ORIGINAL RESEARCH

Adipose Tissue Depots and Their Cross-Sectional Associations With Circulating Biomarkers of Metabolic Regulation

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***Background*—**Visceral adipose tissue (VAT) and fatty liver differ in their associations with cardiovascular risk compared with subcutaneous adipose tissue (SAT). Several biomarkers have been linked to metabolic derangements and may contribute to the pathogenicity of fat depots. We examined the association between fat depots on multidetector computed tomography and metabolic regulatory biomarkers.

***Methods and Results*—**Participants from the Framingham Heart Study (n=1583, 47% women) underwent assessment of SAT, VAT, and liver attenuation. We measured circulating biomarkers secreted by adipose tissue or liver (adiponectin, leptin, leptin receptor, fatty acid binding protein 4, fetuin-A, and retinol binding protein 4). Using multivariable linear regression models, we examined relations of fat depots with biomarkers. Higher levels of fat depots were positively associated with leptin and fatty acid binding protein 4 but negatively associated with adiponectin (all $P<0.001$). Associations with leptin receptor, fetuin-A, and retinol binding protein 4 varied according to fat depot type or sex. When comparing the associations of SAT and VAT with biomarkers, VAT was the stronger correlate of adiponectin ($\beta=-0.28$ [women]; $\beta=-0.30$ [men]; both $P<0.001$), whereas SAT was the stronger correlate of leptin ($\beta=0.62$ [women]; $\beta=0.49$ [men]; both $P<0.001$; $P<0.001$ for comparing VAT versus SAT). Although fetuin-A and retinol binding protein 4 are secreted by the liver in addition to adipose tissue, associations of liver attenuation with these biomarkers was not stronger than that of SAT or VAT.

***Conclusions*—**SAT, VAT, and liver attenuation are associated with metabolic regulatory biomarkers with differences in the associations by fat depot type and sex. These findings support the possibility of biological differences between fat depots. **(*J Am Heart Assoc*. 2016;5:e002936 doi: 10.1161/JAHA.115.002936)**

Key Words: adipokines • adipose tissue • biomarkers • epidemiology • obesity

Obesity is recognized as a heterogeneous condition in which persons with similar body mass index (BMI) may have distinct metabolic and cardiovascular disease risk.[1–3] Differences in ectopic fat (fat deposited in nonclassical locations, such as surrounding the viscera and infiltrating the liver) volume and distribution has been postulated to partially explain this differential risk.[1] Consistent with this premise, ectopic fat depots, such as visceral adipose tissue (VAT) and fatty liver, have been more strongly associated with cardiovascular risk factors and cardiometabolic disorders than subcutaneous adipose tissue (SAT)[4–9]; however, the mechanisms underlying these relationships remain incompletely understood.

Multiple bioactive molecules are believed to contribute to metabolic and vascular disease.[10,11] These include biomarkers

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Accompanying Tables S1 and S2 are available at http://jaha.ahajournals.org/content/5/5/e002936/DC1/embed/inline-supplementary-material-1.pdf

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secreted primarily by adipose tissue only (adiponectin, leptin, leptin receptor, and fatty acid binding protein 4 [FABP-4]), or secreted by both adipose tissue and the liver (fetuin-A and retinol binding protein 4 [RBP-4]). Obesity has been associated with dysregulation of these biomarkers, with upregulation of leptin,[12] FABP-4,[13] and fetuin-A[14] and downregulation of adiponectin[15,16] and the leptin receptor.[17,18] In addition, alterations in these biomarkers have been associated with insulin resistance[15,19–24] and with several components of the metabolic syndrome[23–25] or diabetes.[26]

Levels of certain local and circulating biomarkers have been shown to vary for different adipose tissue depots[27–29]; however, a clear understanding of the development of cardiometabolic disease manifested by dysfunctional ectopic fat depots and metabolic regulatory biomarkers has not been established. Exploring the associations between ectopic adipose tissue and a broad array of metabolic regulatory biomarkers in a large population-based study setting may provide insights into potential links between specific fat depots with cardiovascular and metabolic disease.

In this analysis, we sought to determine the associations of fat depots assessed with multidetector computed tomography (MDCT), including SAT, VAT, and liver attenuation (an MDCT surrogate for the amount of fat in the liver), with a panel of circulating biomarkers previously associated with metabolic regulation, including adiponectin, leptin, leptin receptor, FABP-4, fetuin-A, and RBP-4. We also examined the association of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), potential biochemical surrogates for the presence of nonalcoholic fatty liver disease (NAFLD), and our biomarker panel.

Methods

Study Sample

Participants from the present study were drawn from the Framingham Heart Study Third Generation Cohort, which consists of participants with at least 1 parent enrolled in the Framingham Offspring Study. Participants for the current analysis participated in the MDCT substudy. The study designs were described previously.[30,31] From the Third Generation Cohort, 1994 participants underwent abdominal MDCT from 2002 to 2005. Of these, 1867 had available measures for our panel of circulating biomarkers. Among participants with available biomarker data, 1603 had available adiposity measures. Of those, 1583 had available covariate information and were included in the main analysis. We performed separate analyses examining the associations of ALT and AST (as surrogate biomarkers of NAFLD) with the panel of biomarkers. For these analyses, our sample of 1583 participants was restricted to those who had both ALT and AST data available (n=1546). The institutional review boards of Boston University and Massachusetts General Hospital approved the study protocol, and all participants provided written informed consent.

MDCT Scan Protocol and Adipose Tissue Measurements

The MDCT scan protocol and adipose tissue depot measurements in the Framingham Heart Study were described previously.[32,33] Participants underwent radiographic assessment of the abdomen in the supine position using an 8-slice MDCT scanner (LightSpeed Ultra; General Electric), and 25 contiguous slices were reconstructed with a slice thickness of 5 mm without overlap.

Volumetric assessment of fat depots in the Framingham Heart Study was performed using a dedicated workstation (Aquarius 3D; TeraRecon), as described previously.[32,33] Briefly, the abdominal musculature wall that separates SAT from VAT was manually traced. Pixels of adipose tissue were identified by their characteristic Hounsfield units, and high-resolution volumetric measurements of SAT and VAT were defined as the volumetric fat content inside and outside of this dividing line. Intra- and interreader reproducibility was excellent (>0.99) for SAT and VAT, as reported previously.[32]

For liver attenuation, a calibration phantom (Image Analysis Inc) with a water-equivalent compound (CT-Water, LightSpeed Ultra; General Electric) and calcium hydroxyapatite at 0, 75, and 150 mg/cm^3 was placed under each participant.[33] Three areas from the liver and 1 from the external phantom were measured. The average of the liver measurements was calculated and used to create liver phantom ratios. The liver phantom ratio reflects the relative degree of MDCT liver attenuation and serves as a surrogate for the amount of fat in the liver. The intra- and interclass correlation coefficients were both 0.99, suggesting excellent reproducibility.[33]

Biomarker Measurements

The biomarker panel included those biomarkers secreted primarily by adipose tissue (adiponectin, leptin, leptin receptor, and FABP-4) and those secreted by both adipose tissue and the liver (fetuin-A and RBP-4). Blood samples were drawn in the morning after participants had fasted overnight, were immediately centrifuged, and were stored at −80°C without freeze–thaw cycles until assayed. The average interassay coefficients of variation for the biomarkers were as follows: adiponectin, 2.23%; leptin, 4.97%; leptin receptor, 4.01%; FABP-4, 2.38%; fetuin-A, 2.52%; and RBP-4, 2.18%. Plasma levels of adiponectin, leptin, leptin receptor, and RBP-4 were measured using an enzyme-linked immunosorbent assay (R&D

Systems). Plasma levels of FABP-4 and fetuin-A were measured using sandwich enzyme-linked immunosorbent assay (BioVendor Reasearch and Diagnostic Products). ALT and AST were measured on fasting morning samples using the kinetic method (Liquid-Stat Reagent Kit; Beckman Coulter).[34] Coefficients of variation for ALT and AST were 4.1% to 4.4% and 3.8% to 4.5%, respectively.

Covariate Assessment

BMI was defined as weight (in kilograms) divided by the square of the height (in meters). Waist circumference was measured at the level of the umbilicus. Current smoking was defined as smoking at least 1 cigarette per day in the year preceding the Framingham Heart Study examination. Alcohol use was assessed by physician-administered questionnaires and dichotomized on the basis of consumption of >7 drinks per week for women or >14 drinks per week for men. If menstrual periods had stopped for >1 year, women were considered postmenopausal. A physical activity index score was calculated by summing the reported numbers for each level of activity, weighted by their estimated metabolic expenditure, as described previously.[35] The physical activity index ranges from a minimum score of 24, indicating 24 hours of sleeping, to a theoretical maximum score of 120, indicating 24 hours of heavy physical activity.

Statistical Analysis

The primary independent variables of interest were SAT, VAT, and liver attenuation. The primary dependent variables of interest for all analyses were natural logarithmically transformed adiponectin, leptin, leptin receptor, FABP-4, fetuin-A, and RBP-4. All analyses were stratified by sex, given the known differential association of sex with both fat deposition and biomarker levels[4,36] and because of the significant sex interactions identified in this study. In addition, test for sex interaction was conducted based on the pooled model (women and men combined) to ensure the necessity of presenting sex-stratified results.

Partial Pearson correlations (adjusted for age) were examined to assess relations between adiposity measures (BMI, waist circumference, SAT, VAT, liver attenuation, ALT and AST) and biomarkers. Multivariable linear regressions were performed to assess the association of covariate-adjusted relations between MDCT adiposity measures (independent variables) and levels of circulating biomarkers (dependent variables). A separate model was performed for each association tested. All adipose tissue measurements were standardized within sex to a mean of 0 and an SD of 1. The β coefficients derived from the regression models described the association of metabolic regulatory biomarkers per 1-SD increment in SAT, VAT, or liver attenuation. The multivariable model included age, smoking status, alcohol use (>7 drinks/week for women, >14 drinks/week for men), physical activity index, postmenopausal status (women only), and use of hormone replacement therapy (women only). In the secondary analyses, we also adjusted the models for BMI. The tests for sex interactions were conducted based on the multivariable model.

Given that SAT and VAT are the largest fat depots, and both fat depots were significantly associated with several biomarkers, we formally assessed the relative importance of SAT versus VAT in these associations. We examined models containing both SAT and VAT by using multivariable regression analysis. If only 1 fat depot retained statistical significance ($P<0.008$), we considered that fat depot to have the stronger association. If both fat depots retained statistical significance, we formally tested for the statistical significance of the difference between the SAT and VAT regression coefficients within a multivariable standardized regression model. Similarly, the differences of the associations between VAT and liver attenuation with circulating biomarkers were examined.

For all regression models, the variance inflation factors for all variables in the models were tested prior to conducting the regression analysis. Only the variables with variance inflation factor <3, which is below the standard cutoff point of 10 (generally considered problematic), were included in the regression model because of concerns about multicollinearity.

Secondary Analyses

We explored the associations of ALT and AST as surrogate biomarkers of NAFLD with circulating biomarkers by constructing multivariable linear regression models. We analyzed ALT and AST as exposures (independent variables) and used the same covariates in our multivariable linear regressions as models with MDCT-assessed fat depots. ALT and AST were natural logarithmically transformed and modeled per 1-SD increment. ALT and AST were correlated with each other ($r=0.79$ and $r=0.78$ in women and men, respectively; $P<0.001$).

All analyses were performed with SAS version 9.2 (SAS Institute). To account for the presence of multiple biomarkers, we used a Bonferroni adjusted P value cutoff of <0.008 for statistical significance P value of 0.05 divided by 6 biomarkers for our main regression analysis. In the regression analysis, additional adjustment of the P value for multiple testing is redundant for correlated variables, as it leads the results to be conservative because of the overadjustment. Consequently, we did not adjust the P value for multiple testing for the 3 exposures (SAT, VAT, and the liver), given that the fat depots assessed by MDCT are known to be correlated.[4,9]

Results

Study Sample Characteristics

The study sample included 746 women and 837 men with a mean age of 46.1 and 44.0 years, respectively. Participant characteristics, including mean levels of SAT, VAT, and liver attenuation and median levels of biomarkers, are presented in Table 1.

Table 1. Study Sample Characteristics

Parameters	Women (n=746)	Men (n=837)
Clinical characteristics		
Age, y	46.1 (5.7)	44.0 (6.3)
Smoking (%)		
Never	49.5 (369)	59.4 (497)
Former	36.6 (273)	25.4 (213)
Current	13.9 (104)	15.2 (127)
Alcohol use (%)*	14.7 (110)	15.5 (130)
Postmenopausal status (%)	24.8 (185)	—
Hormone replacement therapy (%)	9.1 (68)	—
Physical activity index†	36.4 (6.1)	38.3 (8.9)
Adiposity measures		
Body mass index, kg/m^2	26.2 (5.9)	27.7 (4.3)
Waist circumference, cm	89.8 (15.5)	98.3 (11.4)
Subcutaneous adipose tissue, cm^3	2937 (1567)	2477 (1166)
Visceral adipose tissue, cm^3	1104 (727)	1924 (872)
Liver phantom ratio‡	0.36 (0.05)	0.36 (0.06)
Biomarkers§		
Adiponectin, μg/mL	10.4 (6.7–15.6)	5.4 (3.5–8.3)
Leptin, ng/mL	13.0 (6.9–26.0)	4.22 (2.6–7.1)
Leptin receptor, ng/mL	18.1 (11.7–24.9)	18.6 (12.0–24.1)
Fatty acid binding protein 4, ng/mL	19.6 (13.9–27.3)	15.1 (11.2–19.4)
Fetuin-A, mg/L	419.7 (313.3–542.7)	396.6 (311.6–508.2)
Retinol binding protein 4, ng/mL	37.0 (30.8–44.5)	43.4 (37.5–50.3)

Unless otherwise indicated, data are shown as means (standard deviations) for continuous variables or proportions (counts) for categorical variables.
*Defined as >7 drinks weekly for women and >14 drinks weekly for men.
†The physical activity index has been previously described.[35] The physical activity index ranges from a minimum score of 24, indicating 24 hours of sleeping, to a maximum score of 120, indicating 24 hours of heavy physical activity.
‡The liver phantom ratio reflects the relative degree of liver attenuation assessed by multidetector computed tomography and thus serves as a surrogate for the amount of fat in the liver.
§Described as medians (25th–75th percentiles) because of their skewed distribution.

Correlations of Adiposity Measures and Biomarkers

In both sexes, there were multiple significant correlations of both anthropometric (BMI, waist circumference) and MDCT (SAT, VAT, liver attenuation) adiposity measures with adiponectin, leptin, leptin receptor, and FABP-4 (Table 2). In general, correlation coefficients between SAT and VAT and these biomarkers tended to be higher than those between liver attenuation or serum transaminases (ALT and AST) and the same biomarkers. Of the biomarkers, leptin and FABP-4 had the highest correlation coefficients with the various fat depots (BMI, waist circumference, SAT, and VAT) with r values ranging from 0.72 to 0.80 for leptin (all $P<0.001$) and from 0.56 to 0.65 for FABP-4 (all $P<0.001$). There were fewer statistically significant correlations between adiposity measures and biomarkers secreted by both liver and adipose tissue (fetuin-A and RBP-4), and the corresponding r values tended to be lower (Table 2).

Multivariable Associations of MDCT Adiposity Measures and Biomarkers

In multivariable models, higher volumes of SAT and VAT were consistently and positively associated with circulating leptin and FABP-4 levels and negatively associated with adiponectin and leptin receptor concentrations (all $P<0.001$) (Table 3). Table 3 shows a separate model performed for each association tested. Results for liver attenuation were similar and directionally consistent, with lower liver attenuation (ie, more negative attenuation) being associated with a more adverse profile for these biomarkers. SAT and VAT, but not liver attenuation, were inversely associated with leptin receptor. For the biomarkers secreted by both adipose tissue and the liver (fetuin-A and RBP-4), several multivariable associations were present, and associations varied according to the specific fat depots and participant sex (Table 3).

Sex-Related Differences in Biomarker Associated With Fat Depots

Associations of the MDCT fat depots with adiponectin, leptin, FABP-4, and RBP-4 were observed in both sexes but tended to be stronger in women compared with men (Table 3), particularly for the associations of SAT and VAT with both adiponectin and leptin, of VAT with FABP-4, and of liver attenuation with RBP-4 (all $P<0.05$ for sex interaction). Associations between fat depots and leptin receptor were similar among women and men. For the biomarkers secreted by both adipose tissue and the liver (fetuin-A and RBP-4), several associations were limited to 1 sex. Liver attenuation, for example, was associated with both fetuin-A and RBP-4 in

Table 2. Age-Adjusted Pearson Correlation Coefficients Between Adiposity Measures and Biomarkers of Metabolic Dysregulation

Log Biomarker	Body Mass Index	Waist Circumference	Subcutaneous Adipose Tissue	Visceral Adipose Tissue	Liver Attenuation*	Log Alanine Aminotransferase†	Log Aspartate Aminotransferase†
Women (n=746)							
Adiponectin	−0.34§	−0.34§	−0.31§	−0.43§	0.23§	−0.12‡	0.07
Leptin	0.76§	0.75§	0.80§	0.69§	−0.17§	0.10‡	−0.10‡
Leptin receptor	−0.24§	−0.23§	−0.23§	−0.23§	0.04	0.04	0.14§
Fatty acid binding protein 4	0.64§	0.63§	0.64§	0.65§	−0.23§	0.23§	0.08
Fetuin-A	0.12‡	0.12‡	0.10‡	0.09‡	−0.11‡	0.10‡	0.03
Retinol binding protein 4	0.04	0.07‡	0.07	0.09‡	−0.13§	0.06	0.09
Men (n=837)							
Adiponectin	−0.26§	−0.23§	−0.13§	−0.35§	0.25§	−0.21§	−0.01
Leptin	0.72§	0.77§	0.79§	0.67§	−0.27§	0.30§	0.08
Leptin receptor	−0.21§	−0.23§	−0.22§	−0.22§	0.03	−0.01	0.10‡
Fatty acid binding protein 4	0.56§	0.60§	0.57§	0.58§	−0.26§	0.26§	0.12§
Fetuin-A	0.09‡	0.10‡	0.05	0.11‡	−0.06	0.11‡	0.10‡
Retinol binding protein 4	0.03	0.06	0.01	0.16§	−0.07	0.19§	0.15§

*Liver attenuation is represented by the natural log-transformed liver phantom ratio.
†The sample size for the alanine and aspartate aminotransferase analyses (n=727 for women and n=819 for men) was slightly smaller because the sample included participants with all available fat depots plus alanine and aspartate aminotransferase measurements.
‡Designates $P<0.01$.
§Designates $P<0.001$.

women only; SAT was associated with fetuin-A in women only; and VAT was associated with fetuin-A in men only.

Comparisons Between SAT and VAT

Given that SAT and VAT are the 2 largest fat depots, we assessed the relative importance of SAT versus VAT in these associations for instances in which both SAT and VAT were associated with a given biomarker. In these analyses, notable differences in associations included the finding that SAT was more strongly associated with leptin, whereas VAT was more strongly associated with adiponectin (Table 4). Associations of SAT and VAT with leptin receptor and FABP-4 tended to be similar.

Comparison Between VAT and Liver Attenuation

When both VAT and liver attenuation were individually associated with biomarkers in multivariable models, we evaluated both fat depots in the same model. Generally, VAT was more likely to remain significantly associated with a given biomarker when both fat depots were included in the model. In some cases, both VAT and liver attenuation remained associated with a given biomarker (adiponectin and leptin in women and adiponectin in men) when both fat depots were included in the same model, and formal comparison demonstrated a stronger association for VAT compared with liver attenuation. In women, the β coefficients for adiponectin were −0.23 (95% CI −0.27 to −0.19) for VAT and 0.06 (95% CI 0.02–0.10) for liver attenuation ($P\leq0.001$ for comparison), and the β coefficients for leptin were 0.67 (95% CI 0.62–0.73) for VAT and 0.07 (95% CI 0.02–0.12) for liver attenuation ($P<0.001$ for comparison). For men, the β coefficients for adiponectin were −0.20 (95% CI −0.25 to −0.16) for VAT, and 0.08 (95% CI 0.04–0.13) for liver attenuation ($P<0.001$ for comparison).

Adjustment for BMI

The associations between VAT and liver attenuation with circulating biomarkers while additionally adjusting for BMI as a measure of generalized adiposity are shown in Table S1. Both VAT and liver attenuation remained significantly associated with several biomarkers, although the β coefficients were generally attenuated, indicating that these associations are not solely due to the contribution of BMI. Models with SAT as the exposure were not adjusted for BMI because of concerns about multicollinearity.[4]

Multivariable Associations of ALT and AST and Biomarkers

In multivariable models, both ALT and AST were associated with several biomarkers (Table S2). In general, ALT was

Table 3. Multivariable* Regression Models for Relations Between Fat Depots and Biomarkers of Metabolic Regulation

	Subcutaneous Adipose Tissue		Visceral Adipose Tissue		Liver Attenuation†	
Log Biomarker	β (95% CI)	*P* Value‡	β (95% CI)	*P* Value‡	β (95% CI)	*P* Value‡
Women						
Adiponectin	−0.18 (−0.23 to −0.14)§	<0.001	−0.25 (−0.29 to −0.21)§	<0.001	0.13 (0.09–0.17)	<0.001
Leptin	0.74 (0.70–0.78)§	<0.001	0.65 (0.60–0.70)§	<0.001	−0.14 (−0.21 to −0.08)	<0.001
Leptin receptor	−0.10 (−0.13 to −0.06)	<0.001	−0.10 (−0.13 to −0.07)	<0.001	0.02 (−0.02 to 0.05)	0.4
Fatty acid binding protein 4	0.30 (0.27–0.33)	<0.001	0.31 (0.28–0.34)§	<0.001	−0.10 (−0.13 to −0.07)	<0.001
Fetuin-A	0.04 (0.01–0.07)	0.004	0.04 (0.01–0.07)	0.008	−0.05 (−0.08 to −0.02)	0.001
Retinol binding protein 4	0.02 (0.004–0.04)	0.02	0.03 (0.008–0.05)	0.006	−0.03 (−0.05 to −0.01)§	<0.001
Men						
Adiponectin	−0.08 (−0.12 to −0.04)§	<0.001	−0.24 (−0.28 to −0.20)§	<0.001	0.17 (0.12 to 0.21)	<0.001
Leptin	0.65 (0.61–0.68)§	<0.001	0.58 (0.53–0.62)§	<0.001	−0.22 (−0.28 to −0.17)	<0.001
Leptin receptor	−0.10 (−0.13 to −0.07)	<0.001	−0.11 (−0.14 to −0.07)	<0.001	0.02 (−0.02 to 0.05)	0.3
Fatty acid binding protein 4	0.25 (0.23–0.28)	<0.001	0.27 (0.24–0.29)§	<0.001	−0.11 (−0.14 to −0.09)	<0.001
Fetuin-A	0.02 (−0.01 to 0.04)	0.2	0.04 (0.02–0.07)	0.001	−0.02 (−0.05 to 0.002)	0.07
Retinol binding protein 4	0.002 (−0.01 to 0.02)	0.7	0.03 (0.02–0.05)	<0.001	−0.01 (−0.03 to 0.004)§	0.2

Data are shown as β coefficients per 1-SD increment of each fat measure. Fat depots were sex standardized to a mean of 0 and an SD of 1.
*Multivariable models adjusted for age, smoking status, alcohol use, physical activity index, postmenopausal status (women only), and hormone replacement therapy (women only).
†Liver attenuation is represented by the natural log-transformed liver phantom ratio.
‡Bonferroni corrected $P<0.008$ *P* value of 0.05 divided by 6 biomarkers was considered statistically significant.
§Significant sex interaction was present ($P<0.05$ for sex interaction) with association stronger in women compared with men.

associated with more biomarkers than AST. For biomarkers secreted by adipose tissue, ALT appeared to generally mirror the associations between MDCT liver attenuation and these biomarkers. In contrast, associations between ALT and AST and biomarkers secreted by both adipose tissue and the liver did not mirror those of liver attenuation.

Table 4. Comparison of the Associations of Subcutaneous Versus Visceral Adipose Tissue and Biomarkers of Metabolic Regulation Based on the Multivariable* Regression Models

	Subcutaneous Adipose Tissue		Visceral Adipose Tissue		
Log Biomarker†	β (95% CI)	*P* Value	β (95% CI)	*P* Value	*P* Difference‡
Women					
Adiponectin	0.04 (−0.03 to 0.10)	0.3	−0.28 (−0.35 to −0.22)	<0.001	§
Leptin	0.62 (0.56–0.69)	<0.001	0.15 (0.08–0.21)	<0.001	<0.001
Leptin receptor	−0.05 (−0.11 to 0.001)	0.05	−0.06 (−0.11 to 0.001)	0.05	‖
Fatty acid binding protein 4	0.16 (0.12–0.20)	<0.001	0.18 (0.14–0.22)	<0.001	0.5
Men					
Adiponectin	0.09 (0.04–0.14)	<0.001	−0.30 (−0.35 to −0.25)	<0.001	<0.001
Leptin	0.49 (0.45–0.53)	<0.001	0.27 (0.23–0.32)	<0.001	<0.001
Leptin receptor	−0.06 (−0.10 to −0.02)	0.002	−0.07 (−0.11 to −0.03)	<0.001	0.9
Fatty acid binding protein 4	0.16 (0.13–0.19)	<0.001	0.17 (0.14–0.20)	<0.001	0.7

Data are shown as β coefficients per 1-SD increment of each fat measure. Fat depots were sex standardized to a mean of 0 and a SD of 1.
*Multivariable models adjusted for age, smoking status, alcohol use, physical activity index, postmenopausal status (women only), and hormone replacement therapy (women only). The subcutaneous adipose tissue model was additionally adjusted for visceral adipose tissue volume, and the visceral adipose tissue model was additionally adjusted for subcutaneous adipose tissue volume.
†Fetuin-A and retinol binding protein 4 were excluded from this analysis because both of those biomarkers were significantly associated only with visceral adipose tissue.
‡The *P* difference reflects formal comparison between the strength of the association of subcutaneous adipose tissue vs visceral adipose tissue and biomarkers.
§Not tested because only visceral adipose tissue was significantly associated with the biomarker in models that contained both fat depots.
‖Not tested because neither fat depot was significantly associated with the biomarker when both fat depots were included in the multivariable model.

Discussion

Principal Findings

In our community-based sample, SAT, VAT, and surrogate biomarkers of NAFLD, including MDCT liver attenuation and circulating transaminases, were associated with multiple biomarkers of metabolic regulation with differences in the associations by fat depot type and sex. SAT and VAT tended to be more strongly associated with our biomarker panel than our surrogate measures for NAFLD or liver attenuation. These stronger associations for SAT and VAT generally extended not only to biomarkers secreted by adipose tissue (adiponectin, leptin, leptin receptor, and FABP-4) but also to those biomarkers secreted by both adipose tissue and the liver (fetuin-A and RBP-4). Comparison of the strength of the associations between SAT and VAT demonstrated several differential associations. Specifically, SAT demonstrated stronger associations with leptin, whereas VAT demonstrated stronger associations with adiponectin. Taken together, these findings support further investigation into potential biological differences between these fat depots.

In the Context of the Current Literature

The sexual dimorphism in respect to the association between fat depots and adipokines are well established by prior studies. Compared with men, higher circulating levels of adiponectin[37,38] and leptin[37,39,40] were observed in women, even in a matched pair of similar age, BMI, insulin sensitivity, and VAT volume[38] or mean fat size.[39] The disparities in the functions of adipokines are also noted in the literature.[41,42] In this current research, significant sex interactions with several biomarkers were identified, with stronger associations noted in women. These circulating levels and the functions of certain adipokines may be sexually dimorphic because of the differences in the amount of total and regional body fat distribution or the relations of sex steroids and circulating biomarkers.[43]

Cross-sectional associations have been reported between fat depots and cardiometabolic risk factors,[4,9,44,45] with associations tending to vary by the type of fat depot.[4,6] Adipokines, bioactive substances secreted by adipose tissue, have been postulated as potential mediators of these associations,[1–3] and several epidemiological studies have examined associations between fat depots and individual adipokines[46–48] and between adipokines and cardiometabolic disease.[15,19–26] No study, however, compared the relative associations of MDCT-derived fat measures (SAT, VAT, and liver attenuation) with a comprehensive list of metabolic regulatory biomarkers. In particular, prior human studies of fatty liver and adipokines have primarily examined individual biomarkers and often have had small sample sizes or been limited to patients with NAFLD and matched controls.[44,49–52] In general, VAT and fatty liver have been more strongly associated with individual biomarkers of metabolic regulation compared with anthropometric measures or subcutaneous fat.[46,53] A recent study of 2215 multiethnic participants showed significant associations between hepatic triglyceride content and VAT with adiponectin (all $P<0.01$).[47] In that study, the association with SAT and adiponectin was not significant after additionally adjustment for hepatic triglyceride content and VAT ($P<0.05$).[47] In another study of 102 healthy Korean women, VAT but not SAT was identified as an independent predictor of RBP-4 based on a multiple regression model.[29]

In this analysis, we observed associations between liver attenuation and multiple biomarkers, including adiponectin, leptin, and FABP-4. We also added to the relatively small number of studies that have compared the relative strength of the associations of measures of liver fat in comparison with VAT. In contrast to the previous studies including the Dallas Heart Study[47] and a study of 242 nondiabetic white participants[54] that measured intrahepatic fat by magnetic resonance spectroscopy, we found a stronger association of VAT with adiponectin compared with liver attenuation. This difference may be related to distinct patient populations and different modalities for assessing intrahepatic fat. Our study showed high precision of abdominal SAT, VAT, and liver attenuation using the computed tomography imaging technique.[32,33] In addition, computed tomography imaging is considered a gold standard for quantifying abdominal SAT and VAT, confirming the accuracy of our abdominal fat measurement. In contrast, the liver phantom ratio that was used in our study as a measure of liver fat is a proxy for liver fat content. Specifically, the Dallas Heart Study measure of intrahepatic fat using magnetic resonance spectroscopy is considered a gold standard among the noninvasive measures of liver fat.[47,55] Collectively, the methodological differences in the assessment of liver fat could lead to misclassification and would tend to bias our results related to liver attenuation toward the null. In addition, the physiological differences between the study participants assessed by different fat assessment techniques may have yielded different findings depending on the characteristics of the study population.

Consistent with experimental and epidemiological studies suggesting differences in local and circulating adipokine levels between SAT and VAT,[27,28,46] we found differential associations of SAT and VAT with certain, but not all, biomarkers. In particular, our results regarding fat depot–specific associations of VAT with adiponectin and SAT with leptin were consistent with the findings of previous studies.[36,38,46,56–60] This present study adds to the growing body of literature supporting the differential associations between specific fat depots and a wide panel of circulating biomarkers based on a population-based setting with a larger sample size. In addition,

we added to the existing literature by examining the associations between measures of fatty liver with adipokines, including those secreted by both adipose tissue and the liver.

Potential Mechanisms

The circulating biomarkers explored in this study modulate a broad range of physiological functions, including glucose homeostasis, lipid metabolism, energy expenditure, hemostasis, and inflammatory and immune responses.[10,11] Higher levels of adiponectin have been associated with hepatoprotective and antisteatotic properties, along with antioxidative, insulin-sensitizing, and antiatherogenic effects.[61,62] Leptin is involved with regulation of a broad property, such as hematopoiesis, maturation, bone metabolism, appetite, and satiety control, which is signaled via binding with leptin receptors.[63] Upregulated leptin is associated with reduction in the hypothalamic levels of leptin receptor and deterioration of the transduction signals of the leptin. FABP-4 is essential for fatty acid trafficking, adipocyte inflammation, and insulin action by binding with fatty acid and interacting with hormone-sensitivity lipase.[64] Fetuin-A is dominantly secreted from the liver, and elevated levels of fetuin-A increase insulin resistance by interfering with the activity of insulin receptor tyrosine kinase in the muscle and the liver.[65] RBP-4 is associated with regulation of insulin responses and lipid hemostasis and is predominantly produced by the liver; however, between SAT and VAT, the release is more active in VAT.[48]

Underlying mechanisms that might explain the differential associations of specific fat depots with circulating biomarkers of metabolic regulation remain speculative. A possibility is that circulating biomarkers may reflect differential local secretion of these biomarkers in specific fat depots. Accumulating evidence supports underlying structural and functional differences between fat depots, including differences in cellularity, adipocyte metabolism, and extracellular matrix composition,[66] that may contribute to differential secretion of adipokines. These local biomarkers may then influence a myriad of pathways important for the development of metabolic and cardiovascular disease, including inflammation and angiogenesis.[10] Nevertheless, this explanation, which links circulating and local biomarkers, may be overly simplistic because circulating biomarkers generally reflect only a proportion of total adipokine secretion and may not reflect their local autocrine and paracrine action. Furthermore, adipose tissue serves as both a source and a target of adipokines, making it difficult to untangle the likely complex biological relationship between fat depots and adipokines.[10] Alternatively, aberrations in circulating adipokine levels may reflect systemic metabolic changes. An emerging body of literature suggests that accumulation of visceral and liver fat may actually serve as a marker of dysfunctional SAT,[67] and it is the inability of SAT to respond to positive energy balance that concomitantly results in dysregulation of adipokines and systemic metabolic changes.[68]

Strengths and Limitations

Strengths of our study include a well-characterized community-based sample with sophisticated assessment adipose tissue depots by MDCT and a broad panel of circulating biomarkers of metabolic regulation. Given our relatively large sample size, we were able to sex-stratify our results and demonstrate several differential associations in women compared with men. These findings are relevant, given the previously reported sex differences in associations between fat depots and cardiovascular risk factors.[4] Some limitations deserve comment. First, our sample is predominantly white, which may limit generalizability to other ethnic groups. Second, the cross-sectional design of the analysis prevents inferences of causality or temporality. Next, repeated measures of MDCT-assessed adipose tissue depots were not available. Accordingly, we were unable to calculate error correction coefficients from the data used in our study. Finally, given that our biomarkers are circulating, the lack of associations between certain fat depots and adipokines does not exclude an association with local levels of these biomarkers.

Conclusion

Multiple adipose tissue depots, including SAT, VAT, and surrogate markers of NAFLD, demonstrated associations with circulating biomarkers of metabolic regulation secreted by adipose tissue only or by both adipose tissue and the liver. The magnitude of these associations varied by fat depot type and sex. These findings support further investigation into potential biological differences among these fat depots.

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Disclosures

Pedley is an employee of Merck & Company, Inc. Caroline S. Fox became an employee of Merck on December 14, 2015.

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SUPPLEMENTAL MATERIAL

Table S1. Multivariable* plus BMI adjusted regression models for relations between fat depots and biomarkers of metabolic regulation. Data are shown as *β* coefficients per 1 standard deviation increment of fat measure. Fat depots were sex-standardized to a mean of 0 and a standard deviation of 1.

Log-Biomarker	Visceral Adipose Tissue		Liver Attenuation†	
	β (95% CI)	*p*-Value	*β* (95% CI)	*p*-Value
Women				
Adiponectin	-0.29 (-0.36, -0.21)	< 0.001	0.10 (0.06, 0.14)	< 0.001
Leptin	0.18 (0.10, 0.26)	< 0.001	0.003 (-0.04, 0.05)	0.9
Leptin Receptor	-0.04 (-0.10, 0.02)	0.2	-0.01 (-0.04, 0.03)	0.7
Fatty Acid Binding Protein-4	0.18 (0.13, 0.23)	< 0.001	-0.04 (-0.07, -0.01)	0.005
Fetuin-A	0.001 (-0.05, 0.06)	0.98	-0.04 (-0.07, -0.01)	0.01
Retinol Binding Protein-4	0.04 (0.01, 0.08)	0.02	-0.04 (-0.05, -0.01)	0.002
Men				
Adiponectin	-0.25 (-0.32, -0.19)	< 0.001	0.12 (0.08, 0.17)	< 0.001
Leptin	0.26 (0.20, 0.32)	< 0.001	-0.02 (-0.07, 0.02)	0.2
Leptin Receptor	-0.07 (-0.12, -0.03)	0.003	-0.02 (-0.05, 0.01)	0.3
Fatty Acid Binding Protein-4	0.16 (0.12, 0.20)	< 0.001	-0.03 (-0.06, -0.01)	0.01
Fetuin-A	0.04 (0.001, 0.08)	0.04	-0.01 (-0.04, 0.01)	0.3
Retinol Binding Protein-4	0.07 (0.04, 0.09)	< 0.001	-0.01 (-0.03, 0.01)	0.2

*Multivariable models adjusted for age, smoking status, alcohol use, physical activity index, postmenopausal status (women only), and hormone replacement therapy (women only).

†Liver attenuation is represented by the natural log-transformed liver-phantom ratio.

Abbreviations: BMI, body mass index; CI, confidence Interval.

Table S2. Multivariable* regression models for relations between natural log-transformed alanine aminotransferase and aspartate aminotransferases with biomarkers of metabolic regulation. Data are shown as *β* coefficients per 1 standard deviation increment of each natural log-transformed aminotransferase.

Log-Biomarker	Alanine Aminotransferase				Aspartate Aminotransferases			
	Multivariable Model		Multivariable + BMI Model		Multivariable Model		Multivariable + BMI Model	
	β (95% CI)	*p*-Value	*β* (95% CI)	*p*-Value	*β* (95% CI)	*p*-Value	*β* (95% CI)	*p*-Value
Women								
Adiponectin	-0.07 (-0.11, -0.02)	0.003	-0.03 (-0.07, 0.02)	0.2	0.04 (-0.004, 0.08)	0.08	0.04 (0.001, 0.08)	0.05
Leptin	0.10 (0.03, 0.17)	0.003	-0.05 (-0.10, -0.004)	0.03	-0.09 (-0.16, -0.02)	0.01	-0.10 (-0.14, -0.06)	< 0.001
Leptin Receptor	0.01 (-0.02, 0.05)	0.4	0.04 (0.003, 0.07)	0.03	0.06 (0.02, 0.09)	0.001	0.06 (0.03, 0.09)	< 0.001
FABP-4	0.11 (0.07, 0.14)	< 0.001	0.05 (0.02, 0.07)	0.001	0.04 (0.002, 0.07)	0.04	0.03 (0.006, 0.060)	0.02
Fetuin-A	0.05 (0.02, 0.08)	0.003	0.04 (0.006, 0.07)	0.02	0.01 (-0.02, 0.04)	0.4	0.01 (-0.02, 0.04)	0.4
RBP-4	0.01 (-0.01, 0.03)	0.1	0.01 (-0.008, 0.03)	0.2	0.02 (-0.003, 0.04)	0.1	0.02 (-0.003, 0.04)	0.1
Men								
Adiponectin	-0.14 (-0.18, -0.09)	< 0.001	-0.09 (-0.14, -0.05)	< 0.001	-0.01 (-0.05, 0.04)	0.8	0.02 (-0.03, 0.06)	0.4
Leptin	0.26 (0.20, 0.31)	< 0.001	0.07 (0.03, 0.11)	0.002	0.07 (0.01, 0.13)	0.02	-0.01 (-0.05, 0.03)	0.5
Leptin Receptor	-0.01 (-0.04, 0.03)	0.7	0.03 (-0.005, 0.06)	0.09	0.04 (0.01, 0.08)	0.01	0.06 (0.03, 0.09)	< 0.001
FABP-4	0.12 (0.09, 0.15)	< 0.001	0.04 (0.01, 0.07)	0.003	0.06 (0.03, 0.09)	<0.001	0.02 (-0.002, 0.05)	0.08
Fetuin-A	0.05 (0.02, 0.07)	<0.001	0.04 (0.01, 0.07)	0.003	0.04 (0.02, 0.07)	0.001	0.04 (0.01, 0.07)	0.003
RBP-4	0.04 (0.02, 0.05)	< 0.001	0.04 (0.02, 0.05)	< 0.001	0.03 (0.01, 0.04)	<0.001	0.03 (0.01, 0.04)	< 0.001

* Multivariable models adjusted for age, alcohol use, smoking status, physical activity index, postmenopausal status (women only), and hormone replacement therapy (women only).

Abbreviations: BMI, body mass index; CI, confidence Interval; FABP-4, fatty acid binding protein-4; RBP-4, retinol binding protein-4.