

1 Original contribution

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3 Intramuscular adipose tissue determined by T1-weighted MRI at 3T primarily reflects  
4 extramyocellular lipids

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20 Running head: Intramuscular adipose tissue

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1 **Abstract**

2 **Purpose:** The purpose of this study was to assess relationships between intramuscular  
3 adipose tissue (IntraMAT) content determined by MRI and intramyocellular lipids  
4 (IMCL) and extramyocellular lipids (EMCL) determined by <sup>1</sup>H-magnetic resonance  
5 spectroscopy (<sup>1</sup>H-MRS) or echo intensity determined by B-mode ultrasonography of  
6 human skeletal muscles.

7 **Methods:** Thirty young and elderly men and women were included. T1-weighted MRI  
8 were taken from the right mid-thigh to measure IntraMAT content of the vastus lateralis  
9 (VL) and biceps femoris (BF) using a histogram shape-based thresholding technique.  
10 IMCL and EMCL were measured from the VL and BF at the right mid-thigh using  
11 <sup>1</sup>H-MRS. Ultrasonographic images were taken from the VL and BF of the right  
12 mid-thigh to measure echo intensity based on grey-scale level for quantitative analysis.

13 **Results:** There was a significant correlation between IntraMAT content by MRI and  
14 EMCL of the VL and BF (VL,  $r = 0.506$ ,  $P < 0.01$ ; BF,  $r = 0.591$ ,  $P < 0.001$ ) and  
15 between echo intensity and EMCL of the VL and BF (VL,  $r = 0.485$ ,  $P < 0.05$ ; BF,  $r =$   
16  $0.648$ ,  $P < 0.01$ ). IntraMAT content was also significantly correlated with echo intensity  
17 of the VL and BF (VL,  $r = 0.404$ ,  $P < 0.05$ ; BF,  $r = 0.493$ ,  $P < 0.01$ ).

18 **Conclusion:** Our study suggests that IntraMAT content determined by T1-weighted  
19 MRI at 3T primarily reflects extramyocellular lipids, not intramyocellular lipids, in  
20 human skeletal muscles. (230 words)

21

22 Key words: intramuscular adipose tissue, spin-echo sequence, extramyocellular lipids,  
23 intramyocellular lipids, ultrasonography, image analysis

24

## 1 **1. Introduction**

2 Adipose tissue infiltrates within the muscle and accumulates as a result of  
3 aging [1-4], inactivity [5, 6], obesity [7-10], or myopathy [11-13]. Although  
4 intermuscular adipose tissue is typically the broadest definition of adipose tissue  
5 infiltration in the muscle, referring to storage of lipids in adipocytes underneath the  
6 deep fascia of muscle [14], this study focuses on adipose tissue within a muscle, that is  
7 intramuscular adipose tissue (IntraMAT). The physiological and biochemical roles of  
8 IntraMAT are not fully understood; however, it is described as a unique adipose tissue  
9 depot that has a similar function to visceral adipose tissue in terms of representing risks  
10 for metabolic impairment such as type 2 diabetes [7, 8, 14-16] and as an index for  
11 progression of muscular dystrophy [11-13].

12 IntraMAT has been evaluated using medical imaging techniques such as  
13 magnetic resonance imaging (MRI) and B-mode ultrasonography (US), and using a  
14 spectrum technique such as <sup>1</sup>H-magnetic resonance spectroscopy (<sup>1</sup>H-MRS). Among  
15 these innovative techniques, MRI is a powerful non-invasive tool to monitor IntraMAT  
16 of the limbs or trunk in humans [11, 17]. Several MRI sequences, such as T1- and  
17 T2-weighted imaging, short-tau inversion recovery imaging, and two- or three-point  
18 Dixon imaging, have been used extensively to acquire anatomical information on the  
19 skeletal muscle and adipose tissue within the skeletal muscle in young and old healthy  
20 subjects as well as patients with neuromuscular disorders [6, 11, 17]. However, it is not  
21 known whether increased IntraMAT is due to an increase in adipocytes inside or outside  
22 of muscle cells, although increased lipids inside of muscle cells, that is, intramyocellular

1 lipids (IMCL), are known to have a negative impact on metabolism [18, 19]. Therefore,  
2 gaining this kind of information would be helpful for understanding the metabolism of  
3 IntraMAT, developing interventions to reduce the risk for type 2 diabetes, or even  
4 determining triggers for and a cure of myopathy [11, 17].

5 US is the less expensive test to estimate IntraMAT compared with other  
6 imaging techniques. If researchers or doctors cannot access an MRI, US is a  
7 substitutable methodology to estimate IntraMAT based on echo intensity [13, 20]. In  
8 earlier studies, Sipilä and Suominen [21, 22] showed that echo intensity of the  
9 quadriceps femoris is lower in elderly athletes than untrained individuals, suggesting  
10 elderly athletes have less IntraMAT content than healthy sedentary individuals. As with  
11 MRI, however, it is not clear that the origin of reflected echo of skeletal muscle  
12 sonography comes from in the muscle cells.

13 To resolve these questions, we used  $^1\text{H}$ -MRS to identify the location of  
14 adipocytes in muscle cells, that is, the IMCL and extramyocellular lipids (EMCL) [23,  
15 24]. The usefulness and validity of physiological and biochemical significance of these  
16 fat components (IMCL and EMCL) using  $^1\text{H}$ -MRS has been extensively reviewed in the  
17 literature [23]. The purpose of this study was to assess relationships between IntraMAT  
18 content determined by MRI and IMCL and EMCL determined by  $^1\text{H}$ -MRS or echo  
19 intensity determined by US. We hypothesized that IntraMAT content determined by  
20 MRI and echo intensity by US would be related to IMCL, because IMCL has been  
21 shown to be a good index of lipid deposits within muscle cells in previous studies [23].

22

1 **2. Materials and Methods**

2 **2.1 Subjects**

3           Fifteen elderly ( $70.7 \pm 3.8$  years, 7 men and 8 women) and 15 young ( $20.9 \pm$   
4  $0.3$  years 8 men and 7 women) men and women volunteered to participate in this study.  
5 The reason of we used elderly and young subjects was to get a wide range of values in  
6 variables to get better correlations. It should be noted that the range of variables in the  
7 two age groups was not polarized, which was as expected (Table 1). Before the  
8 experiment, the procedure, purposes, risks, and benefits associated with the study were  
9 explained and written consent was obtained. This study was approved by the  
10 Institutional Review Board of the local institute, and was conducted in accordance with  
11 the guidelines of the Declaration of Helsinki.

12

13 **2.2 MRI acquisition and analysis**

14           MRI was performed on a 3.0T (MAGNETOM Verio, Siemens Healthcare  
15 Diagnostics K.K., Tokyo, Japan) whole-body scanner. Subjects were placed in a supine  
16 position and images of the thigh were acquired using a body coil. T1-weighted  
17 spin-echo transaxial images of the right-thigh were collected with the following  
18 parameters: TR/TE 604/11 msec; voxel resolution 0.75 mm; optimized field of view  
19 256 x 256 mm; slice thickness 10 mm; and interslice gap 0 mm. Representative images  
20 are shown in Figure 1.

21           Medical Image Processing, Analysis and Visualization software (version  
22 4.4.0; National Institutes of Health, Bethesda, MD) was used to analyze images on a

1 personal computer (MacBook Pro, Apple Inc., Cupertino, CA). The MRI data analysis  
2 procedure was essentially the same as described previously [1, 11]. Briefly, we selected  
3 an image of the mid-thigh according to markers attached at the middle point between  
4 the greater trochanter and lateral condyle of the femur (see Figure 1) and identified  
5 vastus lateralis (VL) and biceps femoris-long head (BF). Contiguous axial images were  
6 used to help identify each of the muscles and the muscle boundaries.

7 We calculated the cross-sectional area (CSA) of skeletal muscle and  
8 IntraMAT content at the mid-thigh as described previously [1, 11]. The first step of the  
9 analysis was to correct for image heterogeneity caused by suboptimal radiofrequency  
10 coil uniformity, or gradient-driven eddy currents, using a well-established  
11 nonparametric nonuniform intensity normalization (N3) algorithm [1, 6, 11, 25]. This  
12 step was essential for subsequent analyses that assume homogenous signal intensity  
13 across images. Optimized image correction parameters (N3) were determined (end  
14 tolerance 0.0001; maximum iterations 100; signal threshold 1; field distance 25 mm;  
15 subsampling factor 4; Kernel full width half maximum of 0.15; Wiener filter noise 0.01),  
16 and the same parameters were applied to all images. The investigator then drew six  
17 region-of-interests (ROIs) of 25 mm<sup>2</sup> each, and three of six ROIs were placed on the VI  
18 and the other three ROIs were placed on the subcutaneous adipose tissue. The VI, which  
19 contains 99% of skeletal muscle tissue [11], was chosen so that we would obtain a pure  
20 skeletal muscle peak in the pixel number-signal intensity histogram. The total number  
21 of pixels within the six ROIs and a frequency distribution and histogram of all pixels  
22 and signal intensities were produced.

1           To separate muscle and adipose tissues in the pixel number-signal intensity  
2 histogram with minimal investigator bias, we implemented the Otsu threshold method, a  
3 reliable histogram shape-based thresholding technique used in medical imaging analysis  
4 [26]. To minimize manual tracing-induced errors on thresholding values, the mean of  
5 five trials was used, and the values were applied to VL and BF. After carefully tracing  
6 the edge of each muscle, the following parameters were calculated: 1) the total number  
7 of pixels within the ROI; 2) the number of pixels with a signal intensity lower than the  
8 threshold value (skeletal muscle), and 3) the number of pixels with a value higher than  
9 the threshold value (IntraMAT). The proportion of skeletal muscle and IntraMAT of VL  
10 and BF muscles was then calculated using the following equations:

$$\begin{aligned} 11 \quad & \text{IntraMAT content (\%)} = (\text{IntraMAT pixel number}) / [(\text{skeletal muscle pixel number}) \\ 12 \quad & + (\text{IntraMAT pixel number})] \times 100 \end{aligned}$$

13           All images were read in random order, and one investigator performed all  
14 analyses. Test-retest reliability of IntraMAT content has been reported elsewhere [1].  
15 Briefly, intraclass correlation coefficients (ICC [2, 1]) in individual muscles at the  
16 mid-thigh for 10 subjects were 0.97 and 1.00 for VL and BF, respectively (all  $p <$   
17  $0.001$ ). The standard error of the measurement was 2.1% and 1.6% for VL and BF,  
18 respectively. The minimal difference between the first measurement and the second  
19 measurement were 5.8% and 4.6% for VL and BF, respectively.

20

### 21 **2.3 <sup>1</sup>H-Magnetic resonance spectroscopy**

22           <sup>1</sup>H-MRS experiments were performed on the same day of MRI experiments

1 using the same MR system. The  $^1\text{H}$ -MRS procedure has been reported elsewhere [24].  
2 Briefly, IMCL of the right-thigh was measured by  $^1\text{H}$ -MRS using a 4-channel flex coil  
3 ( $366 \times 174$  mm). Voxels ( $11 \times 11 \times 20$  mm) were positioned in the VL and BF at the  
4 mid-thigh between the greater trochanter and lateral condyle of the femur to match the  
5 position of MRI measurement. Care was taken to avoid visible vascular structures,  
6 adipose tissue deposits and connective tissues within the voxel.  $^1\text{H}$ -MRS spectra from  
7 regions of interest were acquired using a point-resolved spectroscopy sequence  
8 (PRESS) with the following acquisition parameters: TR/TE 4000/30 msec; 128  
9 averages. The unsuppressed water signal was subsequently measured in the same voxel  
10 under the same shimming conditions. It was used as a reference signal [23].

11 Fitting of all  $^1\text{H}$ -MRS data was performed using LCModel (v.6.2-4A) [27].  
12 The spectroscopic data acquired from the MR scanner were collected in a Linux  
13 computer, and metabolism was quantified using eddy current correction and water  
14 scaling. The concentration of water was assumed to be equal to 42.4 mmol/kg wet  
15 weight on the basis of a mean adult muscle tissue water content of 77% [28].  
16 Concentrations of IMCL ( $-\text{CH}_2$ ) and EMCL ( $-\text{CH}_2$ ) were collected for the T1 and T2  
17 relaxation effects of the unsuppressed water peak using LCModel control parameter  
18 `atth2o`, which were determined using the following equation:  $\exp(-\text{TE}/\text{T2}) [1 - \exp(-\text{TR}/\text{T1})]$  [29], assuming relaxation time  $\text{T1} = 369$  msec,  $\text{T2} = 89.4$  msec for the  
19  $\text{IMCL}_{\text{CH}_2}$  and  $\text{T1} = 369$  msec,  $\text{T2} = 77.6$  msec for the  $\text{EMCL}_{\text{CH}_2}$  [30], respectively. The  
20 concentration of lipid molecules (total lipid content) was computed by the summation of  
21 the  $\text{IMCL}_{\text{CH}_2}$  and  $\text{EMCL}_{\text{CH}_2}$  concentrations and division by factor 31. The value 31

1 follows from the assumption that the average number of methylene protons is 62 per  
2 triglyceride molecule (equivalent to 31 CH<sub>2</sub> groups) [31-33]. The data were converted  
3 from mM to mmol/kg wet weight, assuming a tissue density 1.05 g/mL for skeletal  
4 muscle [32]. We acquired IMCL and EMCL data from the VL and BF of all 15 of the  
5 younger individuals and of 12 and 14 of the elderly individuals, respectively.

6

## 7 **2.4 Ultrasonography measurement and analysis**

8           A real-time B-mode ultrasonography (LOGIQ e, GE Healthcare, USA) with a  
9 8-12 MHz linear-array probe (width of probe, 6 cm) was used to examine the echo  
10 intensity of VL and BF of the right mid-thigh matching the same location of MRI and  
11 <sup>1</sup>H-MRS measurements. US images were scanned using the following acquisition  
12 parameters: frequency 8 MHz; gain 80 dB; depth 8 cm; number of focus point 1 (top of  
13 the image). Body hair at skin surfaces on measurement sites was shaved to minimize  
14 unwanted reflection echo. All measurements for the VL and BF were carried out with  
15 subjects in supine and prone positions, respectively. The probe was coated with an  
16 adequate water-soluble transmission gel to provide acoustic contact without depression  
17 of the dermal surface and was aligned perpendicular to the VL and BF to obtain  
18 transverse images. Five images were obtained for each muscle. Ultrasound images were  
19 stored in an ultrasound machine with the DICOM format for future analysis.

20           Image files were stored on a personal computer and echo intensity was  
21 analyzed using ImageJ (version 1.44; National Institutes of Health, Bethesda, MD). The  
22 first step of analysis involved smoothing to convert originally established grey-scale in

1 an ultrasound image to 256 grey-scale level on ImageJ. The region of interest (ROI),  
2 which included as much muscle as possible but avoided visible bone and fascia, was  
3 determined using polygon selections to calculate echo intensity [34]. Mean grey-scale  
4 levels within a ROI were used for echo intensity, which was expressed in arbitrary units  
5 as a value between 0 and 255. The mean value of echo intensity in five images for each  
6 muscle was used for analysis.

7

## 8 **2.5 Statistical analysis**

9 All values are reported as means and standard deviation. The student paired t  
10 test was used for differences in parameters between the VL and BF. Pearson  
11 product-moment correlation coefficients were used to determine the association  
12 between variables. The level of significance was set at  $P < 0.05$ . All statistical analyses  
13 were performed using IBM SPSS Statistics (version 21.0, IBM Japan, Tokyo, Japan).

14

1 **3. Results**

2 IntraMAT content, EMCL, and echo intensity in the BF were 3.5-, 2.3-, and  
3 1.2-fold, respectively, higher than that of the VL ( $P < 0.001$ ) (Table 1). There was no  
4 significant difference in IMCL between the VL and BF.

5 There was a significant correlation between IntraMAT content and EMCL ( $r$   
6  $= 0.506$ ,  $P < 0.01$ ) in the VL; however, there was no significant correlation between  
7 IntraMAT content and IMCL ( $r = 0.263$ , n.s.) (Figure 2). Similar to the VL, there was a  
8 significant correlation between IntraMAT content and EMCL ( $r = 0.591$ ,  $P < 0.001$ ) in  
9 the BF; however, there was no significant correlation between IntraMAT content and  
10 IMCL ( $r = 0.236$ , n.s.) (Figure 3).

11 Regarding echo intensity, the results were the same as those regarding the  
12 relation between IntraMAT content and IMCL or EMCL in the VL and BF. There was a  
13 significant correlation between echo intensity and EMCL (VL,  $r = 0.485$ ,  $P < 0.01$ ; BF,  
14  $r = 0.648$ ,  $P < 0.001$ ) in the VL and BF; however, there was no significant correlation  
15 between echo intensity and IMCL (VL,  $r = 0.060$  and BF,  $r = 0.341$ ) (Figures 4 and 5).

16 IntraMAT content was correlated with echo intensity in the VL and BF (VL,  $r$   
17  $= 0.404$ ,  $P < 0.05$ ; BF,  $r = 0.493$ ,  $P < 0.01$ ) (Figure 6).

18  
19

## 1 **4. Discussion**

2           The purpose of this study was to assess relationships among IntraMAT  
3 content, echo intensity, and IMCL and EMCL. IntraMAT content or echo intensity was  
4 significantly correlated with EMCL ( $r = 0.458$  to  $0.506$ ,  $P < 0.01$  for the VL and  $r =$   
5  $0.591$  to  $0.648$ ,  $P < 0.001$  for the BF). Contrary to our hypothesis, there was no  
6 significant relation between IntraMAT content or echo intensity and IMCL ( $r = 0.060$  to  
7  $0.341$ ) in any muscle. We also found that IntraMAT content was significantly correlated  
8 with echo intensity. This result clearly showed that information about intramuscular  
9 adipose tissue determined by MRI and US, which are valuable non-invasive  
10 methodologies in research and diagnosis, primarily represent information on lipids  
11 outside of muscle cells, as opposed to inside muscle cells.

### 13 **4.1 IntraMAT and IMCL or EMCL relationship**

14           MRI has been widely used to evaluate quality and quantity of skeletal muscle  
15 as a result of aging [1-4], inactivity [5, 6], obesity [7-10], myopathy [11-13], and  
16 orthopedic-related disuse [35]. MRI is a powerful tool to quantify size of muscle, fat or  
17 other tissues in T1-weighted images using image analysis techniques [1, 4, 6, 11]. To  
18 accomplish this segmentation analysis, several algorithms have been proposed in the  
19 literature [1, 4, 11, 36-38]. In this study, we used a reliable histogram shape-based  
20 thresholding technique that was used in previous studies [1, 11]. Using this technique,  
21 we clearly showed that IntraMAT content was significantly correlated with adipose  
22 tissue accumulated outside of muscle cells, that is, EMCL, but not inside of muscle cells,

1 that is, IMCL, for both the VL and BF. This result did not support our hypothesis;  
2 however, the finding provides crucial information in terms of metabolism of adipose  
3 tissue within skeletal muscle.

4 In this study, IMCL content of our subjects was  $9.4 \pm 4.9$  mmol/kg wet weight  
5 for the VL (younger only:  $8.5 \pm 1.6$  mmol/kg wet weight) and  $10.5 \pm 7.7$  mmol/kg wet  
6 weight for the BF (younger only:  $7.7 \pm 1.1$  mmol/kg wet weight). These values are  
7 comparable with Jacobs et al. [18], who reported that IMCL content of the soleus  
8 muscle in subjects with increased insulin-sensitive (mean age, 32 years) was  $6.4 \pm 0.6$   
9 mmol/kg wet weight. For young individuals, IMCL content in our subjects was similar  
10 to this previous study. In terms of EMCL, only a few studies have reported values. Our  
11 elderly subjects had a 2.7-fold higher EMCL in the VL compared with younger subjects  
12 (young:  $21.9 \pm 3.3$  mmol/kg wet weight). Jacobs et al. [18] showed EMCL content of he  
13 soleus was  $10.7 \pm 1.6$  mmol/kg wet weight, which was approximately half of young  
14 individuals in this study. However, this could be due to physical characteristics,  
15 physical activity level, and/or muscle specific functional roles during daily life. Overall,  
16 we recognized that our IMCL and EMCL content could be within normal ranges.

17 IntraMAT content is positively associated with insulin resistance and  
18 increased levels are a risk factor for type 2 diabetes [7, 8, 39]. Accordingly, EMCL may  
19 have a significant effect on insulin resistance of skeletal muscle. To support this  
20 hypothesis, Sinha et al. [40] reported a significant negative correlation between insulin  
21 sensitivity and EMCL ( $r = -0.53$ ). Interestingly, studies have also shown a significant  
22 positive correlation between IMCL [18, 19] and insulin resistance even though EMCL

1 and IMCL have different associations with IntraMAT content in this study.

2 A correlation study that combined MRI with histochemical analysis showed a  
3 relation between IntraMAT by MRI and the content of adipose tissue in corresponding  
4 muscle biopsy samples. Rossi et al. [41] reported that IntraMAT content was closely  
5 related with adipocyte area by histology of muscle specimens ( $r = 0.84$ ,  $P < 0.001$ ).  
6 However, their study did not identify the exact location of adipocytes on histological  
7 sections under microscope observation.

#### 9 **4.2 Echo intensity and IMCL or EMCL relationship**

10 A novel finding of this study was that the echo intensity determined by  
11 skeletal muscle US was significantly correlated with EMCL, but not IMCL, suggesting  
12 that the reflected echo mainly came from adipose tissue outside of muscle cells, not  
13 inside. This finding may also be strengthened by our finding that the amount of EMCL  
14 was 2.6- to 9.7-fold higher than IMCL in our subjects (Table 1). This result is important  
15 because, similar to MRI, echo intensity could be used as an index of IntraMAT content  
16 as done in previous studies [20, 42, 43]. In these previous studies, increased echo  
17 intensity was thought to be primarily caused by increases in adipose tissue and/or  
18 connective tissue within a muscle [20, 42, 43]; however, it is not well understood  
19 whether echo intensity is associated with adipose and/or connective tissues within a  
20 muscle. This study revealed that increased echo intensity of skeletal US is primarily due  
21 to extramyocellular adipose tissue.

22 In terms of connective tissue within a muscle, Reimers et al. [44] determined

1 that the morphologic basis of increased echo intensity is due to lipomatosis or fibrosis  
2 by histochemical analysis (relative area of fat cells within total cross-sectional area in  
3 transverse sections of muscle samples) of 86 biopsy samples from five lower and upper  
4 limb muscles such as the VL and biceps brachii. They showed that lipomatosis, that is,  
5 increased IntraMAT content, is a major predictor to account for increased echo intensity  
6 of muscle US, but that fibrosis did not significantly affect increased echo intensity.  
7 Furthermore, they showed that there was a significant relation between echo intensity  
8 and IntraMAT content determined by biochemical analysis ( $r = 0.56$ ,  $P < 0.01$ ). Taken  
9 together, echo intensity by US primarily represents adipose tissue outside of the muscle  
10 cells, which is similar results found on MRI.

11

### 12 **4.3 IntraMAT and echo intensity relationship**

13 It is important to note that there was a significant relationship between  
14 IntraMAT content determined by T1-weighted MRI and echo intensity for both the VL  
15 ( $r = 0.404$ ,  $P < 0.05$ ) and BF ( $r = 0.493$ ,  $P < 0.01$ ).

16 The value of quantifying IntraMAT content using MRI or US is that  
17 IntraMAT content is a negatively associated with muscle function in previous studies [6,  
18 13, 14, 24, 42, 45-47]. Rech et al. [45] showed a significant negative correlation  
19 between echo intensity of the VL and maximum voluntary contraction of knee  
20 extension in 55 healthy women ( $r = -0.399$ ,  $P < 0.01$ ), implying individuals with higher  
21 IntraMAT content had lower muscle strength of the quadriceps femoris. Regarding  
22 IntraMAT content by MRI, Tuttle et al. [48] showed that a negative correlation between

1 increased levels of IntraMAT and decreased strength of the calf in healthy obese  
2 individuals, subjects with diabetes mellitus, and subjects with peripheral neuropathy ( $r$   
3 = -0.36,  $P < 0.05$ ). Similarly, the IntraMAT cross-sectional area in the thigh by MRI  
4 was related to mobility function including 6-minute walking distance ( $r = -0.33$ ,  $P <$   
5  $0.01$ ), stair ascent time ( $r = 0.39$ ,  $P < 0.01$ ), stair decent time ( $r = 0.36$ ,  $P < 0.01$ ), and  
6 the timed up-and-go test ( $r = 0.30$ ,  $P < 0.01$ ) in 109 older adults [47].

7           In light of the evidence, skeletal muscle US may be substitutable for MRI for  
8 measurement of IntraMAT if researchers or doctors do not have access to MRI  
9 technology in a facility. In this case, it is important to note that care must be taken to  
10 avoid errors of measurement as suggested by Pillen et al. [13]. They proposed a  
11 generous amount of echo gel should be used to ensure optimal acoustic coupling and  
12 posture and anatomical location of measurement site should be identical for all subjects  
13 to prevent measurement errors. In addition, some other technical aspects such as  
14 pressure and angle of a probe could be influenced on echo intensity. Reflected echo  
15 attenuation by subcutaneous fat should also be accounted for when analyzing images.

16           In conclusion, results of this work demonstrate that IntraMAT content  
17 determined by T1-weighted MRI is related to EMCL and echo intensity of skeletal  
18 muscle on US. Furthermore, lipid deposits within muscle cells may not mainly affect  
19 IntraMAT content. These results suggest that IntraMAT content determined by  
20 T1-weighted MRI primarily reflects extramyocellular lipids.

21

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8

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Table 1 Intramuscular adipose tissue (IntraMAT) content, intramyocellular lipid (IMCL), extramyocellular lipid (EMCL), and echo intensity						
		Mean ± SD			Range	
					Young	Old
<b>Vastus lateralis</b>						
IntraMAT (%)		4.6	±	3.0	0.7 - 5.3	1.8 - 13.7
IMCL (mmol/kg wet weight) <sup>1</sup>		9.4	±	4.9	2.4 - 26.3	6.7 - 14.9
EMCL (mmol/kg wet weight)		40.2	±	30.5	3.3 - 47.5	24.7 - 122.0
Echo intensity (a.u.)		61.1	±	13.3	29.2 - 70.3	50.3 - 88.2
<b>Biceps femoris</b>						
IntraMAT (%)		16.2	±	7.1†	5.1 - 23.0	10.7 - 29.7
IMCL (mmol/kg wet weight)		10.5	±	7.7	1.5 - 16.7	1.9 - 31.9
EMCL (mmol/kg wet weight) <sup>2</sup>		94.0	±	56.2†	13.9 - 143.9	29.5 - 202.3
Echo intensity (a.u.)		74.2	±	14.1†	35.2 - 81.7	77.0 - 100.1
†, P < 0.001 vs. vastus lateralis. 1, n = 12, 2, n = 14.						

1 Figure legends

2

3 Figure 1 Representative T1-weighted MR images (spin-echo; repetition time, 604  
4 msec; echo time 11 msec; field of view 256 x 256 mm; slice thickness 10  
5 mm) of young (a) men and elderly men (b)

6

7 Note that the four markers on the lateral side of the thigh were used to  
8 identify the middle length of the thigh. VL, vastus lateralis; BF, biceps  
9 femoris. Scale 5 cm.

10

11 Figure 2 Relationship between intramuscular adipose tissue content determined by  
12 histogram shape-based thresholding technique of T1-weighted MRI and  
13 extramyocellular lipids (EMCL) or intramyocellular lipids (IMCL)  
14 determined by <sup>1</sup>H-magnetic resonance spectroscopy in the vastus lateralis

15

16 Figure 3 Relationship between intramuscular adipose tissue content determined by  
17 histogram shape-based thresholding technique of T1-weighted MRI and  
18 extramyocellular lipids (EMCL) or intramyocellular lipids (IMCL)  
19 determined by <sup>1</sup>H-magnetic resonance spectroscopy in the biceps femoris

20

21 Figure 4 Relationship between echo intensity determined by B-mode ultrasonography  
22 and extramyocellular lipids (EMCL) or intramyocellular lipids (IMCL)

1 determined by <sup>1</sup>H-magnetic resonance spectroscopy in the vastus lateralis

2

3 Figure 5 Relationship between echo intensity determined by B-mode ultrasonography  
4 and extramyocellular lipids (EMCL) or intramyocellular lipids (IMCL)  
5 determined by <sup>1</sup>H-magnetic resonance spectroscopy in the biceps femoris

6

7 Figure 6 Relationship between intramuscular adipose tissue content determined by  
8 histogram shape-based thresholding technique of T1-weighted MRI and  
9 echo intensity determined by B-mode ultrasonography in the vastus lateralis  
10 (VL) and biceps femoris (BF)

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12

1 Figure 1

2

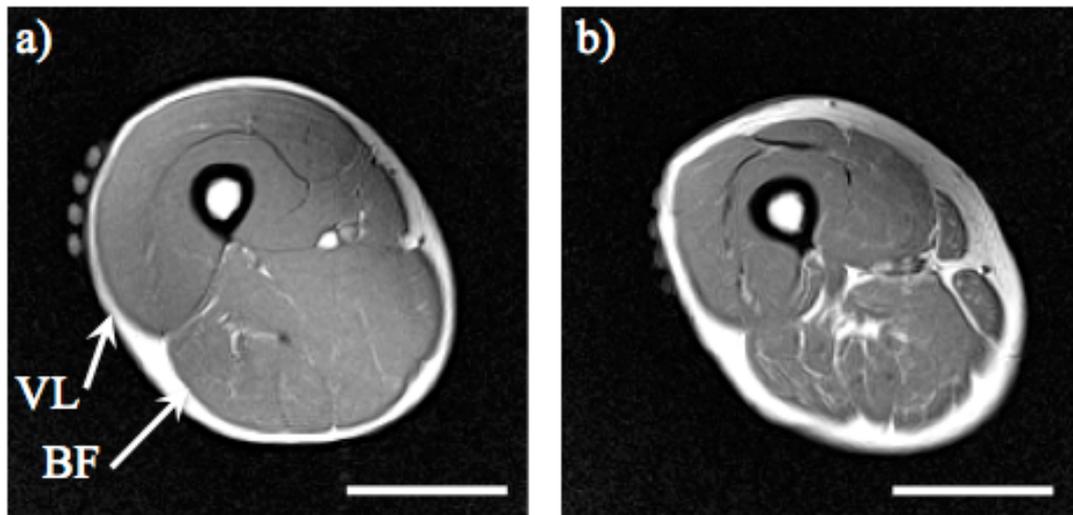


Figure 1

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1 Figure 2

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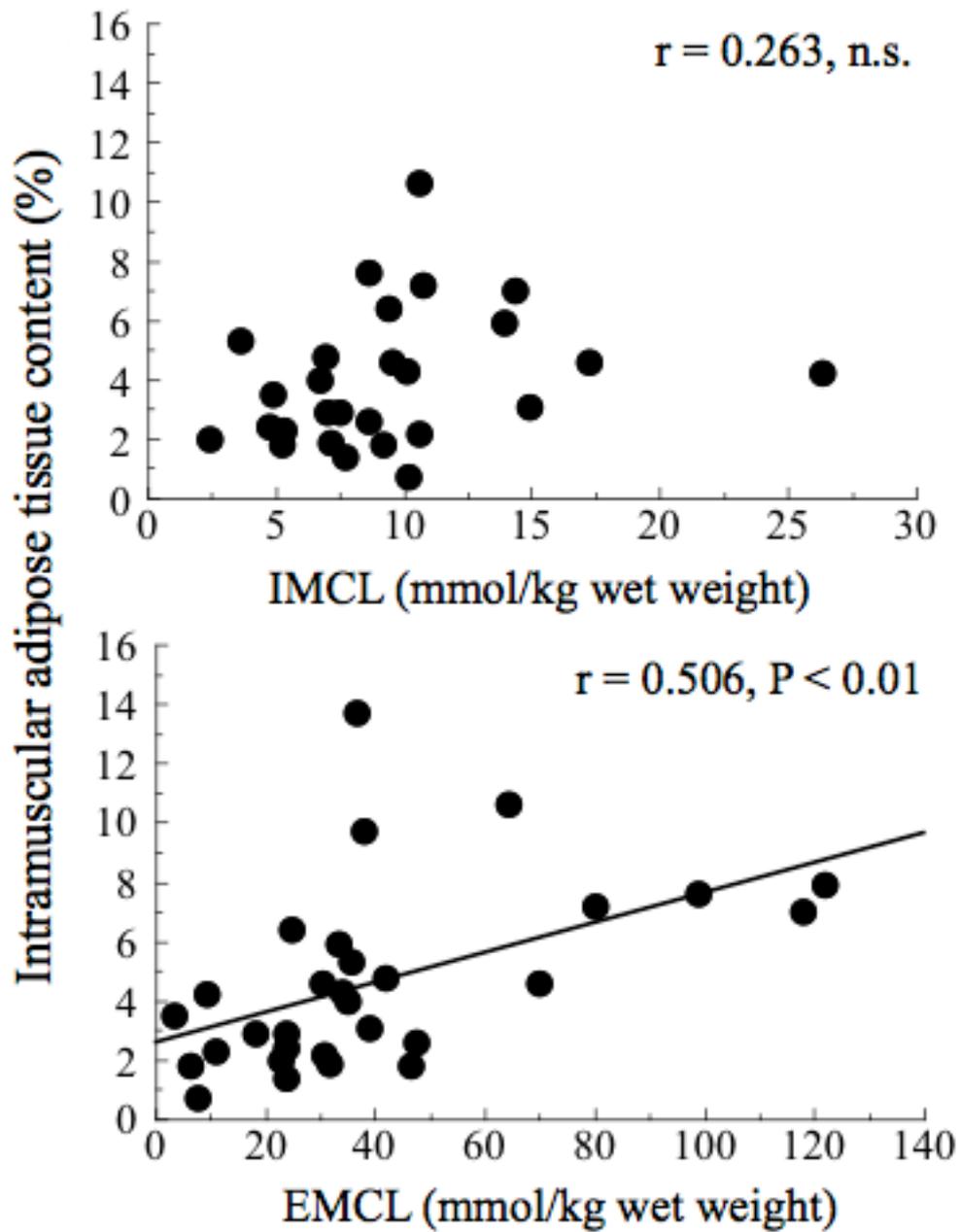


Figure 2

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1 Figure 3

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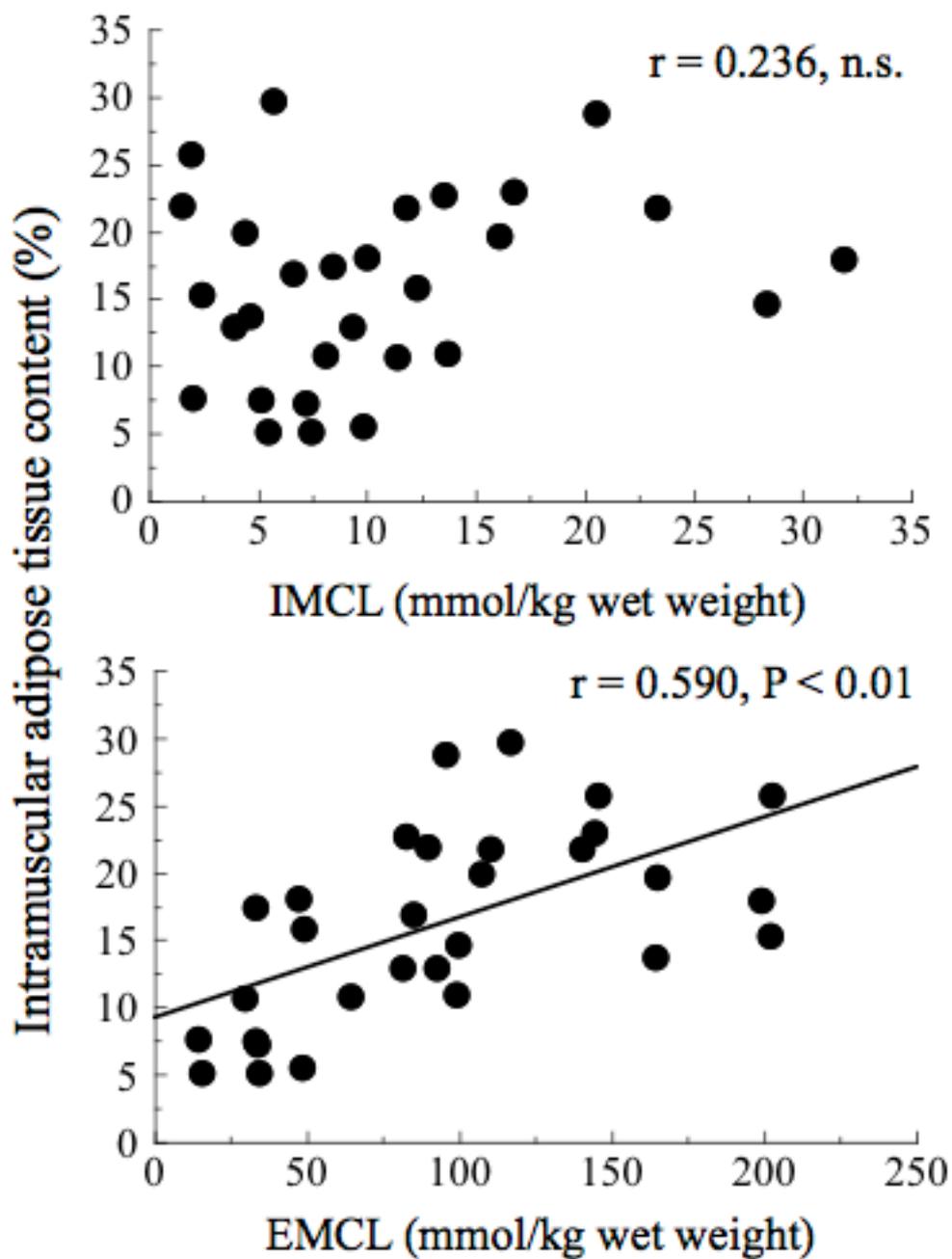


Figure 3

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1 Figure 4

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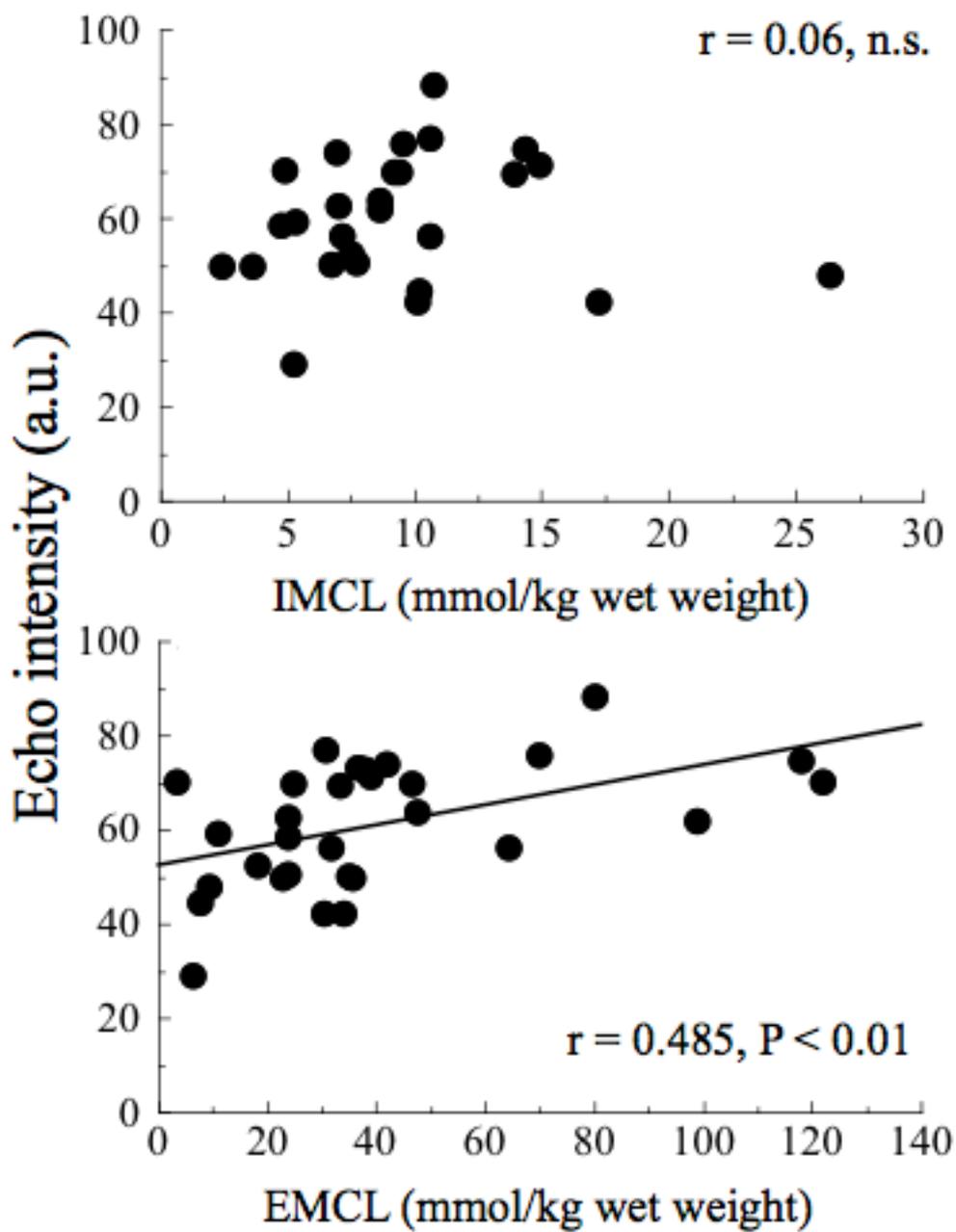


Figure 4

1 Figure 5

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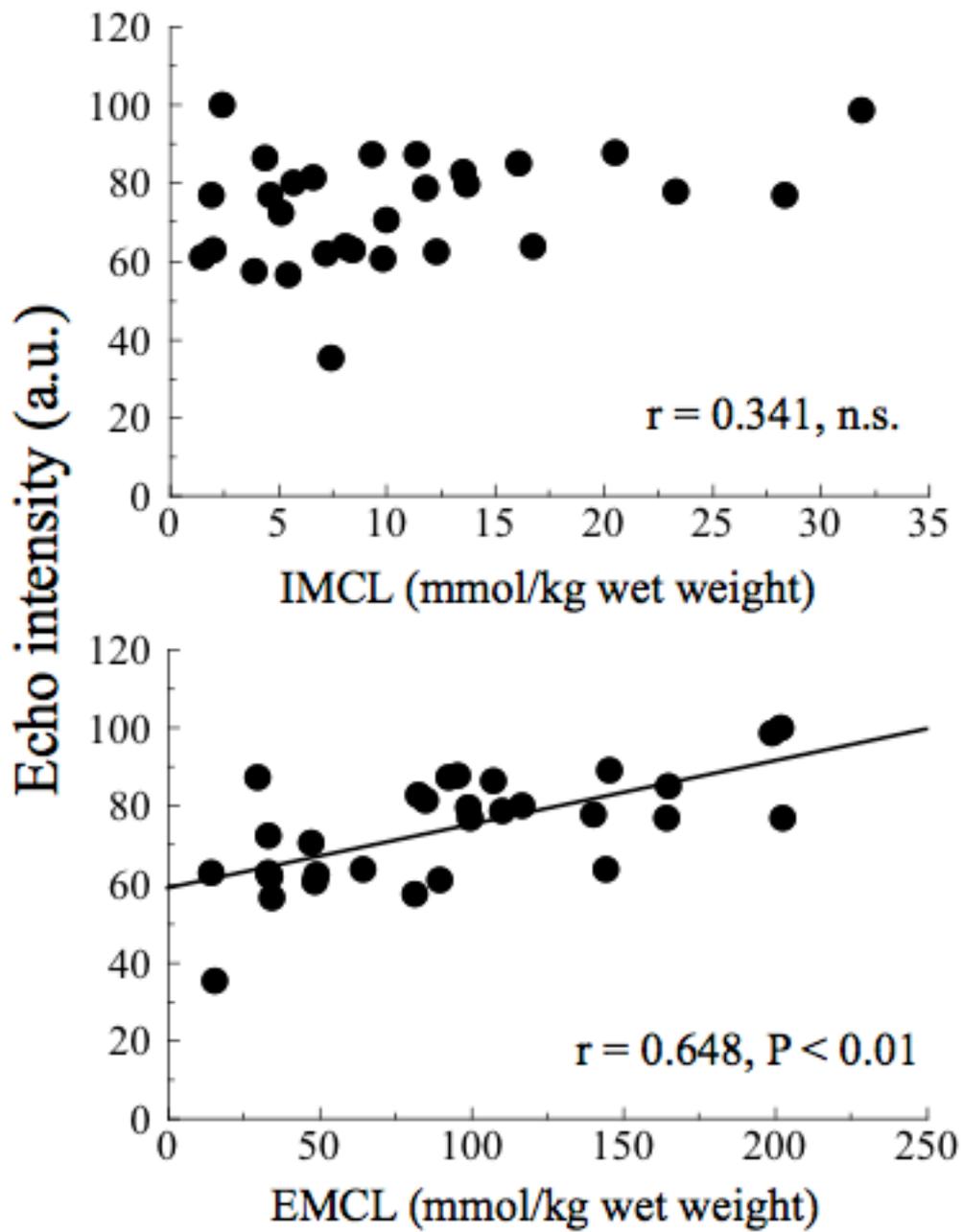


Figure 5

1 Figure 6

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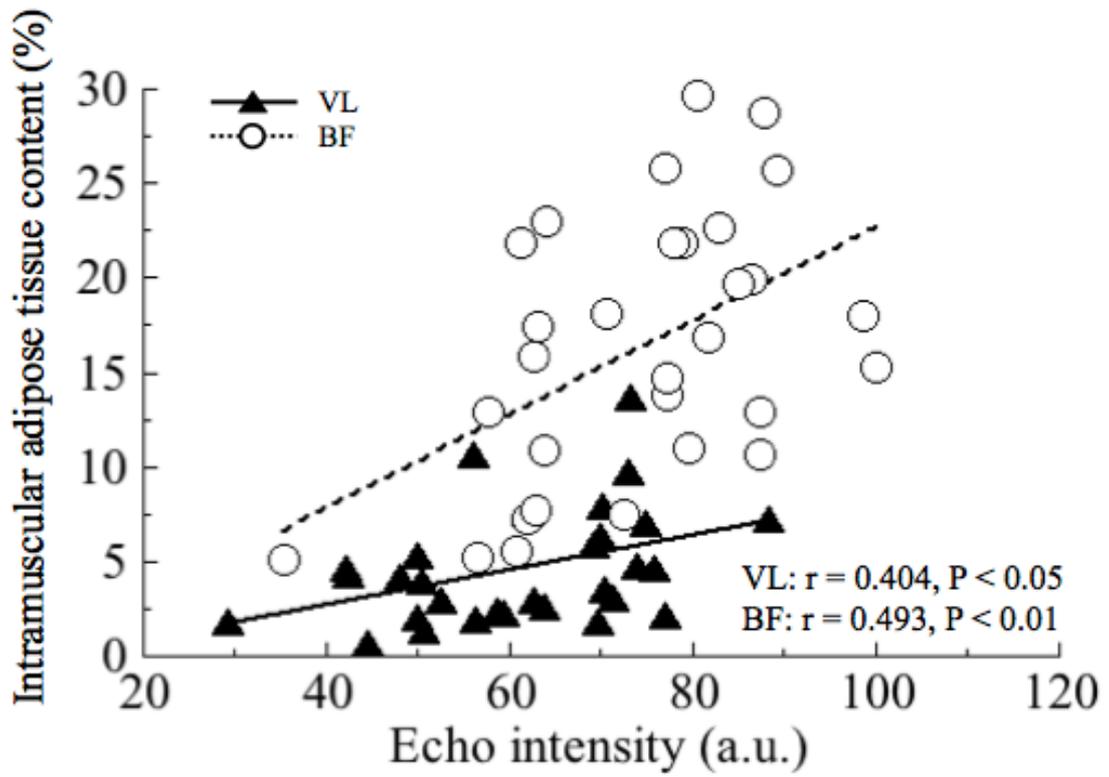


Figure 6