Ectopic Adiposity is Associated with Autonomic Risk Factors and Subclinical Cardiovascular Disease in Young Adults

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Objective: To examine the relationship between ectopic adiposity and markers of cardiometabolic risk, autonomic control, and subclinical cardiovascular disease (CVD).

Methods: Cross-sectional analyses were performed in 324 subjects with overweight and obesity. Single-slice CT images were analyzed to calculate thigh muscle attenuation (MA), a measure of ectopic adiposity. Autonomic control was assessed using low-frequency to respiratory-frequency heart rate variability (LFa/RFa ratio). Carotid intima-media thickness (IMT) was a marker of subclinical CVD.

Results: Among overweight participants, those with low MA had lower HDL-c, higher LFa/RFa ratio, and subcutaneous thigh fat compared to high MA individuals despite no difference in visceral fat or insulin resistance. Significant associations were not observed in the class I obese group. In the class II obese group, those with high MA had higher triglycerides and insulin levels, yet there was no difference in visceral fat compared to the low MA group. Mean IMT was significantly higher in the low MA compared to the high MA overweight group (0.63 mm vs. 0.58 mm, \( P = 0.04 \)) but was similar between the low and high MA class II obese groups.

Conclusions: Excess ectopic adiposity in muscle tissue is associated with metabolic and autonomic risk factors and subclinical CVD, most notably in overweight individuals, independent of insulin resistance and visceral abdominal fat.

Introduction

Abdominal adiposity is a well-established risk factor for cardiovascular events and mortality (1). Adipose tissue in the abdominal visceras is metabolically active, leading to insulin resistance, dyslipidemia, and hypertension, which are known risk factors for cardiovascular disease (CVD) (2-4). However, those with less visceral fat may still be at increased risk for CVD, specifically females and individuals with “metabolic obesity” who have normal body mass index (BMI) and increased cardiometabolic risk (5). Peripheral fat deposits may pose less risk than central deposits, but evidence suggests that increased intramuscular fat is associated with abnormal energy utilization of skeletal muscle in diabetes and obesity (6,7) and may account for some of the CVD risk in normal-weight and overweight individuals.

Excess intramuscular fat is associated with impaired muscle blood flow, reduced insulin diffusion, and increased fatty acids synthesis via altered lipolysis, which may serve as one explanation for its association with metabolic abnormalities and CVD risk (8,9). Low muscle attenuation (MA), a measure of intramuscular ectopic fat, has been linked to insulin resistance in adults with obesity (8). Low MA is independently associated with insulin resistance and greater carotid intima-media thickness (IMT), a marker of subclinical CVD that predicts incident CVD (10,11). These studies support abnormal energy utilization in insulin resistance as a link between intramuscular fat and subclinical CVD. However, recent studies have additionally linked low MA to lower HDL-c, inflammation, and higher blood pressure, suggesting that other factors may be involved in excess ectopic adiposity and increased metabolic risk (12,13).

Elevated sympathetic activity is associated with obesity. Central obesity and elevated sympathetic activity have been reported (14), but a similar association with other adiposity stores is still being elucidated (14,15). We know that elevated sympathetic activity is associated with elevated fatty acids and triglycerides, which occur as a result of abnormal lipolysis in obesity (8,9). Insulin resistance is a proposed explanation for increased sympathetic activity (16). In lean
subjects, an insulin infusion challenge produces a rise in the ratio of low-frequency to high-frequency heart rate variability (LF/HF ratio), an indicator of altered autonomic control; however, in obesity, chronic hyperinsulinemia may blunt this physiologic response (16,17). Thus, excess ectopic adiposity may contribute to alterations in autonomic control and metabolic obesity among individuals with normal BMI. Furthermore, indicators of autonomic control, including the LF/HF ratio, are linked to coronary atherosclerosis; therefore, excess ectopic adiposity may be a source for the increased CVD risk among these individuals (18,19).

Given the gap in the current understanding of peripheral adiposity stores and associated autonomic changes, we examined the relationship between intramuscular ectopic adiposity, autonomic control, and CVD risk. We also tested whether an indicator of autonomic control, the low-frequency to respiratory-frequency heart rate variability ratio (equivalent to the aforementioned LF/HF ratio), may partially account for the associations between skeletal muscle ectopic fat and CVD risk. We predicted that among young adults with overweight and obesity, excess ectopic fat as measured by low MA would be associated with a higher LFa/RFa ratio and thicker IMT, an indicator of subclinical CVD.

Methods

Study design

Cross-sectional analyses were performed using data from the randomized clinical trial, SAVE (the study to Slow Adverse Vascular Effects of excess weight, NCT00366990) which examined the impact of a dietary and activity intervention on measures of subclinical CVD. Recruitment was performed from June 2007 through May 2009 by mail mailing to zip codes in Allegheny County, PA, followed by phone screening to determine initial eligibility, then in-person screen. A total of 349 men and women aged 20-45 years with BMI 25-39.9 kg/m² were included. Inclusion criteria included blood pressure <140/90 mm Hg, a fasting glucose <126 mg/dl, and an inactive lifestyle defined as exercising for <8 months during the past 12 months and for <3 h a week on average. Exclusion criteria included previous weight loss surgery, known CVD, inflammatory disease, or a condition where salt restriction would be harmful. Those who were pregnant/nursing, on lipid lowering medication, or on vasoactive medication were also ineligible for the study. The University of Pittsburgh institutional review board approved research protocols for the SAVE study. All participants provided written informed consent prior to enrollment.

Analyses were generated using baseline data from two ancillary studies to SAVE that additionally assessed heart rate variability (HRV) and adipose tissue distribution. Of the 349 participants in SAVE, 25 participants had missing data for MA (n = 17) and HRV (n = 8), leaving 324 participants for this analysis.

Ectopic adiposity

In this manuscript, we use the term ectopic adiposity to indicate excess adipose tissue in muscle. Single-slice (6 mm) axial CT images of the thigh (15 cm above the patellar apex) and abdomen (between L4 and L5 obtained during suspended respiration) were acquired at baseline using a C-150 Ultrafast CT Scanner (GE Imatron, San Francisco, CA). A pixel range of 30 to 190 Hounsfield units (HU) denoted fat, and 0-100 HU denoted muscle. Areas were calculated by multiplying the number of pixels by the pixel area. MA was calculated by averaging the pixel values of the regions outlined on the images. A line was drawn along the fascial plane of thigh muscles, with fat outside this line considered subcutaneous thigh fat, and fat within this line was considered intramuscular fat. For the abdominal scans, a line was drawn along the fascial plane of the interior abdominal musculature. Fat outside this line was designated as subcutaneous fat, and fat within the line was designated as visceral abdominal fat. The images were quantified using software called Slice-O-Matic v4.3 (Tomovision, Magog, Quebec, Canada).

Heart rate variability

HRV was measured using an ANSAR monitor (ANX-3.0, ANSAR Group Inc, Philadelphia, PA), which provides continuous noninvasive measurements of electrocardiogram signals (for HRV assessment) and bioimpedance plethysmography signals (for respiratory rate variability assessment; RRV). Electrocardiogram electrodes were attached to a participant’s chest in a modified Lead-II configuration, and a blood pressure cuff was placed on the left arm. Participants were asked to sit with their feet flat on the floor and refrain from sudden movements or talking. Resting measures at a normal breathing rate were taken for 5 min. A spectral analysis of the HRV and RRV was generated using ANSAR software to establish low-frequency (LF) and high-frequency (HF) bands. Following established guidelines, the LF band was centered on the HRV spectrum from 0.04 to 0.10 Hz (20.21). From the spectral analysis of the RRV, the frequency of the peak mode was defined as the fundamental respiratory frequency (FRF), then a 0.12 Hz wide window was centered at the FRF to generate the HF band, and its integral was identified as the respiratory-frequency area (RFa) which reflects vagal control over cardiac activity (22-24). During low FRF, the RFa shifts into the LF bandwidth. The area under the spectral curve centered on the FRF was computed as the RFa, and remaining area under the spectral curve within the LF bandwidth was computed as LFa.

Two measures of HRV were used in this analysis: LFa and RFa. Reproducibility analyses for the two HRV measures between three technologists were conducted on 30 participants (10 for each pair). Between technologist reproducibility, intraclass correlation coefficients (ICC) were 0.71 and 0.91 for LFa and RFa, respectively.

IMT measurement

IMT was used as an indicator of subclinical CVD. An Acuson Sonoline Antares high-resolution duplex scanner equipped with digital electronics to provide high-precision gray scale images was used for carotid ultrasound measurements. Images were digitalized from the right and left distal common carotid arteries (CCA, 1 cm proximal to the carotid bulb), the carotid bulbs, and the proximal 1 cm of the internal carotid arteries. IMT was measured by electronically tracing the lumen–intima and media–adventitia interfaces across each segment using a semi-automated edge detection software (AMS system, Sweden) (25). The computer then generated one measurement for each pixel over the area. The mean of all average readings across these locations comprised the average IMT. A reproducibility study of IMT showed that mean differences (SD) between paired measurements of sonographers, readers, and visits were −0.004 mm (0.10), 0.066 mm (0.07), and −0.013 mm (0.13), respectively (26).
TABLE 1 Participant characteristics by body mass index

|                     | Overweight  | Class I obese | Class II obese | P-value |
|---------------------|-------------|---------------|----------------|---------|
|                     | (n = 94)    | (n = 123)     | (n = 107)      |         |
| Age (years)         | 38.2 (6.3)  | 38.0 (5.7)    | 37.5 (6.4)     | 0.73    |
| Male (n, %)         | 16 (21.6)   | 29 (39.2)     | 29 (39.2)      | 0.23    |
| Visceral abdominal fat (cm²) | 90.1 (7.7)  | 100.6 (8.2)   | 109.3 (9.0)    | 0.03    |
| Waist (cm)          | 53.1 (3.0)  | 51.4 (2.7)    | 49.6 (2.9)     |         |
| HDL (mg/dl)         | 123.6 (30.9) | 124.9 (36.7)  | 122.1 (31.3)   | 0.83    |
| Triglycerides (mg/dl) | 97 (70, 143) | 116 (78, 176) | 133 (64, 175)  | 0.01    |
| Glucose (mg/dl)     | 95.7 (7.2)  | 98.7 (9.2)    | 98.5 (7.2)     | 0.01    |
| Weight (kg)         | 58.0 (15.0) | 52.5 (12.9)   | 48.8 (11.8)    | <0.0001 |
| Subcutaneous abdominal fat (mg/dl) | 83.8 (44.3) | 113.3 (49.6)  | 148.3 (52.4)   | <0.0001 |
| Inframuscular thigh fat (cm²) | 316 (84.2)  | 427 (96.5)    | 522 (104)      | <0.0001 |
| Thigh muscle attenuation (HU) | 90.1 (7.7)  | 100.6 (8.2)   | 109.3 (9.0)    | <0.0001 |
| Subcutaneous thigh fat (cm²) | 9.4 (3.1)   | 13.6 (4.7)    | 15.1 (4.6)     | <0.0001 |
| Total activity (MET·h/week) | 96.4 (33.4) | 118.0 (45.5)  | 148.4 (49.6)   | <0.0001 |
| LFa at rest (bpm²)  | 29.0 (11.1, 83.5) | 34.6 (6.6, 78.0) | 23.7 (6.9, 93.4) | 0.92 |
| RFa at rest (bpm²)  | 2.0 (1.1, 3.5) | 1.9 (1.2, 3.9) | 1.8 (1.1, 3.5) | 0.69 |
| LFa/RFa ratio      | 1.0 (0.5, 2.0) | 1.2 (0.7, 2.0) | 1.1 (0.6, 2.0) | 0.28 |
| Intima-media thickness (mm) | 0.58 (0.08) | 0.61 (0.09)   | 0.64 (1.0)     | 0.001   |

Values presented as mean (SD) or n (%). Bolded values indicate statistically significant values (P < 0.05). Variables presented as median (IQR). *P-value generated with chi-square test and one-way ANOVA; where non-normal distributions indicated, P-values were generated with the logarithmic transformed or square root transformed variable.
RFa = respiratory-frequency area; LFa = low-frequency area.

Covariates

Demographic factors (age, gender, and race) were obtained through baseline questionnaires. CVD risk factors consisted of systolic blood pressure (SBP), heart rate, triglycerides, LDL, HOMA index, and physical activity. Blood pressure measurements were collected by a standard sphygmomanometer after a 5-min rest period. Three blood pressure measurements were collected and the last two were averaged. Participants were weighed in light clothing without shoes. Waist circumference was measured to the nearest 0.1 cm at the narrowest portion of the torso. The MAQ questionnaire, a reliable and valid assessment of current leisure and occupational activities, was used to estimate total physical activity (metabolic equivalent hours per week, MET h-week) (27). Serum glucose was quantified by an enzymatic reaction (28). Serum insulin level was measured using an RIA procedure developed by Linco Research, Inc. Insulin resistance was calculated using the homeostasis model assessment of insulin resistance index (HOMA index = fasting glucose (mmol/l) × fasting insulin (μU/ml)/22.5) (29). Triglycerides and HDL-c were determined enzymatically, while LDL-c was estimated using the Friedewald equation (30-32).

Statistical analysis

Analyses were performed using baseline data from the SAVE study. The primary independent variable was MA, and dependent variables included metabolic profiles, adiposity measures, HRV parameters, and carotid IMT measurements. Continuous variable distributions were assessed for normality. Natural logarithmic transformations were performed to approximate a normal distribution for triglycerides, insulin, HOMA index, and the HRV measures (LFa, RFa, and LFa/RFa ratio); a square root transformation was used for total physical activity. All other variables were analyzed without transformation. Race was collapsed into Black and non-Black groups. MA was used as a continuous variable and a categorical variable by categorizing below and above the overall median (51.6 HU, low MA and high MA, respectively). BMI was used as a categorical variable (overweight: 25-29.9, class I obese: 30-34.9, and class II obese: 35-39.9 kg/m²).

One-way ANOVA and chi-square test were utilized to compare participant characteristics between BMI categories. The natural logarithm and square root transformed variables were used to generate P-values in the ANOVA analyses. Bivariate correlations were calculated using Spearman correlation coefficients. Generalized linear regression adjusted for age and gender were performed with subclinical CVD risk factors, measures of adiposity, and IMT as the dependent variables and MA as the independent variable. Interactions between MA and BMI, adjusted for age and gender, were tested for models where beta estimates were in opposite directions between BMI categories. Adjusted least squares means...
TABLE 2 Association between muscle attenuation (MA) and cardiovascular risk factors stratified by body mass index, adjusted for age and gender

| Cardiovascular risk factor                              | Overweight  
|                                                        | (n = 94) |
|                                                        |          |
| SBP (mm Hg)                                             | 0.29 (0.44) |
| LDL (mg/dl)                                             | -0.61 (0.58) |
| HDL (mg/dl)                                             | 0.78 (0.12) |
| Natural logarithm of triglycerides (mg/dl)              | -0.023 (0.18) |
| Glucose (mg/dl)                                         | -0.28 (0.28) |
| Natural logarithm of HOMA index                         | 0.029 (0.05) |
| Natural logarithm of LFa at rest (bpm²)                 | 0.013 (0.65) |
| Natural logarithm of RFa at rest (bpm²)                 | 0.097 (0.01) |
| Natural logarithm of LFa/RFa ratio                      | -0.084 (0.01) |
|                                                        |          |
| Class I obese                                           | (n = 123) |
|                                                        |          |
| SBP (mm Hg)                                             | -0.14 (0.68) |
| LDL (mg/dl)                                             | 0.26 (0.83) |
| HDL (mg/dl)                                             | -0.15 (0.72) |
| Natural logarithm of triglycerides (mg/dl)              | -0.013 (0.48) |
| Glucose (mg/dl)                                         | -0.11 (0.72) |
| Natural logarithm of HOMA index                         | 0.015 (0.36) |
| Natural logarithm of LFa at rest (bpm²)                 | -0.024 (0.35) |
| Natural logarithm of RFa at rest (bpm²)                 | -0.020 (0.49) |
| Natural logarithm of LFa/RFa ratio                      | -0.004 (0.88) |
|                                                        |          |
| Class II obese                                          | (n = 107) |
|                                                        |          |
| SBP (mm Hg)                                             | 0.27 (0.45) |
| LDL (mg/dl)                                             | 1.19 (0.26) |
| HDL (mg/dl)                                             | -0.13 (0.72) |
| Natural logarithm of triglycerides (mg/dl)              | 0.048 (0.002) |
| Glucose (mg/dl)                                         | -0.12 (0.64) |
| Natural logarithm of HOMA index                         | 0.037 (0.02) |
| Natural logarithm of LFa at rest (bpm²)                 | 0.059 (0.033) |
| Natural logarithm of RFa at rest (bpm²)                 | 0.048 (0.12) |
| Natural logarithm of LFa/RFa ratio                      | 0.010 (0.73) |

Values presented as beta estimate (P-value). Bolded values indicate statistically significant values (P < 0.05). Interactions between MA and BMI were also adjusted for age and gender.

Results

Among the 324 subjects included in SAVE, the average age was 37.9 years (SD 6.1), 22.8% were male, 16.1% were black, and median MA was 51.6 HU. Sample characteristics by BMI classifications with median and interquartile ranges are presented in Table 1. Across higher BMI categories, HDL-c and thigh MA are significantly lower (P < 0.0001), whereas systolic blood pressure (SBP), triglycerides, glucose, insulin, HOMA index, all measures of adiposity, and IMT were significantly higher (P < 0.05). Those with missing data (n = 25) did not statistically differ from the participants included in these results.

The associations between MA and CVD risk factors by BMI classifications are reported in Table 2. Among participants with overweight or class I obesity, there was no significant association between MA and BMI, LDL, HDL, or triglycerides. Interestingly, among participants with class II obesity, there was a positive association between MA and triglycerides (beta = 0.048, P = 0.002). For participants with overweight or class II obesity, there was a positive association between MA and HOMA index (P = 0.05 and 0.02, respectively). Among overweight participants, there was an inverse relationship between MA and LFa/RFa ratio (beta = -0.084, P = 0.01). Additionally, we tested the models for interactions between MA and BMI for each CVD risk factor, and we found a significant interaction for HDL-c, triglycerides, and LFa/RFa ratio (P = 0.03, 0.001, and 0.03, respectively).

TABLE 3 Association between muscle attenuation (MA) and measures of adiposity stratified by body mass index, adjusted for age and gender

| Measure of adiposity                  | Overweight  
|                                      | (n = 94) |
|                                      |          |
| Waist (cm)                            | -0.65 (0.002) |
| Visceral abdominal fat (cm²)          | -1.25 (0.34) |
| Intramuscular thigh fat (cm²)         | -0.53 (<0.0001) |
| Subcutaneous abdominal fat (cm²)      | -2.73 (0.35) |
| Subcutaneous thigh fat (cm²)          | 3.42 (<0.0001) |
|                                      |          |
| Class I obese                         | (n = 123) |
|                                      |          |
| Waist (cm)                            | -0.54 (0.02) |
| Visceral abdominal fat (cm²)          | -3.00 (0.04) |
| Intramuscular thigh fat (cm²)         | -1.02 (<0.0001) |
| Subcutaneous abdominal fat (cm²)      | -6.01 (0.04) |
| Subcutaneous thigh fat (cm²)          | 2.31 (0.07) |
|                                      |          |
| Class II obese                        | (n = 107) |
|                                      |          |
| Waist (cm)                            | -0.12 (0.63) |
| Visceral abdominal fat (cm²)          | -1.45 (0.36) |
| Intramuscular thigh fat (cm²)         | -0.86 (<0.0001) |
| Subcutaneous abdominal fat (cm²)      | 0.48 (0.89) |
| Subcutaneous thigh fat (cm²)          | -3.23 (0.02) |

Values presented as beta estimate (P-value). Bolded values indicate statistically significant values (P < 0.05). Interactions between MA and BMI were also adjusted for age and gender.
We then explored the relationship between MA and adiposity distributions (Table 3). Among the overweight and class I obese groups, lower MA was associated with higher waist circumference, which was statistically significant ($P = 0.002$ and 0.02, respectively). Among class I obese group, low MA correlated with greater visceral abdominal fat ($P = 0.04$). Low MA correlated with higher intramuscular fat across all BMI groups ($P < 0.0001$ for all groups). However, the association between MA and subcutaneous fat in the abdomen and thigh varied by BMI (MA and BMI interaction $P = 0.05$ and $P < 0.0001$, respectively). In the overweight group, MA was positively associated with subcutaneous thigh fat. In the class I obese group, MA was negatively associated with subcutaneous abdominal fat. Lastly, in the class II obese group, MA was negatively associated with subcutaneous thigh fat.

Next, the average CVD risk factors and adiposity measures adjusted for age and gender were stratified by MA (above and below the median) for each BMI category (Table 4). In the overweight group, those with low MA had significantly lower HDL-c and higher LFa/RFa ratio compared to those with high MA ($P < 0.05$ for both measures). Additionally they had higher waist circumferences. In the class I obese group, no significant differences were found. In the class II obese group, triglycerides and HOMA index were

![Figure 1](https://www.obesityjournal.org/figure1.png)

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**TABLE 4** Cardiovascular risk factors stratified by relative muscle attenuation and body mass index, adjusted for age and gender

| Cardiovascular risk factor | Low MA ($n = 23$) | High MA ($n = 71$) | Low MA ($n = 59$) | High MA ($n = 64$) | Low MA ($n = 80$) | High MA ($n = 27$) |
|---------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| SBP (mm Hg)               | 108.6 (2.2)       | 111.3 (1.3)       | 115.5 (1.3)       | 122.4 (0.1)       | 127.6 (0.3)       | 118.1 (2.0)       |
| LDL (mg/dl)               | 103.8 (6.9)       | 122.8 (4.7)       | 112.7 (1.6)       | 133.0 (2.5)       | 137.4 (1.1)       | 157.3 (1.7)       |
| HDL (mg/dl)               | 52.4 (2.6)        | 55.6 (1.9)        | 53.5 (1.9)        | 53.5 (1.9)        | 53.5 (1.9)        | 49.0 (0.3)        |
| Triglycerides (mg/dl)     | 119.1 (102.2,165.5) | 122.8 (4.7)       | 112.7 (1.6)       | 133.0 (2.5)       | 137.4 (1.1)       | 157.3 (1.7)       |
| HOMA index                | 2.2 (1.7,3.2)     | 2.2 (1.7,3.2)     | 2.2 (1.7,3.2)     | 2.2 (1.7,3.2)     | 2.2 (1.7,3.2)     | 2.2 (1.7,3.2)     |
| Waist (cm)                | 93.3 (4.4)        | 93.3 (4.4)        | 93.3 (4.4)        | 93.3 (4.4)        | 93.3 (4.4)        | 93.3 (4.4)        |

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**TABLE 5** Association between muscle attenuation and intima-media thickness with progressive multivariable modeling

| Variable                          | Beta-estimate | $P$-value |
|-----------------------------------|---------------|-----------|
| Unadjusted                        | −0.005        | 0.004     |
| Age, gender, race                 | −0.003        | 0.048     |
| LFa/RFa                           | −0.003        | 0.034     |
| BP, heart rate,                   |               | 0.059     |
| triglycerides, HOMA index         |               |           |
| physical activity, LDL            |               |           |
| Adiposity measurements            | −0.002        | −0.218    |

$MA = $ muscle attenuation; $RFa = $ respiratory-frequency area; $LFa = $ low-frequency area.
statistically higher in the high MA group. These participants had less intramuscular thigh fat compared to the low MA group, but no difference in waist circumference was detected.

Lastly, the association between MA and IMT was explored to further understand how differences in MA confer CVD risk, particularly in the overweight and class II obese groups. Unadjusted IMT means were higher across higher BMI categories (Table 1). MA was independently associated with IMT in univariate modeling ($\beta = -0.005, P = 0.004$). In a progressive multivariable model (Table 5), the association between MA and IMT remained statistically significant after adjusting for demographic characteristics ($\beta = -0.003$ and $P = 0.048$) then LFa/RFa ratio ($\beta = 0.003$ and $P = 0.034$). However, this association was weakened after adjusting for other CVD risk factors ($\beta = -0.003, P = 0.059$) and markedly attenuated after adjusting for adipose-specific covariates ($\beta = -0.002, P = 0.22$). When the multivariable regression was stratified by BMI, a significant association between IMT and MA was observed only for the overweight participants ($\beta = -0.007, P = 0.01$). Mean IMT adjusted for the above covariates and stratified by median MA and BMI categories demonstrated a significantly higher IMT in the low MA versus high MA overweight group (0.63 mm vs. 0.58 mm, $P = 0.04$, Figure 1), however IMT did not vary by MA in the class II obese group. Furthermore, mean IMT for the overweight low MA group was similar in magnitude to both the low and high MA class II obese groups (0.63 mm and $P = 0.70$, 0.62 mm and $P = 0.88$, respectively).

**Discussion**

In a population of otherwise healthy young adults, lower MA, a marker of excess ectopic adiposity in skeletal muscle, was associated with more cardiovascular risk factors, greater LFa/RFa HRV ratio, and thicker IMT, which was notable among overweight individuals. The significant interactions between MA and BMI in models predicting lipids, HRV ratios, and adipose tissue distributions suggested varying CVD risk associated with excess ectopic fat between participants with overweight or class II obesity. The altered autonomic control found in the overweight participants with low MA may account for the higher risk of subclinical disease, independent of insulin resistance and visceral adiposity.

Obesity is associated with altered autonomic control. In a previous study, those with higher BMI and fat mass had higher LF/HF HRV (17) (corresponding to higher LFa/RFa ratio). However in our study, overweight participants with low MA, who by definition had the lowest BMIs among all groups, had the highest LFa/RFa ratio (14,15), suggesting that intramuscular adiposity may be as challenging to autonomic control as would be a higher degree of overall obesity. Additionally, elevated sympathetic activity has been linked to insulin resistance in obesity (10,33). However, our results showed normal insulin levels in both low and high MA overweight groups, so the elevated LFa/RFa HRV associated with excess ectopic fat may not be secondary to hyperinsulinemia. Lastly, altered autonomic regulation has been observed with CVD (18,19), and elevated sympathetic activity may be associated with poor vascular adaptation during hemodynamic changes that lead to vascular hypertrophy (34). Kim et al. reported that MA was negatively associated with IMT independent of visceral adiposity and diabetes (10), and together with our findings of higher IMT in the overweight low MA group, altered autonomic function may represent a link between excess ectopic adiposity and increased CVD risk.

The differences in observations by BMI are also likely secondary to differences in adipose tissue deposition, which has been described as a mechanism behind metabolically obese normal-weight and metabolically normal obese phenotypes (5,35,36). The overweight low MA individuals had lower HDL and higher IMT than their high MA counterparts that were not explained by difference in insulin resistance or visceral adiposity, suggesting that excess ectopic fat may account for the higher subclinical CVD. Although their BMI classification would imply lower CVD risk compared to the class II obese group, the overweight low MA group should be classified as metabolically obese. In contrast, an individual with obesity who is metabolically normal without metabolic syndrome may be protected from diabetes and cardiovascular disease (37). Our findings suggest that metabolically normal person with obesity has less ectopic intramuscular fat, higher HDL-c, lower LF/HF HRV, and ultimately lower IMT (22). Of note, some argue against the existence of benign obesity; a Swedish group showed increased CVD events and death in middle-aged men with obesity and without metabolic syndrome or insulin resistances compared to healthy normal-weight individuals (38). Therefore, longitudinal research of various body phenotypes and subclinical CVD is necessary to further aid clinicians in assessing CVD risk.

This study is unique because we identified cardiometabolic risk factors in an otherwise healthy population, and we recognized altered autonomic control as an important contributor in the disease process of CVD, independent of insulin resistance and visceral adiposity. One limitation was the smaller proportion of men and blacks—the proportion of males was concordant with other lifestyle weight loss studies, and the proportion of blacks was representative of the demographics in the study location—as a result, this limited power to further detect difference in risk by gender or race. Also, the clinical significance of IMT differences observed in this study is not clear—the literature shows that 0.1 mm differences in absolute IMT translates to a 10-15% increased risk of MI and 13-18% increased risk of stroke (39), but change in CVD risk with IMT differences less than 0.1 mm has not been addressed. Additionally, by designating 15 cm above the patella as the site of MA measurements, we may be measuring different areas of the thigh depending on the participant’s femur length, and this potentially affects interpretation of results. A caveat of using HRV as a marker of autonomic control is the multifactorial contributions to the variability. While high-frequency HRV is a known indicator of parasympathetic activity, low-frequency HRV is modulated by the sympathetic and parasympathetic nervous systems, adrenomedullary catecholamines, and other circulating hormones that make its interpretation more complex beyond a simple indicator of sympathetic activity (20).

We conclude that in assessing CVD risk, the distribution of fat is as important as the amount of fat that an individual carries. The results strongly suggest that excess ectopic adiposity is associated with multiple cardiometabolic and autonomic markers, which increases CVD risk independent of insulin resistance and visceral abdominal fat. Increased ectopic adiposity within skeletal muscle places overweight individuals into a higher CVD risk category despite their lower overall weight compared to individuals with obesity. Researchers need to identify additional clinical markers that are associated with low MA, rather than BMI alone, to evaluate CVD risk, especially in overweight individuals.
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References

1. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW. Body-mass index and mortality in a prospective cohort of U.S. adults. N Engl J Med 1999;341:1097-1105.
2. Peiris AN, Sotomann MS, Hoffmann RG, et al. Adiposity, fat distribution, and cardiovascular risk. Ann Intern Med 1989;110:867-872.
3. Macor C, Ruggeri A, Mazzonetto P, Federspil G, Cobelli C, Vetto R. Visceral adipose tissue impairs insulin secretion and insulin sensitivity but not energy expenditure in obesity. Metabolism 1997;46:123-129.
4. Lear SA, Humphries KH, Kohli S, Frohlich JJ, Birmingham CL, Mancini GBJ. Visceral adipose tissue, a potential risk factor for carotid atherosclerosis. Stroke 2007;38:2422-2429.
5. Wildman RP, Hunter P, Reynolds K, et al. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). Arch Intern Med 2008;168:1617-1624.
6. Handy O, Porramatikul S, Al-Oraizi E. Metabolic obesity: the paradox between visceral and subcutaneous fat. Curr Diabetes Rev 2006;2:367-373.
7. Kelley DE, Goodpaster BH, Storlien L. Muscle triglyceride and insulin resistance. Annu Rev Nutr 2002;22:325-346.
8. Kelley DE, Goodpaster BH. Skeletal muscle triglyceride. An aspect of regional adiposity and insulin resistance. Diabetes Care 2001;24:933-941.
9. Goodpaster BH, Thaete FL, Kelley DE. Thigh adipose tissue distribution is increased in obesity and decreased by weight loss. Metabolism 2000;49:467-472.
10. Kim SK, Park SW, Hwang JI, Lee YK, Cho YW. High fat stores in ectopic compartments in men with newly diagnosed type 2 diabetes: an anthropometric determinant of carotid atherosclerosis and insulin resistance. Int J Obes (Lond) 2010;34:105-110.
11. van der Meer IM, Bots ML, Hofman A, del Sol AI, van der Kuip DA, Witteman JC. Predictive value of noninvasive measures of atherosclerosis for incident metabolic syndrome in postmenopausal women. Circulation 2001;103:513-519.
12. Dube MC, Lemieux S, Piche ME, et al. Relationship of mid-thigh adiposity to the metabolic syndrome in postmenopausal women. Metab Syndr Relat Disord 2010;8:365-372.
13. Dube MC, Lemieux S, Piche ME, et al. The contribution of visceral adiposity and mid-thigh fat-rich muscle to the metabolic profile in postmenopausal women. Obesity (Silver Spring) 2011;19:953-959.
14. Scherrer U, Randin D, Tappy L, Vollenweider P, Jequier E, Nicod P. Body fat and sympathetic nerve activity in healthy subjects. Circulation 1994;89:2634-2640.
15. Grassi G, Dell'Oro R, Facchini A, Quarti Trevano F, Bolla GB, Mancia G. Effect of central and peripheral body fat distribution on sympathetic and baroreflex function in obese normotensives. J Hypertens 2004;22:2563-2569.
16. Muscelli E, Emdin M, Natali A, et al. Autonomic and hemodynamic responses to insulin in lean and obese humans. J Clin Endocrinol Metab 1998;83:2084-2090.
17. Szajzel J, Golay A, Makoundou V, et al. Impact of body fat mass extent on cardiac autonomic alterations in women. Eur J Clin Invest 2009;39:649-656.
18. Tsuji H, Larson MG, Venditti FJ Jr, et al. Impact of reduced heart rate variability on risk for cardiac events. The Framingham heart study. Circulation 1996;94:2850-2855.
19. Sajadieh A, Nielsen OW, Rasmussen V, Hein HO, Abedini S, Hansen JF. Increased heart rate and reduced heart-rate variability are associated with subclinical inflammation in middle-aged and elderly subjects with no apparent heart disease. Eur Heart J 2004;25:363-370.
20. Bemtson GB, Bigger J, Eckberg D, et al. Heart rate variability: origins, methods, and interpretive caveats. Psychophysiology 1997;34:623-648.
21. Task force of the European society of cardiology and the North American society of pacing and electrophysiology. Heart rate variability standards of measurement, physiological interpretation, and clinical use. Eur Heart J 1996;17:354-381.
22. Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, Cohen RJ. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. Science 1981;213:220-222.
23. Akselrod S, Gordon D, Madwed JB, Snidman NC, Shannon DC, Berger AC. Heart rate variability: methods of physiological measurement and some of its applications to pathophysiology. Circulation 1981;65:411-416.