

RESEARCH ARTICLE

Relationship between adiponectin and intramuscular fat content determined by ultrasonography in older adults

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Abstract

Age-associated intramuscular adipose tissue (IntraMAT) deposition induces the development of insulin resistance and metabolic syndrome. However, the relationship between IntraMAT and biochemical parameters in older adults remains unclear. The purpose of this study, therefore, was to elucidate the relationship between adiponectin and echo intensity-estimated IntraMAT using ultrasonography in normal-weight older adults (men 9, women 13) and examine biochemical parameters. Blood tests were performed to determine fasting levels of glucose, insulin, hemoglobin A1c, total cholesterol (Total-C), high-density-lipoprotein cholesterol, low-density-lipoprotein cholesterol (LDL-C), free fatty acid, triglycerides (TGs), adiponectin, leptin, high-sensitivity C-reactive protein, and high-sensitivity tumor necrosis factor, and homoeostasis model assessment index of insulin resistance (HOMA-IR). Mean gray-scale echo intensity was calculated as the IntraMAT index of the vastus lateralis. Waist circumference was measured at the level of the navel as the visceral adipose tissue (VAT) index. Echo intensity was significantly inversely correlated with adiponectin or LDL-C, and that was significantly positively correlated with TG. Adiponectin level was inversely correlated with waist circumference. Partial correlation analysis with waist circumference as the control variable revealed that adiponectin was inversely correlated with echo intensity, independent of waist circumference, whereas no such correlation was observed after controlling for LDL-C and TG levels. When biochemical parameters were grouped in the principal component analysis, among men, Total-C, insulin, and HOMA-IR or hemoglobin A1c, and high-sensitivity tumor necrosis factor- α were grouped with the same distribution for factors 1 and 2. Among women, glucose, insulin, HOMA-IR, and Total-C or TGs were grouped with the same distribution for factors 1 and 2. These data suggest that adiponectin level is related to IntraMAT content, independent of VAT in normal-weight older

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adults. The dynamics of adiponectin might not be similar to those of other circulating biochemical parameters in older men and women.

Introduction

The lipotoxic effects of over-nutrition and corresponding increased incidence of obesity and type 2 diabetes have become a critical public health problem, particularly in developed countries [1]. Although triglycerides (TGs) are redistributed from hepatocytes and muscle cells to subcutaneous adipose tissue with advancing age, TGs can accumulate in adipose tissue in harmful ectopic locations (e.g., in the abdomen, liver, heart, and skeletal muscle). Such ectopic adipose deposits in organs can cause metabolic dysfunction [2]. Ectopic adipose tissue deposition in skeletal muscle is particularly important in humans because skeletal muscle is the largest organ. Muscular ectopic adipose tissue consists of extra-myocellular adipocytes and is designated intramuscular adipose tissue (IntraMAT), which accumulates as adipose tissue within a single muscle. Excessive IntraMAT accumulation may also lead to the development of insulin resistance and other features of metabolic syndrome [3]. Moreover, IntraMAT infiltration that occurs with aging can induce dysfunctions such as loss of muscle strength in older adults [4], thus increasing the risk of falls and fractures and the chance an individual could become bedridden.

Ultrasound imaging is a widely available, non-invasive imaging technique frequently used for the quantitative measurement of IntraMAT. Echo intensity–estimated IntraMAT content agrees with that determined using magnetic resonance imaging (MRI) and ^1H -magnetic resonance spectroscopy, which are considered gold-standard methods for IntraMAT quantification. However, muscle echo intensity measures not only IntraMAT but also connective tissue [5]. Many IntraMAT studies have investigated the quadriceps femoris component, particularly the vastus lateralis (VL), using muscle biopsy or imaging diagnostic methods [6]. Because the quadriceps femoris muscle is susceptible to age-related defects and the VL occupies approximately 30% of the quadriceps femoris in young adults [7], this study used ultrasound to quantify the IntraMAT of the VL.

Previous studies have demonstrated a relationship between IntraMAT and various blood biochemistry parameters in older adults. For example, our previous MRI study showed that IntraMAT in the thigh muscles, including the quadriceps femoris, hamstrings, and adductors, is correlated with levels of blood lipoproteins (TGs or low-density-lipoprotein cholesterol [LDL-C]) [8]. However, the relationship between IntraMAT in VL and adiponectin remains unclear. Adiponectin and leptin play roles in maintaining energy homeostasis and regulating glucose and lipid metabolism. Leptin is an adipose-derived adipokine hormone that regulates fat storage and plays a role in regulating food intake as a satiety signal [9]. Leptin levels are positively correlated with body composition (body mass index, weight, and waist circumference) as well as intermuscular adipose tissue (IMAT) [10]. An animal study demonstrated that leptin induces an increase in fatty acids in skeletal muscle and a decrease in TG accumulation [11]. Adiponectin has attracted considerable research attention because of its antidiabetic and anti-atherogenic effects and is expected to become a therapeutic target in the treatment of type 2 diabetes, metabolic syndrome, and cardiovascular diseases [12].

Adiponectin plays a role in decreasing the accumulation of ectopic fat such as visceral adipose tissue (VAT), intrahepatic lipids, intramyocellular lipids (IMCLs), and IMAT (which includes both IntraMAT and adipose tissue of the subfascia between muscles) [10, 13–15].

Hence, adiponectin may also be closely related to the accumulation of IntraMAT in a manner similar to that of VAT, IMCL, and IMAT; however, the relationship between adiponectin and IntraMAT remains poorly understood. Adiponectin could play a role in determining healthy longevity in older adults. For example, the genotype and allele frequencies of the rs1501299 single-nucleotide polymorphism in the adiponectin gene was found to be associated with longevity in men [16], and higher adiponectin levels were associated with lower risk of type 2 diabetes [17]. The purpose of this study was primarily to determine whether adiponectin is correlated with echo intensity–estimated IntraMAT in non-obese older men and women and secondarily to and examine biochemical parameters. We hypothesized that adiponectin levels are inversely correlated with IntraMAT.

Material and methods

Participants

We recruited participants in the age range of 60 to 80 years at local exercise clubs designed specifically for older adults. Exclusion criteria were explained to potential participants in the local exercise clubs. Participant eligibility was assessed using a questionnaire (n = 27), which resulted in the exclusion of a total of 5 participants for the following reasons: did not meet inclusion criteria (n = 3), or were excluded for other reasons (n = 2). No participants who were recruited for the study declined to participate. Finally, a total of 22 participants (9 men, 13 women) between the age of 62 and 77 years were decided for the study. All participants were living independently. The clinical history of each participant was assessed using a questionnaire. Participants with a history of heart disease (myocardial infarction, angina pectoris, cardiac insufficiency), cerebrovascular disease (cerebral infarction, hemorrhage), extreme hypertension (systolic blood pressure ≥ 180 mm Hg; diastolic blood pressure ≥ 110 mm Hg), neuromuscular disorders, or limb surgery were excluded. Of the three participants with type 2 diabetes, one was receiving mitiglinide calcium hydrate, one glibenclamide and mitiglinide calcium hydrate/voglibose, and one metformin. All participants provided written informed consent prior to enrollment. The study was approved by the Ethics Committee of the Graduate School of Medicine of Nagoya University, and all protocols were in accordance with the guidelines of the Declaration of Helsinki. A portion of the data was previously reported by Hioki et al. [18].

Study protocol

The study protocol included measurement of body composition, ultrasonography, and blood tests in the morning for all participants. Participants visited the laboratory twice to ensure reproducibility of the ultrasonography measurements, with an interval of approximately 1 week between measurements. Blood tests and dietary habit assessments were performed on different dates.

Anthropometric characteristics

According to the U.S. National Institutes of Health recommendations, waist circumference was measured at the level of the navel as the VAT index [19]. Participants were advised to take relaxed and natural breaths before the actual waist measurement to minimize the inward pull of the abdominal contents during measurement [20]. Hip circumference was taken as the greatest circumference of the pelvis.

Biochemical parameters

Blood tests were performed to determine the fasting levels of glucose, insulin, hemoglobin A1c, total cholesterol (Total-C), high-density-lipoprotein cholesterol (HDL-C), low-density-lipoprotein cholesterol (LDL-C), free fatty acid, triglycerides (TGs), adiponectin, leptin, high-sensitivity C-reactive protein (hs-CRP), and high-sensitivity tumor necrosis factor (hs-TNF α). Homoeostasis model assessment index of insulin resistance (HOMA-IR) was calculated as fasting glucose (mg/dL) \times fasting insulin (μ IU/mL)/405 [21].

Ultrasonography

Participants visited the laboratory twice for ultrasonography testing and re-testing. Ultrasonography measurements were performed by a single investigator (MH) using a LOGUQ e instrument (GE Healthcare, Boston, MA). B-mode with a multi-frequency linear transducer (8.0–12.0 MHz) was used. The transducer frequency and gain were adjusted to 8.0 MHz and 80 dB, respectively. Scanning depth was set to 8.0 cm. Participants were assessed in the prone position with the leg fully extended and relaxed. Ultrasonographic images of the VL (lateral) were obtained at the mid-thigh between the greater trochanter and lateral condyle of the femur. Five images were collected, and all images were stored in the ultrasonographic device in DICOM format for future analysis. Ultrasonographic images were allocated serial numbers to prevent individual identification before subsequent analysis.

Intramuscular fat measurement by echo intensity

As an index that reflects IntraMAT, intramuscular fat content was measured based on ultrasonography echo intensity. A region of interest (ROI) was selected in the image of each VL, including as much of the muscle as possible, and bone and surrounding fascia were excluded. The smoothing function was applied to decrease noise in the ROI. Mean echo intensity of the ROI was calculated (8-bit resolution, resulting in a value between 0 and 255; scale: black = 0; white = 255). Mean echo intensity within the ROI in five images was measured for the VL, and five images with highest and lowest echo intensity values were excluded to minimize variations resulting from technical errors. The echo intensity of three remaining images was averaged for future analysis.

Muscle thickness and lateral subcutaneous thickness

Muscle and subcutaneous tissue thicknesses were measured with electronic calipers placed at the middle of the ultrasound image. Muscle thickness of the VL was measured between the superficial and ventral muscle fascia, and subcutaneous tissue thickness was measured between the uppermost part of the skin and the superficial fascia of the muscle at the lateral site. Three images were scanned for each part of the thigh, and these images were averaged for future analysis.

Physical activity levels and dietary habits

Time spent performing physical activities and total steps taken over a 10-day period were estimated from three-dimensional ambulatory accelerometer (Lifecorder; Suzuken Co., Nagoya, Japan) records, and intensity was categorized as light (<3.0 metabolic equivalents [METs]), moderate (3.0–6.0 METs), or vigorous (>6.0 METs). Physical activity level was calculated as the product of METs and time spent performing physical activities (MET h) at each intensity level (see previous studies [22, 23] for additional details). The dietary habits of the participants were assessed by a nutritionist. Dietary habits were estimated using a food frequency

questionnaire [24], which included 39 food and beverage items. The questionnaire asked participants about their average intake and frequency of consumption of each food. Consumption was categorized as small, medium, or large. Five categories were used to describe consumption frequency (almost always, often, sometimes, rarely, or never).

Statistical analyses

Differences between the male and female participants were evaluated using the unpaired Student's *t* test. Differences in biochemical parameters (glucose, insulin, HbA1c, Total-C, HDL-C, LDL-C, FFAs, TGs, adiponectin, leptin, hs-CRP, hs-TNF α , and HOMA-IR) were then assessed using principal component (PC) analysis (PCA). To simplify interpretation of the biochemical profile data for men or women, we used PCA because it best explained the variance of the data. Data were arranged into a $P \times N$ matrix A, where $P = 13$ biochemical parameters, and $N = 9$ or 13 (i.e., number of subjects). The covariance matrix B was calculated from the data A, and the PC weightings were determined from the eigenvectors of covariance matrix B. Extracted components with eigenvalues >1 were considered significant. Similarity of biochemical parameters was evaluated for men and women by calculating the PC weighting of the first (PC1) and second (PC2) PCs. A PC weighting >0.8 was considered significant [25]. The relationships between echo intensity, adiponectin, leptin, body composition, and biochemical parameter values were examined using Pearson's correlation analysis. When a significant correlation between adiponectin or echo intensity and body composition or biochemical parameter values was found, partial correlations adjusted for these factors were used to quantify the correlation between adiponectin and echo intensity. Ultrasound test-retest data (the first and second measurements) and the interrater reliability of echo intensity measurements were analyzed using coefficients of variation (CVs) and 1-way intra-class correlation coefficients (ICCs). All statistical analyses were performed using SPSS software, version 24.0 (SPSS Inc., Chicago, IL). Data are presented as mean \pm SD. $P < 0.05$ was considered significant.

Results

The physical characteristics, blood biochemistry data, and skeletal muscle parameters of the participants are provided in Table 1. Height and weight were higher in men than women. The percentages of body fat, leptin, and lateral subcutaneous thickness were higher in women than men.

PCA identified 4 factors (84.4%) in men and 5 factors (88.5%) in women. Fig 1 shows a significant PC weighting for factor 1 in the positive Total-C or negative insulin and HOMA-IR (28.2%) and for factor 2 in the positive HbA1c or negative hs-TNF α (26.1%) among the men, and for factor 1 in positive glucose, insulin, and HOMA-IR or negative Total-C (42.2%) and for factor 2 in positive TGs among the women (16.6%).

The physical activity and dietary habit characteristics of the participants are summarized in Table 2.

The CV and ICC of the first and second echo intensity measurements were $5.3 \pm 3.7\%$ (mean \pm SD) and 0.88, respectively.

Table 3 shows Pearson correlation coefficients for echo intensity, adiponectin, leptin, and body composition or biochemical parameter values. Echo intensity was correlated with Total-C, HDL-C, LDL-C, TGs, and muscle thickness but not other body composition or blood biochemistry factors. Adiponectin was correlated with waist circumference and lateral subcutaneous thickness but not other body composition or blood biochemistry factors. Leptin was correlated with body mass index (BMI), percent body fat, and hs-CRP but not other body composition or blood biochemistry factors.

Table 1. Participant characteristics.

	Men	Women	P-value
No. of participants	9	13	
Physical characteristics			
Age (years)	69.4 ± 4.3	68.2 ± 4.6	0.54
Height (cm)	167.6 ± 7.2	151.5 ± 4.2	0.00
Weight (kg)	62.1 ± 7.9	51.5 ± 7.6	0.00
BMI (kg·m ⁻²)	22.0 ± 1.6	22.4 ± 2.7	0.70
Waist circumference (cm)	83.9 ± 4.2	81.0 ± 9.5	0.42
Hip circumference (cm)	92.7 ± 4.0	91.4 ± 5.6	0.57
WHR	0.9 ± 0.0	0.9 ± 0.1	0.39
Fat (%)	23.7 ± 2.5	33.6 ± 4.8	0.00
Blood biochemistry			
Fasting glucose (mg/dL)	103.4 ± 24.2	90.4 ± 10.5	0.09
Fasting insulin (μIU/mL)	8.0 ± 7.5	5.6 ± 2.6	0.28
HbA1c (%)	5.8 ± 0.4	6.0 ± 0.4	0.42
Total-C (mg/dL)	220.1 ± 31.6	214.8 ± 29.5	0.68
HDL-C (mg/dL)	60.6 ± 15.1	60.5 ± 14.5	0.98
LDL-C (mg/dL)	133.4 ± 26.3	130.8 ± 22.5	0.80
FFAs (μEq/L)	728.7 ± 203.1	731.5 ± 223.4	0.97
TGs (mg/dL)	111.3 ± 89.4	87.1 ± 38.3	0.39
Adiponectin (μg/mL)	10.1 ± 4.7	14.0 ± 10.7	0.32
Leptin (ng/mL)	4.3 ± 1.1	8.1 ± 4.0	0.01
hs-CRP (ng/mL)	741.1 ± 772.4	4678.5 ± 13403.6	0.39
hs-TNFα (pg/mL)	1.6 ± 0.9	1.2 ± 0.3	0.20
HOMA-IR	2.1 ± 2.0	1.3 ± 0.7	0.19
Skeletal muscle profiles			
Echo intensity (a.u.)	70.3 ± 5.9	70.0 ± 6.7	0.92
Muscle thickness of VL (cm)	2.0 ± 0.2	1.7 ± 0.4	0.06
Lateral subcutaneous thickness (cm)	0.4 ± 0.1	0.7 ± 0.3	0.00

Value are mean ± SD. BMI, body mass index; FFAs, free fatty acids; HbA1c, hemoglobin A1c; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homoeostasis model assessment index of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; hs-TNFα, high-sensitivity tumor necrosis factor-α; LDL-C, low-density-lipoprotein cholesterol; TGs, triglycerides; Total-C, total cholesterol; VL, vastus lateralis; WHR, waist-to-hip ratio. Only one male subject had a hs-CRP value <50 ng/mL; therefore, the value was taken as 50 ng/mL.

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The relationship between echo intensity and adiponectin is shown in Fig 2. Echo intensity was significantly correlated with adiponectin but not leptin ($r = 0.04$, $P = 0.86$).

Table 4 shows partial correlation coefficients between adiponectin and echo intensity, with waist circumference, TGs, LDL-C, lateral subcutaneous thickness, and muscle thickness of VL as control variables. After controlling for waist circumference, a significant inverse association between adiponectin and echo intensity remained, whereas after controlling for TGs, LDL-C, lateral subcutaneous thickness, and muscle thickness, this correlation was not observed.

Discussion

In the present study, we demonstrated that adiponectin level was inversely correlated with echo intensity-estimated IntraMAT in the VL in normal-weight older men and women (age range 62–77 years). After controlling for waist circumference (VAT index), there remained a significant inverse association between adiponectin level and echo intensity. Furthermore, our

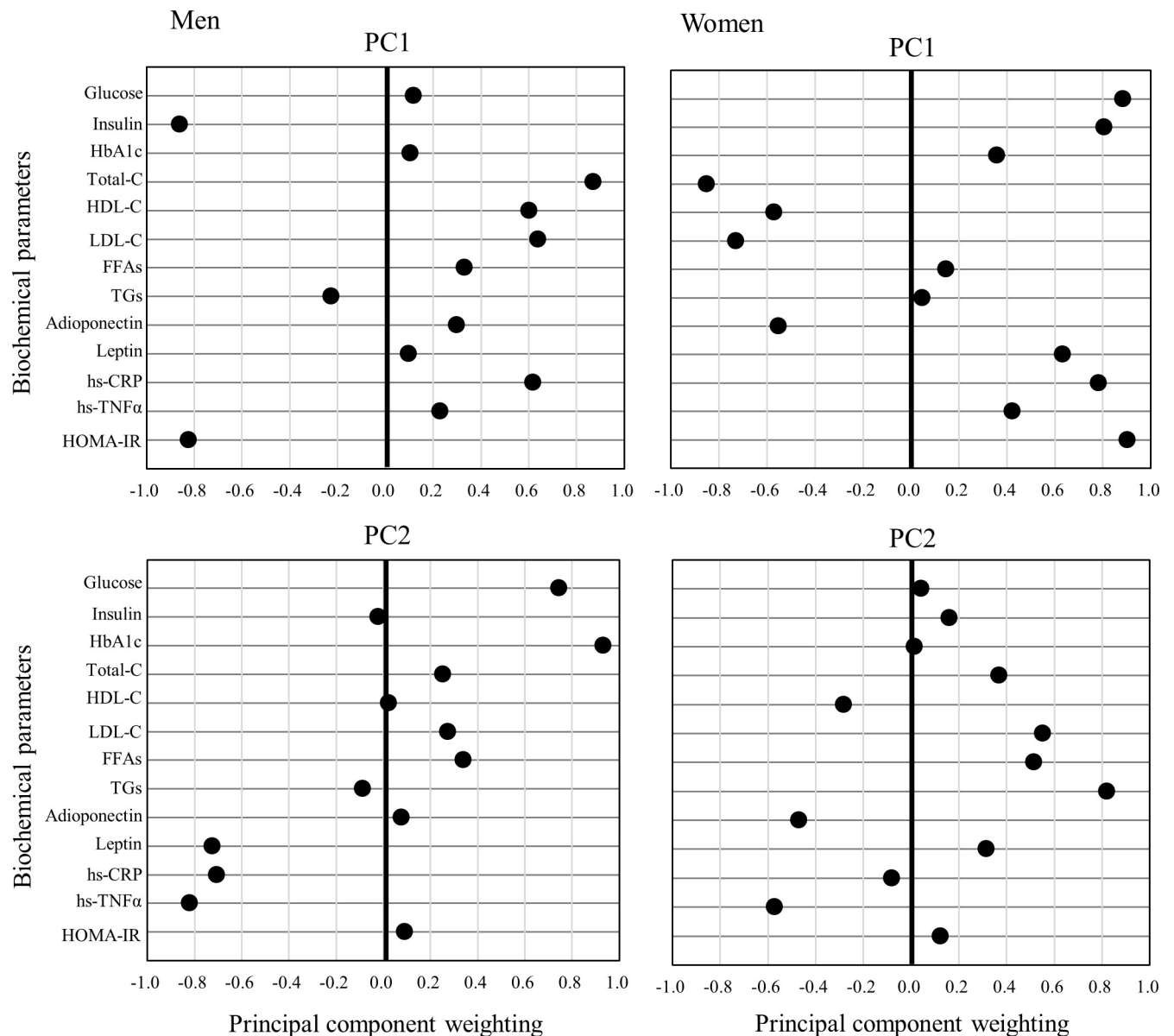


Fig 1. PC weighting in men (a, left side) and women (b, right side). FFAs, free fatty acids; HbA1c, hemoglobin A1c; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homoeostasis model assessment index of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; hs-TNF α , high-sensitivity tumor necrosis factor; LDL-C, low-density-lipoprotein cholesterol; TGs, triglycerides; Total-C, total cholesterol. PC weighting was significant (>0.8).

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study used PCA to examine biochemical parameters. Among men, Total-C, insulin, and HOMA-IR or HbA1c and hs-TNF α were grouped with the same distribution for factors 1 and 2. Among women, glucose, insulin, HOMA-IR, and Total-C or TGs were grouped with the same distribution for factors 1 and 2. Our results suggest that adiponectin is related to Intra-MAT content, independent of VAT, and that the dynamics of adiponectin might not be similar to those of other biochemical parameters in older men and women.

Our analyses revealed an inverse correlation between adiponectin and echo intensity, independent of waist circumference. This result is comparable with reports indicating that adiponectin is inversely correlated with IMCL, independent of VAT and total body fat mass in

Table 2. Physical activity and dietary habit characteristics.

	Men	Women	P-value
No. of participants (men/women)	9	13	
Physical activity			
Number of steps	10389.9 ± 2465.5	9663.4 ± 2684.1	0.52
Light (min)	67.1 ± 12.2	70.2 ± 17.8	0.66
Moderate (min)	35.9 ± 22.5	29.2 ± 17.0	0.43
Vigorous (min)	2.8 ± 3.1	1.5 ± 1.3	0.26
Total (min)	105.8 ± 20.2	100.8 ± 26.7	0.64
Light (MET h)	2.6 ± 0.5	2.6 ± 0.7	0.79
Moderate (MET h)	2.5 ± 1.5	2.0 ± 1.2	0.46
Vigorous (MET h)	0.3 ± 0.4	0.2 ± 0.1	0.24
Total (MET h)	5.3 ± 1.4	4.8 ± 1.5	0.41
Habitual dietary intake			
Energy (kcal/body weight)	33.7 ± 5.8	36.4 ± 8.6	0.41
Carbohydrates (g/body weight)	4.6 ± 0.6	4.9 ± 1.1	0.51
Protein (g/body weight)	1.2 ± 0.3	1.4 ± 0.4	0.20
Fat (g/body weight)	0.9 ± 0.3	1.2 ± 0.3	0.08

Value are mean ± SD. MET h, metabolic equivalent × hours.

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obese women [13]. Adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) mediate increased AMP-activated kinase and PPAR activity, thereby regulating glucose and lipid metabolism [12]. In an animal study, peripheral administration of adiponectin attenuated

Table 3. Pearson correlation coefficients for echo intensity, adiponectin, and leptin and body composition or biochemical parameter values.

	Echo intensity	Adiponectin	Leptin
Age	0.10	0.15	-0.07
BMI	0.01	-0.28	0.51*
Waist circumference	0.03	-0.46*	0.15
% Fat	0.15	-0.12	0.66**
Glucose	0.20	-0.34	-0.05
Insulin	0.18	-0.31	0.02
HbA1c	0.30	-0.21	0.02
Total-C	-0.43*	0.26	-0.29
HDL-C	-0.49*	0.40	-0.19
LDL-C	-0.47*	0.12	-0.15
FFAs	0.15	-0.15	0.18
TGs	0.52*	-0.32	-0.07
hs-CRP	0.17	-0.15	0.77**
HOMA-IR	0.20	-0.33	0.03
hs-TNF α	-0.07	-0.08	-0.09
Lateral subcutaneous thickness	-0.33	0.42*	0.34
Muscle thickness	-0.56**	0.24	-0.12

*, $p < 0.05$

** $p < 0.01$.

BMI, body mass index; FFAs, free fatty acids; HbA1c, hemoglobin A1c; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homoeostasis model assessment index of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; hs-TNF α , high-sensitivity tumor necrosis factor α ; LDL-C, low-density-lipoprotein cholesterol; TGs, triglycerides; Total-C, total cholesterol.

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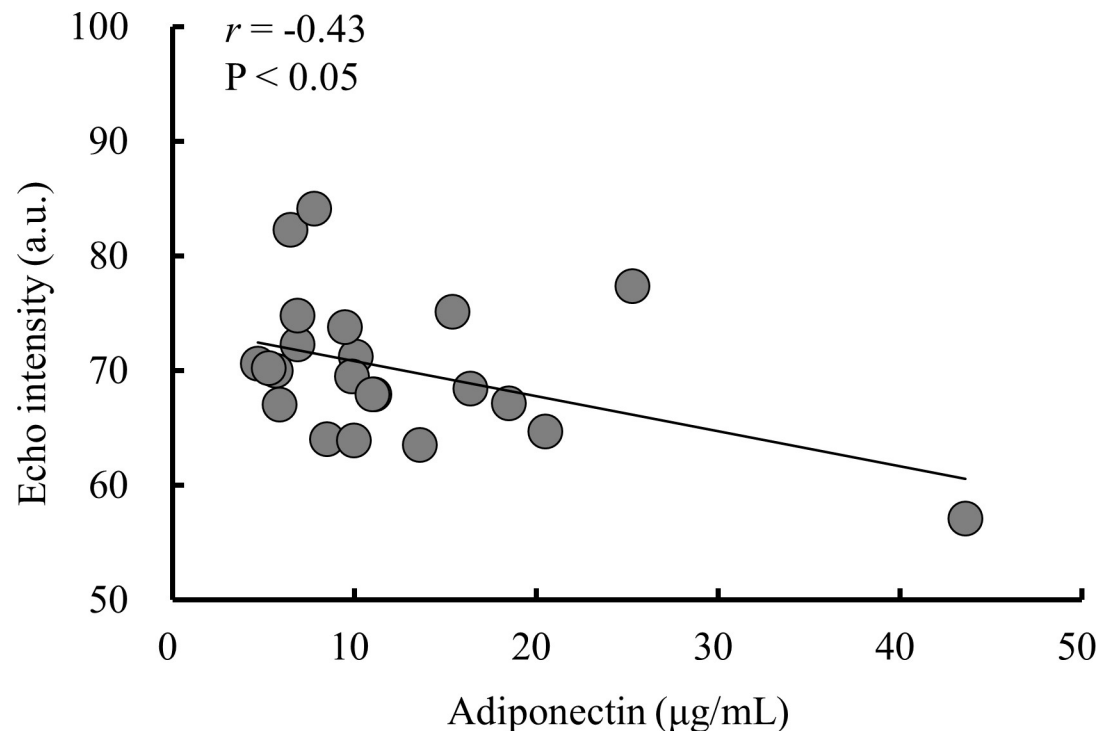


Fig 2. Relationship between echo intensity and adiponectin.

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body weight gain and reduced VAT [26]. Moreover, a single injection of purified recombinant human adiponectin in diabetic swine produced a 2- to 3-fold elevation in the circulating adiponectin level, in turn triggering a transient decrease in basal glucose level, independent of insulin level [27]. These results suggest that adiponectin mediates accumulation of VAT.

According to the Framingham cohort study, adiponectin level is inversely related to ectopic fat deposition (visceral, epicardial, and mediastinal fat) [28]. Our findings thus suggest that adiponectin level is related to IntraMAT content in normal-weight older men and women, with adiponectin exhibiting a similar influence on ectopic fat (i.e., IMCL, VAT, IMAT, epicardial and mediastinal fat) [10, 13, 29]. By contrast, according to the Cardiovascular Health Study, an epidemiologic investigation (participants >80 years of age; N = 988), adiponectin levels increase with age, which is associated with increased physical disability and mortality. This phenomenon may be associated with age-related homeostatic dysregulation [30]. Adiponectin resistance can occur in persons ≥ 80 years old. The age of participants differed between the present

Table 4. Partial correlation coefficients between adiponectin and echo intensity.

Control variables	<i>r</i>	<i>P</i> -value
Waist circumference	-0.47	0.03
TGs	-0.33	0.14
LDL-C	-0.43	0.05
Lateral subcutaneous thickness	-0.34	0.12
Muscle thickness	-0.37	0.09

LDL-C, low-density-lipoprotein cholesterol; TGs, triglycerides.

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study (range 62 to 77 years) and the previous study (>80 years). Therefore, our result might not agree with those of Kizer et al. [30].

The level of methylation of adiponectin-gene DNA in subcutaneous adipose tissue and VAT was found to be positively associated with LDL-C level, suggesting a common epigenetic regulation mechanism that is independent of the biological roles of these factors [31]. Moreover, adiponectin might serve as a starvation signal released by adipocytes, thereby inducing local expansion of TG stores in adipose tissue [32] and effectively redistributing TGs from hepatocytes and muscle cells to subcutaneous adipose tissue. The results of these two previous studies indicate that adiponectin controls lipogenesis and lipolysis of TGs and LDL-C. We found that adiponectin was inversely correlated with echo intensity-estimated IntraMAT, but this correlation became insignificant after adjusting for TG and LDL-C levels. These results suggest that TGs and LDL-C affect the relationship between adiponectin and IntraMAT content. When biochemical parameters were grouped in the PC analysis, among men, Total-C, insulin, and HOMA-IR or HbA1c and hs-TNF α were grouped with the same distribution for factors 1 and 2. Among women, glucose, insulin, HOMA-IR, and Total-C or TG were grouped with the same distribution for factors 1 and 2. These results indicate that factors differ between men and women. As adiponectin did not belong to the same group of factors, it might function independently.

Adiponectin is expressed more in VAT than subcutaneous adipose tissue, and VAT adipocytes are more metabolically active and more sensitive to lipolysis than subcutaneous adipose tissue adipocytes [33]. An epidemiologic investigation of Japanese middle-aged men reported that adiponectin levels were inversely correlated with VAT and subcutaneous adipose tissue in a regression model that concomitantly included these factors. VAT exhibited a significant inverse and subcutaneous adipose tissue a significant positive association [34]. These results indicate that VAT and subcutaneous adipose tissue are differentially correlated with adiponectin concentration. We also found that adiponectin level was positively correlated with subcutaneous adipose tissue; however, adiponectin level was inversely correlated with echo intensity-estimated IntraMAT. Therefore, our results are congruent with those reported by Nakamura et al. [34].

In general, older adults reportedly have lower circulating levels of leptin compared with young adults. Obesity has also been associated with leptin resistance, which can result in increased circulating leptin levels but decreased leptin signaling [35]. Moreover, plasma leptin is correlated with body fat content in young men and women, but no such correlation has been observed in middle-aged and older men and women [36]. According to Vella et al. [37], physical activity may positively affect levels of select adiposity-associated inflammatory markers (leptin, IL-6, and resistin), irrespective of total and/or central adiposity. The level of physical activity (number of steps) among older participants in the present study (men, mean 10389.9 steps; women, mean 9663.4 steps) was similar to that of young adults in our previous study (young men and women, mean 9097.4 steps). These results indicate that the older adults in our present study were quite physically active. In line with previous observations in young adult men and women [36], our present study identified a correlation between leptin and BMI or percent body fat in the older men and women enrolled in the study.

Our study has some limitations. First, VAT was estimated from waist circumference as the VAT index. Notably, we estimated this value and did not measure it. Waist circumference tended to correlate more significantly with MRI-measured VAT ($n = 1192$; $r = 0.80$) [19]. Waist circumference is utilized as an index of central obesity, as recommended by the U.S. National Institutes of Health, World Health Organization [20], American Heart Association, and International Diabetes Foundation. The waist-to-hip ratio and waist measurements can be utilized as a “central obesity or visceral fat index” for screening [38]. Second, our study

included both men and women as a single group. Circulating levels of adiponectin and leptin are known to exhibit sexual dimorphism [12, 39]. Levels of circulating adiponectin and leptin tend to be higher in women than men, suggesting that sex hormones regulate the production of adiponectin and leptin. The PCA clearly indicated that Total-C, insulin, and HOMA-IR or HbA1c and hs-TNF α were grouped with the same distribution for factors 1 and 2 among the men, and glucose, insulin, HOMA-IR, and Total-C or TGs were grouped with the same distribution for factors 1 and 2 among the women. Biochemical parameters also likely differ between adult men and women. Our results also indicated that adiponectin and leptin levels were higher in women than men, although the difference in adiponectin levels was not significant. Third, the transmission of ultrasound beams through tissue can be attenuated due to reflection, dispersion, or absorption of the sound waves, thus reducing the echo intensity of deeper structures [40]. The non-invasive tauonic ultrasonography technique is widely used to evaluate muscle quality and quantity (i.e., muscle mass or echo intensity–estimated IntraMAT content) in both research and medical settings. However, the range of ultrasonographic images covers only a part of the muscle; therefore, IntraMAT content must be estimated from a narrow range of images.

Conclusion

In conclusion, we assessed biochemical parameters and echo intensity–estimated IntraMAT in the VL via ultrasonography among normal-weight older adult men and women. Partial correlation analysis with waist circumference as a control variable revealed that adiponectin is inversely correlated with echo intensity, independent of waist circumference (measured as the VAT index); however, after controlling for LDL-C and TGs, this correlation was not observed. Our results thus suggest that adiponectin is related to IntraMAT content, independent of VAT. Furthermore, our study used PCA to examine biochemical parameters. Among men, Total-C, insulin, and HOMA-IR or HbA1c and hs-TNF α were grouped with the same distribution for factors 1 and 2. Among women, glucose, insulin, HOMA-IR, and Total-C or TGs were grouped with the same distribution for factors 1 and 2. The dynamics of adiponectin might not be similar to those of other circulating biochemical parameters in older men and women. The prevalence of both type 2 diabetes and obesity increases with age, and therefore, the risk of metabolic diseases increases with fat accumulation in ectopic fat deposits (i.e., IMCL, IMAT, visceral, epicardial, and mediastinal fat). IntraMAT might also be an adiponectin target tissue, suggesting that adiponectin positively affects insulin sensitivity in older adults by decreasing IntraMAT content. In addition, adiponectin might function differently in men and women.

Supporting information

S1 Fig. PC weighting among men (left side; PC1-PC4) and women (right side; PC1-PC5). FFAs, free fatty acids; HbA1c, hemoglobin A1c; HDL-C, high-density-lipoprotein cholesterol; HOMA-IR, homoeostasis model assessment index of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; hs-TNF α , high-sensitivity tumor necrosis factor–alpha; LDL-C, low-density-lipoprotein cholesterol; TGs, triglycerides; Total-C, total cholesterol. PC weighting is significant (>0.8).
(TIF)

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