Visceral fat and liver fat are independent predictors of metabolic risk factors in men

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Duy, Thanh-Binh Nguyen, Milton Z. Nichaman, Timothy S. Church, Steven N. Blair, and Robert Ross. Visceral fat and liver fat are independent predictors of metabolic risk factors in men. Am J Physiol Endocrinol Metab 284: E1065–E1071, 2003. First published January 28, 2003; 10.1152/ajpendo.00442.2002.—We examined the independent associations among abdominal adipose tissue (AT), liver fat, cardiorespiratory fitness (CRF), and lipid variables in 161 Caucasian men who had a wide variation in adiposity. We measured AT and liver fat by computed tomography and CRF by a maximal exercise test on a treadmill. Visceral AT remained a significant \( P \leq 0.05 \) predictor of plasma triglycerides (TG), high-density-lipoprotein cholesterol (HDL-C), and total cholesterol (TC)/HDL-C ratio (TC/HDL-C) after statistical control for abdominal subcutaneous AT, CRF, and alcohol consumption. Abdominal subcutaneous AT was not a significant \( P > 0.05 \) correlate of any lipid variable after control for visceral AT and CRF. Furthermore, subdivision of subcutaneous AT into deep and superficial depots did not alter these observations. Visceral AT was the strongest correlate of liver fat and remained so after control for abdominal subcutaneous AT, CRF, and alcohol consumption \( r = -0.34, P < 0.01 \). In contrast, abdominal subcutaneous AT and CRF were not significant \( P > 0.10 \) correlates of liver fat after control for visceral AT. Visceral AT remained a significant \( P < 0.01 \) correlate of TG, HDL-C, and TC/HDL-C independent of liver fat. However, liver fat was also a significant correlate \( P \leq 0.05 \) of fasting glucose and TG independent of visceral AT. These observations reinforce the importance of visceral obesity in the pathogenesis of dyslipidemia in men, and they suggest that visceral AT and liver fat carry independent health risk.

Abdominal adipose tissue; cardiorespiratory fitness; metabolic risk

THE CONTRIBUTION of abdominal subcutaneous and visceral adipose tissue (AT) to the established association between abdominal adiposity and health risk remains a subject of debate (14). Initial attempts to resolve the confusion by subdivision of abdominal subcutaneous AT into its metabolically determined components have failed to provide consistent results. For insulin resistance, some suggest that the deep metabolically active depot of subcutaneous AT is a strong marker independent of visceral AT (21, 42); others provide evidence to the contrary (40, 43a). Whether subdivision of subcutaneous AT provides insight into the relationships between abdominal obesity and lipid profile is unknown. Given the strong association between elevated free fatty acid (FFA) levels and lipid metabolism (26), it is reasonable to think that the isolation of deep from superficial subcutaneous AT would provide insight into the relationships between abdominal adiposity and dyslipidemia. Indeed, DeNino et al. (9) report that both visceral and deep subcutaneous AT abolish the age-related differences in lipid and lipoprotein levels in women. Whether the association between deep subcutaneous AT and lipid profile remained significant independent of visceral AT was not reported. Furthermore, the relationships between visceral AT, subdivisions of abdominal subcutaneous AT, and lipid metabolism in men are unknown.

Several recent studies provide compelling evidence that fatty liver, or hepatic steatosis, is another characteristic of fat distribution that is strongly associated with the metabolic syndrome in both obese (29) and normal-weight subjects (30). Although the pathogenesis of steatosis remains unclear, it is repeatedly suggested that sustained exposure of the liver to an increased flux of FFA would lead to an increase in lipid deposition consequent to a failure to adequately oxidize the excess lipid (13). The strong association between abdominal obesity, elevated FFA levels, and hepatic steatosis would support this notion (24), and, given direct exposure of the liver to FFAs in portal circulation, suggest a primary role for visceral AT. Few studies have considered whether a relationship exists between radiologically measured abdominal obesity, liver fat, and metabolic risk factors (4, 7). Banerji et al. (4) report that visceral AT is associated with computed tomography (CT)-measured liver fat and that both visceral AT and liver fat are correlates of plasma triglycerides and glucose disposal in a small cohort of African-American men. However, in that study only triglycerides were measured, the influence of abdominal...
subcutaneous AT was not considered, and the authors did not report whether visceral AT and liver fat remained correlates of triglyceride values independent of each other. Furthermore, because physical activity is associated with a lower prevalence of fatty liver (20), insight into the association between abdominal obesity and fatty liver would be gained by control for variation in physical activity. Indeed, it is possible that physical activity has an influence on liver composition and, hence, hepatic lipid metabolism that is independent of abdominal obesity, a finding that would provide insight into the mechanisms by which exercise improves lipid profile without a corresponding change in total adiposity (43).

This study was undertaken to investigate the independent relationships among abdominal AT, liver fat, cardiorespiratory fitness, and lipid profile. To evaluate these relationships, we examined a large group of healthy men characterized by wide variation in adiposity and cardiorespiratory fitness (CRF).

METHODS

Subjects. Subjects included 162 men selected from a larger sample who received a medical examination at the Cooper Clinic in Dallas, Texas, between 1995 and 2001. Inclusion criteria required the subjects to be Caucasian and nonsmokers and to have received a CT scan of the abdominal region. Subjects were primarily from middle to upper socioeconomic background. Exclusion criteria included persons with personal history of diabetes mellitus, hyperlipidemia, cardiovascular disease, stroke, hypertension, or cancer; abnormal resting heart rate or exercise electrocardiograms (ECG); or failure to reach ≥85% of their age-predicted maximal heart rate during the treadmill test. Subjects taking medication to treat diabetes mellitus, hypertension, or high cholesterol were also excluded. All subjects gave their informed written consent before 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. Study participants completed medical examinations in the morning after an overnight fast of ≥12 h. The evaluation included a self-reported personal and family health history, a physical examination, a questionnaire on demographic factors and health habits (alcohol use, cigarette smoking habits, physical activity, and medication use), 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 body mass index was calculated (weight in kilograms/height in meters squared). Waist circumference was measured at the level of the umbilicus with a plastic tape measure.

Maximal treadmill test. CRF was evaluated with a maximal exercise test performed on a treadmill according to a modified Balke procedure (3). Initial treadmill speed was 88 m/min. The grade was 0% for the first minute, was 2% for the second minute, and then was increased by 1% every minute until 25 min. Once this point was reached, the grade remained constant, and the speed was increased 5.4 m/min each minute until test termination. Total treadmill endurance time with this protocol has been shown to correlate highly with maximal oxygen uptake (\(V_{O_2\text{max}}\)) (\(r = 0.92\)) (36).

Total treadmill time was converted to \(V_{O_2\text{max}}\) with a standard prediction equation (36).

Biochemistry analyses. We obtained venous blood samples from an antecubital vein, and samples were analyzed by automated methods in a laboratory that participates in and meets quality control standards of the Centers for Disease Control and the Prevention Lipid Standardization Program. Measures included total serum triglyceride (TG), cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and fasting blood glucose levels. Low-density lipoprotein (LDL) cholesterol levels were estimated using the Friedewald equation (16).

Measurement of abdominal AT distribution. Axial images of the abdominal region were obtained with electron-beam computed tomography (CT, Imatron; General Electric, Milwaukee, WI) by use of a standard protocol (39). Subjects were examined in a supine position with their arms extended above their heads. Approximately 40 transverse images (3 mm thickness, 6 mm spacing) were acquired from the midregion of the 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 obtained in Dallas were transferred electronically to the laboratory in Kingston for analysis by specially designed image analysis software (Tomovision, Montreal, Canada).

For each subject, the CT images corresponding to the L4-L5 and L3-L4 vertebral disc spaces were selected by using anatomic landmarks. Abdominal adipose tissue areas (cm²) were computed using an attenuation range of −190 to −30 Hounsfield units. Visceral AT was determined by delineating the intra-abdominal cavity at the innermost aspect of the abdominal and oblique wall musculature and the posterior aspect of the vertebral body. Abdominal subcutaneous AT area was defined as the area of AT between the skin and the outermost aspect of the abdominal muscle wall. By use of the subcutaneous fascia, abdominal subcutaneous AT was further subdivided at the level of L4-L5 into deep and superficial depots. Because the subcutaneous fascia is not always clearly visible, subcutaneous AT was also divided into anterior and posterior compartments according to methods previously described (40). With this method, it is assumed that the anterior and posterior compartments act as surrogates for superficial and deep depots, respectively (31).

The interobserver reliability error for subcutaneous AT area (cm²) measurements (all depots) was determined by comparing the results of two observers’ analyses of the same L4-L5 image for 70 men. As expected, the variability between observers subdividing subcutaneous AT into anterior and posterior compartments was low (coefficients of variation (CV): 0.5 and 0.3%, respectively). The interobserver error for partitioning subcutaneous AT into deep and superficial subcutaneous AT was slightly higher (CV: 6 and 5%, respectively).

Measurement of liver fat. CT is capable of distinguishing different tissue types on the basis of their attenuation characteristics, which in turn are a function of tissue density and chemical composition. Because of the normally higher density of the liver compared with the spleen, a lower mean liver attenuation value relative to that of the spleen indicates fatty infiltration of the liver (35). A ratio of mean liver-to-spleen attenuation values (CTL/CTS) was used as an index of liver fat. Lower density values for the liver relative to the spleen (i.e., CTL/CTS <1) indicate excess infiltration of lipid or fatty liver (35). Furthermore, CTL/CTS <1 has been validated as an index of fatty change of the liver (28, 35) and is routinely used to diagnose fatty liver (35, 38).
From a single CT image that included both the liver and the spleen, CTL and CTS were obtained by identifying two regions of interest within each organ and then calculating the corresponding average density value. The regions of interest were consistently placed in the parenchyma of the right lobe of the liver and in a similar region in the spleen. In choosing regions of interest, blood vessels, artifacts, and areas of inhomogeneity were carefully avoided. The variability for determination of liver fat according to image selection was assessed by analyzing three contiguous CT images that included both the liver and spleen. Average CTL, CTS, and CTL/CTS scores were compared among the three images in 15 men. No significant differences were observed among the three images for determination of CTL, CTS, and corresponding CTL/CTS values (data not shown). Therefore, one image was arbitrarily chosen for determination of liver fat content. The same image was consistently identified in every subject according to specific criteria and anatomic landmarks.

The reliability for liver fat measurements was examined in 10 men. Intraobserver error was determined by analysis of the same image on two separate occasions separated by 2 days. The CV for repeat CTL, CTS, and CTL/CTS measurements by the same observer were 5.2, 2.8, and 6.3%, respectively. The interobserver variability was determined from the results of two observers’ analysis of the same image. The interobserver CVs for CTL, CTS, and CTL/CTS measurements were 5.1, 4.3, and 4.5%, respectively.

To determine whether visceral and subcutaneous AT areas (cm$^2$) related to liver fat differently from visceral AT and subcutaneous AT volumes (liters), area measurements were obtained at L$_4$-L$_5$ and L$_3$-L$_4$ levels in a random sample of 25 men. On these same men, visceral AT and subcutaneous AT volumes were derived by a series of 25 contiguous, abdominal CT images extending from the iliac crest to the anterior crest of the kidneys. The results revealed that visceral and subcutaneous AT area values (e.g., cm$^2$) at both levels were highly correlated with visceral and subcutaneous AT volume values (e.g., liters), respectively (0.92 $\leq r < 0.97$ and 0.94 $< r < 0.99$; P $< 0.01$). Given that the magnitude of the relationships between abdominal AT volumes, areas, and liver fat were comparable, CT values obtained at the L$_4$-L$_5$ level were used to represent abdominal adiposity for all subsequent analyses.

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 transformation was used to normalize the distribution for TG, HDL, and TC/HDL. All other variables were normally distributed. Simple correlation coefficients were used to determine the relationships among regional AT areas, volumes, and liver fat. Intra- and interobserver reliability data for duplicate measurements of subcutaneous AT areas and liver fat were compared using a paired t-test. A one-way analysis of variance was conducted to compare CTL, CTS, and CTL/CTS values among the three selected images. All statistical analyses were performed using SPSS (Chicago, IL).

RESULTS

Subject characteristics. Subject characteristics are presented in Table 1. The cohort was characterized by wide variation in age, total and abdominal obesity, CRF, liver fat, and metabolic variables. Self-reported alcohol consumption averaged 15 g/day (range 0–125 g/day), with 143 subjects consuming <30 g/day. Alcohol consumption was a significant correlate of fatty liver fat, with alcohol consumption averaged 15 g/day (range 0–125 g/day), with 143 subjects consuming <30 g/day. Alcohol consumption was a significant correlate of fatty liver (r = 0.22, P < 0.01) but was not related to any metabolic variable (P > 0.10). Absolute liver density values (29.5–75.3 HU) were within a normal range.

Among abdominal subcutaneous AT depots, anterior abdominal subcutaneous AT showed strong and similar associations with both deep and superficial subcutaneous AT depots (r = 0.90; P < 0.01). Posterior abdominal subcutaneous AT was also a strong correlate of both deep (r = 0.95; P < 0.01) and superficial AT (r = 0.90; P < 0.01).

Relationships between abdominal obesity, CRF, liver fat, and metabolic variables. Visceral AT was the strongest correlate of liver fat (r = −0.43; P < 0.01) and remained so after statistical control for abdominal subcutaneous AT, CRF, and alcohol consumption (r = −0.34; P < 0.01; Table 2, Fig. 1). In contrast, abdominal subcutaneous AT and CRF did not remain significant (P > 0.10) correlates of liver fat after control for visceral AT (Table 2). However, CRF remained a significant correlate (P = 0.01) of liver fat after control for abdominal subcutaneous AT (Table 2).

Total abdominal, visceral, and abdominal subcutaneous AT were significantly associated (P < 0.05) with all metabolic variables by a similar magnitude, with the exception of TC and LDL-C (Table 2). Subdivision of abdominal subcutaneous AT into deep and superficial depots (Table 2) or anterior and posterior depots (data not shown) did not substantially alter the strength of the associations observed between abdominal subcutaneous AT and metabolic variables. CRF was significantly (P < 0.05) associated with all lipid and lipoprotein variables with the exception of LDL-C.
Liver fat was also correlated (P < 0.01) with TG, HDL-C, TC/HDL-C, and fasting glucose.

Partial correlation analyses revealed that abdominal AT remained a significant (P < 0.05) predictor of fasting glucose, TG, HDL-C, and TC/HDL-C independent of CRF (Table 2). CRF did not remain a significant (P > 0.05) correlate of lipid and lipoprotein parameters after control for visceral AT, although it did remain a significant correlate of TG and TC/HDL-C after control for abdominal subcutaneous AT (Table 2). Visceral AT was significantly (P ≤ 0.05) associated with TG, HDL-C, and TC/HDL-C after control for abdominal subcutaneous AT and CRF (Table 2). Subcutaneous AT did not remain a significant (P ≥ 0.05) correlate of any lipid variable after control for visceral AT and CRF (Table 2).

After control for liver fat alone, visceral AT remained a significant (P < 0.01) correlate of TG, HDL-C, and TC/HDL-C (Table 3). Liver fat also remained a significant correlate (P < 0.05) of fasting glucose and TG independent of visceral AT, abdominal subcutaneous AT, and CRF (Table 3).

**DISCUSSION**

The findings of this study reinforce and extend the observation that the established relationship between abdominal obesity and lipid profile is explained in large measure by visceral AT, as this depot remained a correlate of lipid values after control for abdominal subcutaneous AT, its subdivisions, and CRF. Subcutaneous AT did not remain a significant correlate of the lipid profile after control for visceral AT, and the isolation of deep and superficial depots did not alter this observation. That visceral AT remained a significant correlate of lipid-related metabolic risk independent of liver fat further underscores a primary role for visceral obesity in the pathogenesis of dyslipidemia in men. However, that liver fat remained a significant correlate of fasting glucose and TG independent of visceral AT is a novel finding and suggests that visceral AT and liver fat carry independent health risk.

Our finding that visceral AT was a significant predictor of lipid profile independent of abdominal subcutaneous AT strengthens the notion that visceral adiposity is a strong marker of metabolic risk (11, 37). However, in previous studies no attempt was made to isolate the metabolically active, deep subcutaneous AT depot. In doing so, the presumption is that segmentation of deep AT would isolate the adipocytes primarily responsible for the elevated FFA levels and consequent increase in VLDL-TG and decrease in HDL-C that are typical of obesity (26). Accordingly, inclusion of the superficial or storage depot (e.g., abdominal subcutaneous AT per se) would mask the contribution of the deep AT depot alone. Despite this rationale, we were unable to demonstrate that segmentation of the deep and superficial depots provides additional insight. This agrees with earlier reports from our group (40) and...
Despite this, others (43a) suggesting that subdivision of abdominal subcutaneous AT does not alter the relationships observed between subcutaneous AT per se and insulin resistance. A plausible explanation is that, unlike served between subcutaneous AT per se and insulin subcutaneous AT does not alter the relationships observed between subcutaneous AT and insulin resistance. The lipolytic rate of superficial AT within the anterior abdomen in men measured by microdialysis is higher than deep AT located in the posterior abdominal wall. Studies are clearly needed to confirm the metabolic heterogeneity and consequent metabolic implications of abdominal subcutaneous AT in humans.

Several studies now report that fatty liver is a characteristic of fat distribution that is strongly associated with several metabolic risk factors in both obese (29) and normal-weight subjects (30) and thus argue that it is an additional feature of the metabolic syndrome (23). Although the pathogenesis of fatty liver remains unclear, abdominal obesity is likely among the explanations (29, 30). Because the development of fatty liver requires a net retention of lipids within hepatocytes (2), the close association between abdominal obesity and fatty liver may be partially explained by a sustained exposure of the liver to an increased flux of FFA consequent to the accumulation of visceral AT (5). For this reason, we hypothesized that visceral AT would be a strong correlate of liver fat independent of other AT depots. Indeed, in this study, visceral AT was a robust marker of liver fat and remained so independent of all subcutaneous AT depots, CRF, and alcohol consumption. This finding extends preliminary reports based on small samples wherein a strong association between visceral AT and liver fat was observed in black men with type 2 diabetes and nondiabetic Japanese men (4, 18, 28). Together, these observations suggest a primary role for visceral AT in the etiology of fatty liver and thus reinforce the importance of visceral AT in the pathogenesis of lipid-related metabolic risk.

Our observation that visceral AT and liver fat were independently related to selected metabolic risk factors is a novel finding that argues against a common mechanism. Accordingly, although sustained exposure of the liver to elevated levels of FFAs from visceral AT may explain the net increase in liver fat, extension of this hypothesis (e.g., the portal theory) to explain the increase in VLDL-TG and circulating glucose appears tenuous. Alternatively, recent observations have established that visceral AT is a secretory organ that releases many factors that could influence liver metabolism and metabolic risk factors (45). Adipocytokines such as interleukin-6 and plasminogen-activator-inhibitor 1 are more highly secreted by visceral AT than abdominal subcutaneous AT (1, 15) and may contribute to metabolic complications associated with obesity (22, 45). Furthermore, it has been suggested that the regulation of transcription factors such as the peroxisome proliferator-activated receptors (PPAR) involved with the expression of genes related to lipid and lipoprotein metabolism may be important (17, 34, 46). Indeed, expression of PPARγ is relatively increased in visceral AT compared with abdominal subcutaneous AT in morbidly obese subjects (25).

Our finding that fatty liver was associated with fasting glucose and plasma TG values independent of visceral and subcutaneous AT is not readily explained. However, we did note that the relationship between alcohol intake and fatty liver remained after control for visceral and subcutaneous AT (data not shown). Thus, although alcohol consumption was not directly related to any metabolic variable, it is plausible that alcohol intake affects metabolic risk indirectly via its influence on liver fat. Alternatively, we cannot rule out that other factors with a known influence on liver tissue composition, including diet and weight loss (2), may have influenced the observed relationship between fatty liver and metabolic risk.

In this study, CRF remained a significant predictor of circulating TG and TC/HDL independent of abdominal subcutaneous AT, but not visceral AT. Furthermore, the association observed between CRF and liver fat (for an increase in CRF associated with a decrease in liver fat, see Fig. 1) was abolished after control for visceral AT. Given our finding that both visceral AT and liver fat accumulation were independent predictors of lipid and lipoprotein values, these observations provide insight into earlier studies, wherein it was reported that exercise has a modest effect on lipid and lipoprotein parameters when body weight is maintained (43). In other words, to the extent that CRF reflects participation in regular exercise, our findings suggest that the effects of exercise on lipid profile observed in the absence of weight loss are likely explained by the ability of exercise to induce a marked reduction in visceral adiposity and liver fat independent of a corresponding reduction in abdominal subcutaneous or total adiposity (33, 41).

Limitations in this study warrant mention. First, the cross-sectional design does not allow us to deduce a cause-effect relationship. Furthermore, we were not able to correct for variation in diet composition, which may confound interpretation (8). On the other hand, to minimize the acute effects of exercise on blood lipid levels, all participants were asked to refrain from ab-

| Table 3. Correlations among visceral AT, liver fat, and metabolic variables |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Visceral AT      | Liver Fat       |                  |                  |
|                                | Un   | Adj*            | Un   | Adj‡           | Adj‡           |
| Fasting glucose                | 0.16 | 0.29            | 0.23 | 0.23           |                |
| TG                             | 0.35 | 0.26            | 0.32 | 0.17           | 0.17           |
| HDL-C                          | −0.34| −0.27           | −0.24|                |                |
| TC/HDL-C                       | 0.33 | 0.24            | 0.28 |                |                |

All listed correlations are significant (*P* ≤ 0.05); * after control for liver fat; ‡ after control for visceral AT; † after control for visceral AT, abdominal subcutaneous AT, CRF, and alcohol consumption.
normal exertion 24 h before blood sampling. Finally, whether the observations here can be extended to women and nonwhite men remains to be determined.

In summary, the findings of this study reinforce the importance of abdominal AT, in particular visceral AT, as an independent predictor of lipid-related metabolic risk. In contrast, abdominal subcutaneous AT was not independently related to the plasma lipids and lipoproteins measured, and subdivision of abdominal subcutaneous AT into deep and superficial depots did not alter the observed relationships. That visceral AT and liver fat were related to metabolic risk factors independent of each other argues against a common mechanism and suggests that both depots carry independent health risk.

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