Subcutaneous adipose tissue distribution and serum lipid/lipoprotein in unmedicated postmenopausal women: A B-mode ultrasound study

Takashi Abe, Vickie Wong, Zachary W. Bell, Robert W. Spitz, Scott J. Dankel, Jeremy P. Loenneke

1 Department of Health, Exercise Science, & Recreation Management, Kevser Ermin Applied Physiology Laboratory, The University of Mississippi, University, MS 38677, USA

2 Department of Health and Exercise Science, Rowan University, Glassboro, NJ 08028, USA

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Contact Information: Takashi Abe, PhD, 224 Turner Center, University, MS 38677, USA, Phone: +1 (662) 915-5521, Fax: +1 (662) 915-5525, E-mail: t12abe@gmail.com

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Abstract

Background: It has been observed that gluteal-femoral adipose tissue has a protective effect against risk factors for cardiovascular disease but has not yet been concluded how different evaluation methods of fat distribution affect the results.

Methods: To test the hypothesis that B-mode ultrasound-measured subcutaneous adipose tissue distribution is associated with cardiovascular risk factors, 326 Japanese unmedicated postmenopausal women aged 50-70 years were analyzed. Subcutaneous adipose tissue thickness at 6 sites (anterior and posterior aspects of trunk, upper-arm, and thigh) and serum total (TC) and high-density lipoprotein cholesterol (HDLC) was measured, and a ratio of HDLC to TC (HDLC/TC) was calculated. We used Bayesian linear regression with 4 separate models with each model predicting HDLC/TC.

Results: Our first model provided evidence for an inverse correlation ($r = -0.23$) between ultrasound measured body fat (6 site measurement) and HDLC/TC. The second model noted evidence for an inverse correlation between trunk fat and HDLC/TC and found evidence for the null with respect to the correlation between thigh fat and HDLC/TC. Therefore, we added thigh fat to the null model to produce Distribution Model 2. Within this model, we noted an inverse correlation ($r = -0.353$) between trunk fat and HDLC/TC. Our last model determined that within the trunk fatness, the abdominal area (anterior trunk) was a larger predictor than the subscapular site (posterior trunk).

Conclusion: These results support the evidence that ultrasound-measured abdominal subcutaneous adipose tissue thickness is a non-invasive predictor for monitoring the risk for dyslipidemia in postmenopausal women.
Keywords: body fat distribution, high-density lipoprotein cholesterol, subcutaneous fat thickness, ultrasonography

Introduction

Obesity is increasingly prevalent in the aging process and excess body fat is an independent predictor of cardiovascular disease and its risk factors [1, 2]. However, where that fat is distributed on the person may ultimately dictate the risk of disease. For example, gluteal-femoral adipose tissue has specific functional properties that are correlated with a reduced cardiometabolic risk profile in childhood [3, 4] and middle-aged and older adults [5-13], but has not yet been concluded how different evaluation methods of fat distribution affect the results. Total and regional (abdominal and gluteal-femoral) adipose tissue can be estimated by dual-energy X-ray absorptiometry (DXA) with a whole-body scan and by computed tomography (CT) and magnetic resonance imaging (MRI) based on a single abdominal scan. However, these imaging techniques are not always available for use and ultrasonography may offer another imaging technique of assessment with virtually no inherent risks associated with other measurements relying on radiation (albeit low levels of radiation).

In the human body, approximately 85% of total adipose tissue is subcutaneous regardless of lean or obese, while the remaining 15% constitutes intra-abdominal fat, including both visceral and retroperitoneal adipose depots [14, 15]. Therefore, subcutaneous adipose tissue distribution of the body, i.e. fat accumulation in the lower-body (gluteal-femoral region) or upper-body (abdominal region), may have a large impact to classification of body fat distribution pattern,
which may associate with lipid and lipoprotein metabolism and cardiovascular disease risk [16]. B-mode ultrasound has been used to assess body composition including measurements of the different subcutaneous adipose tissue depots. Previously, measurement of visceral fat has attracted heavy attention, and ultrasound has been used for evaluating an index of visceral fat accumulation such as preperitoneal fat thickness or intra-abdominal thickness [17-19]. Hence, it is unknown whether ultrasound-measured subcutaneous adipose tissue distribution associates with cardiovascular risk factors in healthy adults. Thus, the aim of this study was to examine the effect of ultrasound-measured subcutaneous adipose tissue distribution on selected cardiovascular risk factors in postmenopausal women.

**Methods**

**Participants**

Participants were recruited from a community-based general health examination. A total of 672 Japanese women were screened for this study; those exhibiting a history of cardiac, pulmonary, renal or malignant disease and those who were currently taking medications were excluded by physicians. Because it was very difficult to measure adipose tissue thickness by ultrasound, the participants with higher body mass index (>30 kg/m²) were excluded. Pre-menopausal women (amenorrhea of >6 months) were also excluded. As a result, a total of 326 unmedicated women aged 50-70 years (mean age 59.0 years) were used for data analysis (Table 1). Prior to obtaining written informed consent, a written description of the purpose of the study and its safety was distributed to potential participants, along with a lifestyle questionnaire. The study was conducted according to the Declaration for Helsinki and was approved by the Ethics Committee for Human Experiments of Tokyo Metropolitan University, Japan.
Subcutaneous adipose tissue thickness

Subcutaneous adipose tissue layer (AT) thickness was measured according to a previous study [20, 21], using a B-mode ultrasound apparatus (Aloka SSD-500, Tokyo, Japan). For each segment (trunk, upper arm, and thigh), two sites were selected for covering anterior and posterior surface of the body. The measurements were taken while the participants stood with their elbows and knees extended. The sonographer applied a water-soluble transmission gel to each measurement site, and a 5-MHz ultrasound probe was placed on the measurement site without depressing the dermal surface. The skin surface and subcutaneous AT-muscle interface were identified and the distance between the two interfaces was recorded as subcutaneous AT thickness. All ultrasound measurements were performed by a single observer. Test-retest reliability measures of intraclass correlation coefficient (ICC_{3,1}), standard error of measurement (SEM), and minimal difference (MD) was determined from middle-aged and older adults (n=12) for anterior (0.972, 0.05, and 0.10 cm) and posterior (0.959, 0.06, and 0.17 cm) upper-arm, anterior (0.993, 0.08, and 0.22 cm) trunk, and anterior (0.984, 0.06, and 0.15 cm) and posterior (0.994, 0.04, and 0.08 cm) thigh.

Anthropometry

Standing height (in m) was measured barefoot using a stadiometer (YL-65, Yagami Inc, Nagoya, Japan). Body mass (in kg) was measured with minimal clothing using a calibrated digital weight scale (WB-150, Tanita, Tokyo, Japan), and body mass index (kg/m^2) was calculated as body mass divided by height squared. Waist and hip circumferences were measured using a flexible tape measure during normal breathing, and waist to hip ratio was calculated.

Total and high-density lipoprotein cholesterol
A venous blood sample was obtained from the antecubital vein after fasting overnight 12 h. The blood was drawn into a vacutainer tube containing no anticoagulants and centrifuged for 20 min at 1500x after clotting. Serum total cholesterol (TC) was measured by the enzymatic technique (Eiken Chem. Co., Tokyo), and high-density lipoprotein cholesterol (HDLC) was measured by the same procedure after separation of HDL fraction. HDLC to TC ratio was calculated to estimate an index of dyslipidemia because the HDLC/TC is a useful and simple index of ischemic heart disease [22].

Lifestyle questionnaire

Selected lifestyle information included weekly alcohol consumption [none, light (less and 2 drinks per week), or moderate to high (more than 2 drinks per week)], smoking status (current, former, or never), and regular sport activity (<1 time a week or ≥1 time a week). Participants self-reported medical conditions and had assessment of prescription medication use.

Statistical analysis

We used Bayesian linear regression with 4 separate models with each model predicting HDLC/TC. A default JZS prior of $r=0.354$ was used for each model. The first model was used to analyze the effects of the sum of 6 sites to assess the impact of global fatness on HDLC/TC. The next two models included distribution of fat to determine the impact of fat location on HDLC/TC. Distribution model 1 included trunk fat and leg fat and model 2 included trunk fat with leg fat added to the null model. The second distribution model was included because leg fat was not meaningfully contributing to the overall model. The last model was investigating the individual components of the trunk fat model, to determine which variable was having the greatest impact. Bayes Factors ($BF_{10}$) were used to quantify evidence for ($BF_{10} \leq 0.33$) or against
(BF<sub>10</sub> ≥ 3.0) the null hypothesis. All statistical analyses were conducted using JASP version 0.9.0.1.

**Results**

The prevalence of current and former smokers was 3% (n=9). Thus, approximately 97% of the participants had no smoking experience. Only 8% (n=25) of the participants had habitual alcohol consumption. The prevalence of regular sports activity (≥1 time a week for at least one year) was 18% (n=58).

To address the purpose of this study, we first analyzed two separate models (total fat model vs. distribution of fat model). Our total fat model provided evidence for an inverse correlation (r=-0.23) between ultrasound measured body fat (6 site measurement) and HDLC/TC. Our second model looked at distribution of fat by separating the body into two regions of interest; the trunk (abdominal plus subscapular fat) and the thigh (anterior plus posterior fat). We noted evidence for an inverse correlation between trunk fat and HDLC/TC and found evidence for the null with respect to the correlation between thigh fat and HDLC/TC. Therefore, we added thigh fat to the null model to produce Distribution Model 2. Within this model, we noted an inverse correlation (r= -0.353) between trunk fat and HDLC/TC. This model would be interpreted as follows:

\[
\text{HDLC/TC} = -0.149X + 25.5, \text{ where } X \text{ is the difference from the mean in mm with respect to trunk fat.}
\]

Simply stated, for every 1 mm increase in trunk fat, there is a predicted 0.149 decrease in the HDLC/TC. Our last model determined that within the trunk fatness, the abdominal area was a larger predictor than the subscapular site.

**Discussion**
The primary findings of the current study were that trunk fat assessed by the ultrasound (Figure 1), especially the abdominal region, showed an inverse relationship with serum HDLC/TC. However, there was no evidence of an association between ultrasound-measured thigh subcutaneous adipose tissue thickness and HDLC/TC.

The results in this study indicate that abdominal subcutaneous adipose tissue thickness measured by ultrasound may provide a non-invasive predictor for risk factors associated with dyslipidemia in postmenopausal women. Our findings support the results obtained using DXA scans in adolescents [3] and middle-aged and older adults [6, 9, 11]. For instance, Van Pelt et al. [11] examined whether DXA-derived trunk fat mass is a good predictor of dyslipidemia and insulin resistance in healthy postmenopausal women (n=166). The authors found that there was an inverse correlation ($r=-0.40$, $p<0.001$) between trunk fat mass and serum HDLC. Similarly, Boorsma et al. [6] reported significant associations between DXA-derived trunk fat mass and HDLC in middle-aged and older men ($r=-0.50$, $p<0.01$) and women ($r=-0.55$, $p<0.01$) after adjusting for other body compartments and age. Recently, Hetherington-Rauth et al. [3] investigated the relationships between DXA-derived body fat mass distribution and cardiometabolic risk factors in Hispanic girls (n=232). They reported that total body and trunk adiposity were significantly related with HDLC ($r=-0.37$ and $r=-0.38$, both $p<0.001$) and triglycerides ($r=0.32$ and $r=0.29$, both $p<0.001$) after adjusting for maturation. The results from the present and previous studies together suggest that abdominal adiposity is an associative factor of cardiovascular disease risk. Of note, trunk fat is comprised of more than just visceral fat but the DXA is unable to differentiate directly between subcutaneous and visceral fat mass in the abdominal region. Although adipose tissue thickness by ultrasound has a similar limitation, the ultrasound has the following advantages: 1) portable, 2) no radiation exposure, and 3) often can
be assessed at a lower cost (compared with DXA and MRI). However, further research is needed on whether ultrasound measurements of abdominal adipose tissue thickness can be used as a routine clinical examination.

In the present study, our results showed that accumulating thigh subcutaneous adipose tissue may not associate (favorably or negatively) with a known risk factor for dyslipidemia in postmenopausal women. Previous cross-sectional studies have proposed that subcutaneous thigh fat is associated with more favorable levels of lipids and glucose in adolescents [3, 4] and adults [9-11, 23, 24]. However, other studies did not observe this favorable association [6, 7, 24]. Our findings support the results of these previous studies and suggest that this site not be used as a predictor of HDLC/TC in postmenopausal women.

Several limitations to this study warrant mention. First, the participants were non-obese Japanese women between 50-70 years in age who were not taking medication. Therefore, it is unclear if similar results can be seen in other ethnicities and different age groups. Second, our findings were only women, so it is unknown if results pertain to men who have different fat distributions. Last, we did not consider other cardiovascular risk factors such as insulin resistance, blood pressure, or markers of inflammation.

In conclusion, our linear regression models provided evidence that there was an inverse correlation between ultrasound-measured total body (6 sites) subcutaneous fat thickness and HDLC/TC ratio. This corroborates previous findings which found an association between body fatness and risk for cardiovascular disease. The next model noted evidence for an inverse correlation between trunk fat and HDLC/TC and found evidence for the null with respect to the correlation between thigh fat and HDLC/TC. This supports previous findings which suggest that where the fat is located may be an important factor to consider when assessing risk. The last
model determined that within the variable of trunk fatness, the abdominal area was the largest predictor, suggesting that abdominal obesity (rather than subscapular fat) is driving the relationship with HDLC/TC. These results support the evidence that ultrasound measurement of abdominal subcutaneous fat thickness is a non-invasive predictor for monitoring the risk for dyslipidemia in postmenopausal women.

**Author contributions**: Conceived and designed the study: TA, VW, ZWB, RWS, SJD, and JPL. Performed the data collection: TA. Analyzed the data: TA, SJD, and JPL. Wrote the manuscript: TA. Reviewed and critically revised the manuscript: VW, ZWB, RWS, SJD, and JPL. All authors approved the final version of the manuscript.

**Conflict of interest**: None of the authors had financial or personal conflict of interest with regard to this study.

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Figure Legend

Figure 1

The inverse association between trunk fat (mm) and HDLC/TC (%). Each dot represents a single individual (n=326).
Table 1 – Descriptive characteristics of the participants in this study

| n = 326 | Mean (SD) |
|---------|-----------|
| Age, yrs | 59 (5) |
| Body mass, kg | 51 (7) |
| Height, cm | 148 (5.2) |
| Body Mass Index, kg/m^2 | 23.2 (2.7) |
| Total cholesterol, mg/dl | 198.4 (33.2) |
| High-density lipoprotein cholesterol, mg/dl | 49.5 (10.3) |
| HDLC / TC, % | 25.5 (6.0) |
| Sum of 6 sites, mm | 82.4 (19.7) |
| Trunk (Abdominal + Subscapular), mm | 42.1 (14.1) |
| Thigh (Anterior + Posterior), mm | 21.4 (5.5) |
| Abdominal, mm | 28.8 (10.8) |
| Subscapular, mm | 13.2 (4.5) |
| Anterior thigh, mm | 11.6 (3.2) |
| Posterior thigh, mm | 9.8 (3.0) |

HDLC, high-density lipoprotein cholesterol; TC, total cholesterol
Table 2 - Bayesian linear regression with 4 separate models with each model predicting HDLC/TC

| HDL/TC (25.5) | Models |
|---------------|--------|
| **Total Fat Model** | |
| Coefficient | $\beta$ | 95% Credible Interval | BF$_{10}$ | $R^2$ |
| Null | | | | |
| Sum of 6 | -0.068 | -0.100, -0.036 | 558.405 | 0.053 |
| **Distribution Model 1** | |
| Coefficient | $\beta$ | 95% Credible Interval | BF$_{10}$ | $R^2$ |
| Null | | 1.000 | 0.000 |
| Trunk Fat | -0.149 | -0.195, -0.104 | 990509.454 | 0.096 |
| Leg Fat | 0.184 | 0.07, 0.298 | 0.281 | 0.005 |
| Trunk Fat + Leg Fat | | | 2.058e +7 | 0.125 |
| **Distribution Model 2** | |
| Coefficient | $\beta$ | 95% Credible Interval | BF$_{10}$ | $R^2$ |
| Null (+ Leg Fat) | | 1.000 | 0.005 |
| Trunk Fat | -0.149 | -0.195, -0.104 | 7.328e +7 | 0.125 |
| **Trunk Model** | |
| Coefficient | $\beta$ | 95% Credible Interval | BF$_{10}$ | $R^2$ |
| Null | | 1.000 | 0.000 |
| Abdominal Fat | -0.158 | -0.233, -0.116 | 1.511 e+6 | 0.099 |
| Subscapular Fat | -0.042 | -0.141, 0.072 | 165.681 | 0.045 |
| Abdominal Fat + Subscapular Fat | | | 242578.725 | 0.100 |