HDL-C. Association between abdominal subcutaneous AT mass and (B1) TC, (B2) LDL-C, and (B3) TC/HDL-C. Association between liver fat and (C1) TC, (C2) LDL-C, and (C3) TC/HDL-C. AT, adipose tissue; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; CTL, mean liver attenuation value; CTS, mean spleen attenuation value.

bohydrate metabolism at levels well below those previously reported.

It has also been suggested that the deep compartment of the abdominal subcutaneous AT is an important predictor of metabolic risk. We[3] and others[18] have argued that failure to segment the superficial or storage depot from abdominal subcuta-

Table 2. Relationships Between Abdominal Obesity, CRF, and Metabolic Variables

	Abdominal AT Depots (kg)*							
	Total		Visceral			Subcutaneous		CRF
	UnAdj	Adj[1]	UnAdj	Adj[1]	Adj[2]	UnAdj	Adj[1]	Unadj
TG	0.22	—	0.22	—	—	—	—	—
Cholesterol	—	—	0.28	0.27	0.24	—	—	—
HDL-C	−0.22	—	—	—	—	−0.22	—	0.23
LDL-C	0.31	0.26	0.31	0.30	0.23	0.29	—	—
TC/HDL-C	0.37	0.26	0.31	0.23	—	0.33	0.22	−0.27
LDL-C/HDL-C	0.37	0.28	0.31	0.23	—	0.34	0.24	−0.26
Systolic blood pressure	—	—	—	—	—	—	—	—
Fasting glucose	—	—	—	—	—	—	—	—

NOTE. All listed Pearson correlations significant at $P < .05$. No associations were significant after control for visceral AT.

Abbreviations: Adj[1], after control for CRF; Adj[2], after control for subcutaneous AT.

*AT masses were measured from the level of L3-L4 to L4-L5.

Table 3. Relationships Between Various Abdominal Subcutaneous AT Depots and Metabolic Variables

	Abdominal Subcutaneous AT Area at L4-L5					
	ASAT		Superficial		Deep	
	UnAdj	Adj	UnAdj	Adj	UnAdj	Adj
TG	—	—	—	—	—	—
Cholesterol	—	—	—	—	0.31	—
HDL-C	−0.23	—	−0.24	—	—	—
LDL-C	0.32	—	0.23	—	0.41	0.29
TC/HDL-C	0.36	—	0.31	—	0.38	0.27
LDL-C/HDL-C	0.36	—	0.32	—	0.39	0.30
Systolic blood pressure	—	—	—	—	—	—
Fasting glucose	—	—	—	—	—	—

NOTE. All Pearson correlations significant ($P < .05$), N = 84. After control for VAT area at L4-L5

neous AT per se may mask the contribution of the metabolically active deep subcutaneous AT.[18,19] In theory, segmentation of abdominal subcutaneous AT would isolate the adipocytes primarily responsible for the elevated free fatty acid (FFA) levels and consequent increase in VLDL-TG and decrease in HDL-C that is typical of excess accumulation of subcutaneous AT.[20] Indeed, the findings here support that the relationship between abdominal subcutaneous AT and lipid-related metabolic risk is strengthened by the isolation of the adipocytes within the deep subcutaneous depot, although this contrary to previous observations.[3,21] Unlike the total abdominal subcutaneous AT, the deep subcutaneous AT remained a significant predictor of lipid-related metabolic risk independent of visceral AT. This is a novel observation that has not previously been observed in studies with overweight and obese populations.[3,4]

This discrepancy may highlight metabolic differences in lean and obese populations. In lean individuals, as with overweight and obese populations, the abdominal subcutaneous AT and/or deep subcutaneous AT may act as a surrogate for total adiposity,[22] itself known to be a risk factor.[18] However, in lean populations with visceral AT levels well below those thought to be associated with frank dyslipidemia, visceral AT accumulation may also be below levels required to maintain its independent influence on metabolism and the metabolic profile. Conversely, in obese individuals, significant accumulation of visceral AT may introduce multiple mechanisms, such as cytokines[23] and increased portal FFA flux,[20] that overshadow the independent contributions of the deep or abdominal subcutaneous AT observed in lean populations.

The limitations of this study warrant mention. We did not control for the stage of menstrual cycle when obtaining the fasting blood sample. It is reported that lipid variables will vary at due to altered hormonal levels consequent with menstrual phase changes.[24] We did not correct for plasma volume or control for diet composition prior to blood sampling.[25] However, to limit the acute effects of exercise on lipid levels, the participants were asked to refrain from strenuous activity at least 24 hours prior to blood sampling. Finally, although the predominantly white, middle-to-upper class study population limits the generalizability of the results of our study, it should not affect the internal validity. In fact, the homogeneity of our study group on socioeconomic factors is a benefit because it reduces the likelihood of confounding by these factors.

In summary, the findings of this study suggest that liver fat is not a significant predictor of the metabolic risk in lean premenopausal women. Conversely, both visceral and abdominal subcutaneous AT are significant correlates of metabolic risk. Furthermore, subdivision of abdominal subcutaneous AT according to its metabolic characteristics is important for elucidating the independent contributions of the deep subcutaneous AT depot towards the development of metabolic risk in lean women. It is apparent that accumulation of visceral and abdominal subcutaneous AT, and in particular the deep abdominal subcutaneous AT is associated with increased metabolic risk prior to the development of obesity as measured by body mass index. Accordingly, these findings reinforce once more the health risk associated with abdominal obesity and the importance of efforts to prevent or reduce this obesity phenotype.

ACKNOWLEDGMENT

The authors would like to extend a special thanks to Elisa Priest for all her hard work.

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