Associations of intramyocellular lipid in vastus lateralis and biceps femoris with blood free fatty acid and muscle strength differ between young and elderly adults

Short running title: Triglyceride in thigh muscles and ageing

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Summary

The present study aimed to determine relationships between intramyocellular lipid and biochemical profiles or muscle strength in elderly (n = 15; mean age, 71 years) and young (n = 15; mean age, 21 years) male and female adults. Levels of intramyocellular lipid in the vastus lateralis and biceps femoris muscles were determined using ^{1}H-magnetic resonance spectroscopy. Fasting blood samples were collected to measure levels of glucose, insulin, hemoglobin A1c, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, free fatty acid, triglyceride, adiponectin, and high sensitivity C-reactive protein. Muscle strength was assessed as maximal voluntary contraction during isometric knee extension. Muscle cross-sectional area in the vastus lateralis was measured using magnetic resonance imaging. Specific force (N/cm^2) indicating force generation capacity was calculated as muscle strength (N) divided by the muscle cross-sectional area of the vastus lateralis (cm^2). The intramyocellular lipid content was similar in both muscles in both groups. The intramyocellular lipid content in the biceps femoris significantly correlated with serum free fatty acid levels ($r = 0.62$, $P < 0.05$), and that in the vastus lateralis significantly and inversely correlated with specific force ($r = -0.58$, $p < 0.05$) in the young, but not in the elderly adults. The relationship between the intramyocellular lipid content in the thigh muscles and biochemical profiles, or specific force differed between elderly and young adults. Age-associated changes in morphology, function and metabolic factors apparently influence intramyocellular lipid metabolism in the thigh muscles.

Key words: ageing; biochemical profile; function; metabolism; morphology; ^{1}H-magnetic resonance spectroscopy; magnetic resonance imaging

Introduction

The increasing prevalence of type 2 diabetes and obesity is causing a critical healthcare problem worldwide, particularly in developed countries (Morley 1998; Wild *et al.,* 2004). Type 2 diabetes and obesity are characterized by insulin resistance in adipose tissue, liver and skeletal muscle. Intramyocellular lipid (IMCL), a primary cause of skeletal muscle insulin resistance, has been investigated in detail (Virkamäki *et al.,* 2001). Intracellular adiposity has been studied in vivo using ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) (Schick *et al.*, 1993). Intramyocellular lipid is partly metabolized to ceramide and diacylglycerol (DAG), both of which can inhibit the insulin signal pathway in skeletal muscles (Coen & Goodpaster 2012).

A cross-sectional study using ^{1}H-MRS has found a higher IMCL content in the soleus of elderly, than young individuals (Cree *et al.,* 2004; Nakagawa *et al.,* 2007; Petersen *et al.,* 2003), and intramyocellular triglyceride (IMTG) accumulation in individuals with sarcopenia, which is age-related skeletal muscle loss (Poggi *et al.*, 1987). Atrophy is induced in the antigravity quadriceps femoris muscles compared with the non-antigravity hamstrings as a result of ageing (Overend *et al.*, 1992). Thus, the IMCL content in the quadriceps femoris might differ from that in the hamstrings.

Compared with the triceps surae, less is known about relationships among blood profiles, IMCL content and function in the quadriceps femoris and hamstrings. Because these muscles play key roles in walking, running and climbing steps, they are critical for elderly individuals to live independently (Overend *et al.* 1992).

Disrupted biochemical profiles such as increases in free fatty acid (FFA), triglycerides (TG), and

in the proportion of small dense low density lipoprotein cholesterol (LDL-C), are features of patients with type 2 diabetes or cardiovascular disease (Kelley *et al.*, 2001; McFarlane *et al.*, 2001). Both TG and LDL-C correlate with the IMCL content in elderly and young adults (Nakagawa *et al.* 2007), suggesting that increase in circulating lipids affect IMCL content. However, whether the relationship between IMCL and biochemical profiles differs between elderly and young adult humans remains unknown.

The role of IMCL has not been established. Resistance training enhances IMCL content and lipid oxidative capacity in sedentary young men (Shepherd *et al.*, 2014), indicating that IMCL can function as an energy substrate. However, a higher skeletal muscle lipid content can negatively affect muscle strength in elderly individuals (Goodpaster *et al.*, 2001). Accordingly, IMCL might play physiological and functional roles that could significantly impact the ageing process.

The present study aimed to determine the relationship between the IMCL content in the thigh muscles and biochemical profiles, and between IMCL content and muscle strength in elderly and young adults. Ageing naturally causes changes in the quality and quantity of skeletal muscle. Therefore, we postulated that the IMCL content correlates with biochemical profiles, and that the IMCL content in the VL correlates with muscle strength in young, but not in elderly adult humans.

Methods

Subjects

Thirty physically active adults (young: male, n = 8; female, n = 7; mean age, 21.0 ± 0.0 years; elderly: male, n = 7; female, n = 8; mean age, 70.7 ± 3.8 years) who lived independently participated in this study. The clinical histories of elderly adults were assessed using questionnaires. Those with a history of heart disease (myocardial infarction, angina pectoris, cardiac insufficiency), cerebrovascular disease (cerebral infarction, hemorrhage), or extreme hypertension (systolic blood pressure ≥ 180mmHg; diastolic blood pressure ≥ 110mmHg) were excluded. Among four with type 2 diabetes, one was medicated with an α-glucosidase inhibitor (α-GI) and thiazolidinedione (TZD), one was medicated with dipeptidyl peptidase inhibitor (DPP-4), and two were medicated with metformin (MET).

All 30 individuals who met the study criteria provided written informed consent to participate in this study, which was approved by the Ethics Committee of the Graduate School of Medicine, Nagoya University, and proceeded in accordance with the guidelines in the Declaration of Helsinki.

Experimental protocol

The experimental protocol consisted of measuring body composition and waist and hip circumference, collecting blood samples, assessment by ^{1}H-MRS and magnetic resonance imaging (MRI) in the morning, and measuring maximal voluntary contraction (MVC) in all participants.

The subjects continued life as usual, but refrained from eating high-fat foods, participating in

sport and consuming more than one alcoholic drink per day for 2 days before the ^{1}H-MRS experiment. A nutritionist calculated total energy intake and the composition of daily food intake from food diaries during the 3 days before ^{1}H-MRS assessment (young: 61.0% carbohydrate, 24.8% fat, and 14.2% protein; mean total energy intake, 28.7 kcal/body weight; elderly: 57.9% carbohydrate, 24.9% fat, and 17.2% protein; mean total energy intake, 33.0 kcal/body weight). Dietary habits were estimated using a food frequency questionnaire (FFQ), and the rate of fat intake in the regular daily diet was similar between the groups. Mean physical activity was estimated using an ambulatory accelerometer (Lifecorder, Suzuken, Nagoya, Japan) for ten days.

Menstrual cycle histories of the young women were assessed using questionnaires. ^{1}H-MRS assessments proceeded during the early, to follicular phase of the menstrual cycle in the young women as described (Larson-Meyer *et al.,* 2002). None of the women were under estrogen replacement therapy.

Blood collection and analysis

Blood samples were collected after an overnight fast. Samples were refrigerated 2-15 °C until serum levels of FFA, TG, total cholesterol (T-C), high density lipoprotein cholesterol (HDL-C), and LDL-C were measured using an enzymatic method. Glucose, hemoglobin A1c (HbA1c), and insulin were measured using the hexokinase method, latex immunoagglutination and chemiluminescence enzyme immunoassays, respectively. Serum high sensitivity C-reactive protein (hs-CRP) and adiponectin were measured using latex immunity nephelometry.

Insulin resistance was estimated using the homeostasis model assessment index (HOMA-IR) derived from fasting glucose and insulin concentrations and calculated as fasting plasma insulin concentration (μIU/mL) × fasting plasma glucose concentration (mg/dL) divided by 405 (Matthews *et al.*, 1985).

Anthropometric

Waist circumference was measured at the level of the navel, hip circumference was taken as the largest circumference of the pelvis, and then the waist-to-hip ratio (WHR) was calculated.

^{1}H-Magnetic resonance spectroscopy

Intramyocellular lipid in the right thigh was assessed by ^{1}H-MRS using a 3.0 T MAGNETOM Verio whole body system (Siemens, Munich, Germany) with a 4-channel flex coil (366 × 174 mm). Voxels (11 × 11 × 20 mm) positioned in the VL and long head of biceps femoris (BF) at the mid-thigh between the greater trochanter and lateral condyle of the femur, starting at the greater trochanter. Care was taken to avoid visible vascular structures, adipose tissue deposits and connective tissues within the voxel. We acquired ^{1}H-MRS spectra from regions of interest using a point-resolved spectroscopy sequence (PRESS) with the following acquisition parameters: repetition time/echo time, 4000/30 ms, 128 averages. The unsuppressed water signal was subsequently measured in the same voxel under the same shimming conditions as a reference signal (Boesch *et al.*, 2006).

Post processing

All ^{1}H-MRS data were fit using LCModel software v. 6.2-4A (Provencher 1993) (Stephen Provencher, Inc., Oakville, Ontario, Canada). The spectroscopic data acquired from the MR scanner were collected in a Linux computer, and metabolism was quantified using eddy current correction and water scaling. The water concentration was assumed to be equal to 42.4 mmol/kg wet weight based on a mean adult muscle tissue water content of 77% (Sjogaard & Saltin 1982). The IMCL ($-CH_2$) and extramyocellular lipid (EMCL) ($-CH_2$) concentration were collected for the T1 and T2 relaxation effects of the unsuppressed water peak using LCModel control parameter atth2o, which were determined using the following equation: exp (-TE/T2) [1-ext (-TR/T1)] (Drost *et al.*, 2002), assuming relaxation time T1 = 369 ms, T2 = 89.4 ms; T1 = 369 ms, T2 = 77.6 ms for the $IMCL_{CH2}$ and $EMCL_{CH2}$ (Krssak *et al.,* 2004). The concentration of lipid molecules (total lipid content) was computed by dividing the sum of the $EMCL_{CH2}$ and $IMCL_{CH2}$ concentrations by 31, a value that was derived from the assumption that the average number of methylene protons is 62 per TG molecule, which is equivalent to 31 CH_2 groups (Boesch *et al.,* 1999; Szczepaniak *et al.,* 1999; Weis *et al.,* 2009). The data were converted from mM to mmol/kg wet weight, assuming a tissue density 1.05 g/mL for skeletal muscle (Szczepaniak *et al.* 1999). We acquired IMCL data from the VL and BF of all 15 of the younger individuals and from 12 and 14 of the elderly individuals, respectively.

Muscle cross-sectional area

The right mid-thigh of each subject was assessed by MRI using the 3.0 T whole body system to

determine muscle cross-sectional area (mCSA). T1-weighted spin echo, axial-plane imaging proceeded under the following conditions, repetition time/echo time, 604/11 ms, matrix 256 × 256, field of view 256 mm, slice thickness 10 mm, and interslice gap 0 mm. Prone subjects were imaged with pillows placed under the buttocks and leg to minimize tissue compression in the thigh. The mCSA of the VL was estimated using one axial image, which was transferred to a Let's Note personal computer (Panasonic, Osaka, Japan) equipped with the NIH Image software (National Institute of Health, Bethesda, MD, USA). Moreover, the muscle mass of the VL and BF was estimated using a modified mCSA according to Kanehisa et al. (Kanehisa *et al.*, 1994).

Muscle strength

The subjects were familiarized with the experiments at the laboratory at least 1 week before testing. We measured MVC force during unilateral isometric knee extension using a custom-made dynamometer (Takei Scientific Instruments Co. Ltd., Tokyo, Japan), as we previously described (Akima *et al.*, 2008). The hip was strapped to the dynamometer and the knee joint was flexed at 90° (0° = fully extended). The length of the vertical lever arm was adjusted for the leg length of the subjects who were fixed with straps during three or four MVC tests of the right leg at about 3 minute intervals. We considered the maximal attempt as the test that yielded the highest force. Isometric knee extension force is expressed both as absolute value (N) and as the ratio of force to mCSA of the VL, namely, specific force (N/cm^2). We selected the mCSA of VL, because this is the largest among the quadriceps femoris group of muscles (Akima *et al.*, 2007). Therefore, specific force was used as an index of the

force generation capacity of the quadriceps femoris.

Statistics analysis

All data are shown as means and standard deviation (SD). Differences between the young and elderly individuals were evaluated using a two-tailed Student's t test. Relationships between IMCL and biochemical profiles, or specific force were assessed using Pearson's correlation analyses. The level of significance was set at $p < 0.05$.

Results

Physical and biochemical profiles and characteristics of skeletal muscle

Table 1 shows the physical and biochemical profiles, as well as the skeletal muscle characteristics of the two groups. The elderly group was significantly shorter and had significantly higher systolic blood pressure (BP) and WHR than the young group, whereas weight, body mass index (BMI), diastolic BP and physical activity did not significantly differ. Fasting glucose, HbA1c, T-C and LDL-C, TG and hs-CRP values were significantly higher in the elderly, than in the young group, whereas fasting insulin, HDL-C, FFA, adiponectin and HOMA-IR did not significantly differ. The mCSA of the VL and BF, MVC and the muscle mass of the VL were significantly lower in the elderly, than in the young group, whereas the muscle mass of BF did not significant differ.

Findings of IMCL

Figure 1 shows that the IMCL contents in the VL and BF did not significantly differ between the young and elderly groups ($p = 0.32$ and $p = 0.06$, respectively). The IMCL contents also did not significantly differ between the VL and BF of these groups ($p = 0.68$ and $p = 0.30$, respectively).

Relationship between IMCL and serum FFA or biochemical profiles

The IMCL content significantly correlated with serum FFA levels ($r = 0.62$, $p < 0.05$) in the BF, but not in the VL ($r = 0.50$, $p = 0.06$) in the young group (Fig. 2a, b). IMCL and serum FFA did not significantly correlate in the VL or BF ($r = -0.33$, $p = 0.30$ and $r = -0.05$, $p = 0.86$, respectively) of the

elderly group (Fig. 2c, d).

Table 2 shows the relationship between IMCL and biochemical values from both muscles in the young and elderly groups. The values for glucose, insulin, HbA1c, T-C, HDL-C, LDL-C, TG, adiponectin and hs-CRP, as well as HOMA-IR, did not significantly correlate with IMCL in both muscles in the young and elderly groups.

Relationship between IMCL in VL and specific force

Figure 3 shows the relationship between IMCL in VL and specific force in the two groups. Intramyocellular lipid in the VL inversely correlated with specific force in the young ($r = -0.58$, $p < 0.05$), but not in the elderly ($r = 0.01$, $p = 0.97$) groups.

Discussion

We compared relationships between IMCL contents in VL and BF and biochemical profiles, and between IMCL contents in the VL and specific force in elderly and young adult humans. We discovered that the IMCL contents in the BF correlated with serum FFA, whereas that in the VL inversely correlated with specific force in the young, but not in the elderly group. The findings suggest that changes due to ageing influence these relationships.

The IMCL content in both muscles did not significantly differ between the elderly and young groups. This finding contradicts those of Petersen et al. (Petersen *et al.* 2003), Cree et al. (Cree *et al.* 2004), Nakagawa et al. (Nakagawa *et al.* 2007), who found higher IMCL content in soleus muscles from elderly, than from young individuals using ^{1}H-MRS (Cree *et al.* 2004; Nakagawa *et al.* 2007; Petersen *et al.* 2003). We anticipated that more IMCL would be present in the thigh muscles of elderly, than young individuals, but, the difference in the type of muscle might explain our findings. Type I muscle fibers contain threefold more IMCL than type II (Saltin & Gollnick 1983). Because the VL or BF contains more type II fibers than the soleus, which predominantly comprises type I fibers, a difference in the IMCL content between elderly and young individuals might be less detectable.

We considered that the IMCL content would be lower in the BF, a non-antigravity muscle, than in the VL, an antigravity muscle, especially in the elderly group. Ageing is associated with IMTG accumulation in atrophied muscles (Poggi *et al.* 1987; Stein & Wade 2005), and atrophy is more remarkable in the quadriceps femoris than in the hamstrings (Overend *et al.* 1992). The muscle mass of the VL was significantly lower in the elderly, than in young group, whereas that of the BF did not

significantly differ. However, the IMCL content in VL and BF was similar between the two groups.

We discovered a significant correlation between IMCL in BF and serum FFA in the young group ($r = 0.62$, $p < 0.05$), which is consistent with the results of a previous study of the influence of FFA on IMCL (Boesch *et al.* 2006). Circulating FFA is re-esterified in myocytes into IMCL while fasting (Jensen *et al.*, 2001). According to Stannard et al. (Stannard *et al.*, 2002), both circulating FFA and IMCL levels increase in young individuals during fasting. The levels of circulating FFA and IMCL might be mutually dependent. However, such a correlation was not found in the elderly group. An imbalance between circulating FFA and IMCL can be caused by an age-related decrease in the amount of mitochondria and/or lower enzyme activities involved in lipid oxidation. Such age-related changes can cause increases in the IMCL content of muscles in elderly individuals (Crane *et al.*, 2010).

We found that IMCL in VL was inversely correlated with specific force in the young group ($r = -0.58$, $p < 0.05$). According to Shepherd et al. (Shepherd *et al.* 2014), resistance training induces increases in lipid oxidative capacity and IMTG breakdown in both type I and II fibers during moderate-intensity exercise. That report indicates that young individuals in high muscle strength have high IMCL oxidative capacity. Therefore, our results suggest that the correlation between IMCL and muscle strength reflects the characteristics of muscles trained by daily activities in young individuals. In contrast, we did not find such an association in the elderly group. Ageing causes changes in IMCL uptake (Crane *et al.* 2010) and in the neuromuscular system (Clark & Manini 2008), and such changes might influence the association between IMCL and specific force in elderly individuals.

In conclusion, the relationship between IMCL content in the thigh muscles and biochemical

profiles or specific force differed between elderly and young individuals. These results suggest that age-associated changes in morphology, function and metabolic factors influence IMCL metabolism in the thigh muscles (VL and BF).

Acknowledgements

This project is supported by a Grant-in-Aid for challenging Exploratory Research from the Ministry of Education, Culture, Sports and Science and Technology Grant (#23650432) to HA, the Descente and Ishimoto Memorial Foundation for the promotion of Sport Science to YO. We are grateful to Haruo Isoda, MD, Atsushi Fukuyama PhD, and Akira Ishizuka RT at the Nagoya University Brain & Mind Research Center, to Naoji Yasue MD and Masumi Morita RN at the Yasue clinic and to Yuko Shibata PhD at the Nagoya University sports club for help with this project.

Conflict of interest

The authors declare that they have no conflict of interest.

Reference

Akima H, Hioki M and Furukawa T. Effect of arthroscopic partial meniscectomy on the function of quadriceps femoris. *Knee Surg Sports Traumatol Arthrosc* (2008); **16**: 1017-1025.

Akima H, Ushiyama J, Kubo J, Fukuoka H, Kanehisa H and Fukunaga T. Effect of unloading on muscle volume with and without resistance training. *Acta Astronautica* (2007); **60**: 728-736.

Boesch C, Décombaz J, Slotboom J and Kreis R. Obeservation of intramyocellular lipids by means of 1H magnetic resonance spectroscopy. *Proc Nutr Soc* (1999); **58**: 841-850.

Boesch C, Machann J, Vermathen P and Schick F. Role of proton MR for the study of muscle lipid metabolism. *NMR Biomed* (2006); **19**: 968-988.

Clark BC and Manini TM. Sarcopenia =/= dynapenia. *J Gerontol A Biol Sci Med Sci* (2008); **63**: 829-834.

Coen PM and Goodpaster BH. Role of intramyocelluar lipids in human health. *Trends Endocrinol Metab* (2012); **23**: 391-398.

Crane JD, Devries MD, Safdar A, Hamadeh MJ and Tarnopolsky MA. The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure. *J Gerontol A Biol Sci Med Sci* (2010); **65A**: 119-128.

Cree MG, Newcomer BR, Katsanos CS, Sheffield-Moore M, Chinkes D, Aarsland A, Urban R and Wolfe RR. Intramuscular and liver triglycerides are increased in the elderly. *J Clin Endocrinol Metab* (2004); **89**: 3864-3871.

Drost DJ, Riddle WR and Clarke GD. Proton magnetic resonance spectroscopy in the brain: report of AAPM MR Task Group #9. *Med Phys* (2002); **29**: 2177-2197.

Goodpaster BH, Carlson CL, Visser M, Kelley DE, Scherzinger A, Harris TB, Stamm E and Newman AB. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *J Appl Physiol* (2001); **90**: 2157-2165.

Jensen MD, Ekberg K and Landau BR. Lipid metabolism during fasting. *Am J Physiol Endocrinol Metab* (2001); **281**: E789-793.

Kanehisa H, Ikegawa S and Fukunaga T. Comparison of muscle cross-sectional area and strength between untrained women and men. *Eur J Appl Physiol Occup Physiol* (1994); **68**: 148-154.

Kelley DE, Williams KV, Price JC, McKolanis TM, Goodpaster BH and Thaete FL. Plasma fatty acids, adiposity, and variance of skeletal muscle insulin resistance in type 2 diabetes mellitus. *J Clin*

Endocrinol Metab (2001); **86**: 5412-5419.

Krssak M, Mlynarik V, Meyerspeer M, Moser E and Roden M. 1H NMR relaxation times of skeletal muscle metabolites at 3 T. *MAGMA* (2004); **16**: 155-159.

Larson-Meyer DE, Newcomer BR and Hunter GR. Influence of endurance running and recovery diet on intramyocellular lipid content in women: a 1H NMR study. *Am J Physiol Endocrinol Metab* (2002); **282**: E95-E106.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF and Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* (1985); **28**: 412-419.

McFarlane SI, Banerji M and Sowers JR. Insulin resistance and cardiovascular disease. *J Clin Endocrinol Metab* (2001); **86**: 713-718.

Morley JE. The elderly Type 2 diabetic patient: special considerations. *Diabet Med* (1998); **15 Suppl 4**: S41-46.

Nakagawa Y, Hattori M, Harada K, Shirase R, Bando M and Okano G. Age-related changes in intramyocellular lipid in humans by in vivo H-MR spectroscopy. *Gerontology* (2007); **53**: 218-223.

Overend TJ, Cunningham DA, Paterson DH and Lefcoe MS. Thigh composition in young and elderly men determined by computed tomography. *Clin Physiol* (1992); **12**: 629-640.

Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, DiPietro L, Cline GW and Shulman GI. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* (2003); **300**: 1140-1142.

Poggi P, Marchetti C and Scelsi R. Automatic morphometric analysis of skeletal muscle fibers in the aging man. *Anat Rec* (1987); **217**: 30-34.

Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Reson Med* (1993); **30**: 672-679.

Saltin B and Gollnick PD. Skeletal muscle adaptability: significance for metabolism and performance. *In : skeletal muscle (Peachey, L D ed), Baltimore, Waverly Press, Inc* (1983): 555-631.

Schick F, Eismann B, Jung WI, Bongers H, Bunse M and Lutz O. Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. *Magn Reson Med* (1993); **29**: 158-167.

Shepherd SO, Cocks M, Tipton KD, Witard OC, Ranasinghe AM, Barker TA, Wagenmakers AJ and Shaw CS. Resistance training increases skeletal muscle oxidative capacity and net intramuscular triglyceride breakdown in type I and II fibres of sedentary males. *Exp Physiol* (2014); **99**: 894-908.

Sjogaard G and Saltin B. Extra- and intracellular water spaces in muscles of man at rest and with dynamic exercise. *Am J Physiol* (1982); **243**: R271-280.

Stannard SR, Holdaway MA, Sachinwalla T and Cunningham CW. Fasting for 72 h increases intramyocellular lipid content in nondiabetic, physically fit men. *Am J Physiol Endocrinol Metab* (2002); **283**: E1185-E1191.

Stein TP and Wade CE. Metabolic consequences of muscle disuse atrophy. *J Nutr* (2005); **135**: 1824S-1828S.

Szczepaniak LS, Babcock EE, Schick F, Dobbins RL, Garg A, Burns DK, Mcgarrt JD and Stein DT. Measurement of intracellular triglyceride stores by 1H spectroscopy: validation in vivo. *Am J Physiol Endocrinol Metab* (1999); **276**: 977-988.

Virkamäki A, Korsheninnikova E, Seppälä-Lindroos A, Vehkavaara S, Goto T, Halavaara J, Häkkinen AM and Yki-Järvinen H. Intamyocellular lipid is associated with resistance to in vivo insulin actions on glucose uptake, antilipolysis, and early insulin signaling pathways in human skeletal muscle. *Diabetes* (2001); **50**: 2337-2343.

Weis J, Johansson L, Ortiz-Nieto F and Ahlstrom H. Assessment of lipids in skeletal muscle by LCModel and AMARES. *J Magn Reson Imaging* (2009); **30**: 1124-1129.

Wild S, Roglic G, Green A, Sicree R and King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* (2004); **27**: 1047-1053.

Table 1. Physical biochemical characteristics and body composition of subjects.

	Young (n = 15)	Elderly (n = 15)
Physical characteristics		
Age (year)	21.0 ± 0.0	70.7 ± 3.8
Height (cm)	167.2 ± 10.9	157.2 ± 6.4‡
Weight (kg)	62.3 ± 10.8	55.8 ± 7.7
BMI (kg/m^2)	22.2 ± 2.7	22.5 ± 2.1
Resting systolic BP (mmHg)	118.5 ± 9.6	131.2 ± 10.3‡
Resting diastolic BP (mmHg)	73.1 ± 8.5	76.3 ± 8.6
WHR	0.8 ± 0.1	0.9 ± 0.1††
Physical activity level (kcal/day)	266.7 ± 103.0	220.5 ± 76.4
Blood biochemistry		
Fasting glucose (mg/dL)	86.1 ± 5.7	101.1 ± 16.9‡
Fasting insulin (μIU/mL)	6.4 ± 3.0	4.5 ± 2.3
$HbA1_C$ (%) (NGSP)	5.4 ± 0.2	6.1 ± 0.8‡
Total cholesterol (mg/dL)	174.7 ± 26.4	206.5 ± 28.5‡
HDL-cholesterol (mg/dL)	62.9 ± 7.9	57.0 ± 11.8
LDL-cholesterol (mg/dL)	97.6 ± 26.9	124.9 ± 25.2‡
FFA (μEq/L)	489.3 ± 261.3	618.6 ± 137.8
TG (mg/dL)	62.6 ± 19.4	98.1 ± 45.0‡
Adiponectin (μg/mL)	11.0 ± 3.5	12.2 ± 6.2
hs-CRP (μg/mL)	0.3 ± 0.2	0.6 ± 0.6†
HOMA-IR	1.3 ± 0.5	1.1 ± 0.6
Skeletal muscle		
mCSA of VL (cm^2)	21.0 ± 3.4	14.5 ± 4.0††
mCSA of BF (cm^2)	9.8 ± 2.1	8.3 ± 1.2†
Muscle mass of VL (mCSA$\cdot l^{-2}(\times 10^{-4})$)	158.9 ± 26.8	126.3 ± 26.5‡
Muscle mass of BF (mCSA$\cdot l^{-2}(\times 10^{-4})$)	73.7 ± 14.9	73.4 ± 11.0
MVC during isometric knee extension (N)	316.8 ± 118.7	178.8 ± 66.0§

All values are means ± SD. BF, long head of biceps femoris; BMI, body mass index; BP, blood pressure; FFA, free fatty acid; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; hs-CRP, high sensitivity C-reactive protein; *l*, length of thigh; LDL, low density lipoprotein; mCSA, muscle cross sectional area; MVC, muscle voluntary contraction; TG, triglyceride; VL, vastus lateralis; WHR, waist-to-hip ratio. Only three young subjects had hs-CRP value < 0.05, therefore these tree values were taken as being as 0.05. †$p < 0.05$; ‡$p < 0.01$; §$p < 0.001$; ††$p < 0.0001$ versus young adults.

Table 2. Correlation coefficients between IMCL and biochemical values from VL and BF in the young and elderly groups.

	Young		Elderly	
	VL (n = 15)	BF (n = 15)	VL (n = 12)	BF (n = 14)
Fasting glucose (mg/dL)	-0.39	0.40	0.26	-0.33
Fasting insulin (μIU/mL)	-0.15	0.13	0.24	-0.09
$HbA1_C$ (%) (NGSP)	-0.18	-0.03	0.46	0.04
Total cholesterol (mg/dL)	-0.13	0.03	-0.17	-0.34
HDL-cholesterol (mg/dL)	-0.28	0.18	-0.47	-0.40
LDL-cholesterol (mg/dL)	-0.03	0.00	-0.07	-0.24
TG (mg/dL)	-0.26	-0.08	0.37	0.31
Adiponectin (μg/mL)	-0.11	0.28	-0.32	-0.25
hs-CRP (ng/mL)	0.50	0.22	0.39	-0.15
HOMA-IR	-0.09	0.34	0.34	-0.17

BF, long head of biceps femoris; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment index; hs-CRP, high sensitivity C-reactive protein; IMCL, intramyocellular lipid; LDL, low density lipoprotein; TG, triglyceride; VL, vastus lateralis. No significant correlation was observed between IMCL and all biochemical values (excluded free fatty acid).

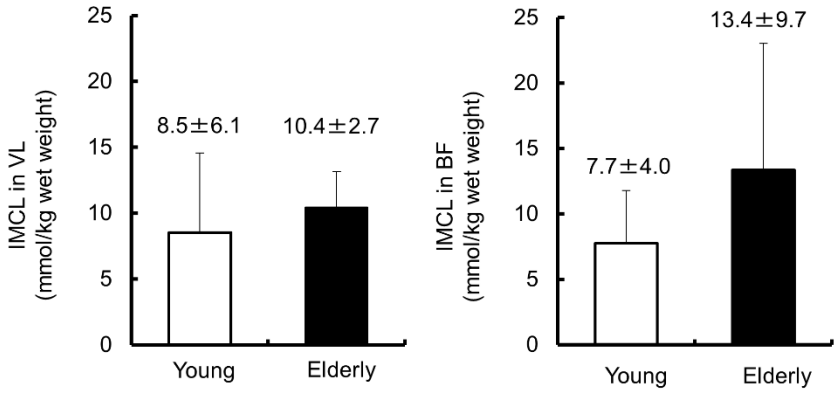

Figure 1 Comparison of IMCL contents of VL and BF between young and elderly individuals. BF, long head of biceps femoris; IMCL, intramyocellular lipid; VL, vastus lateralis.

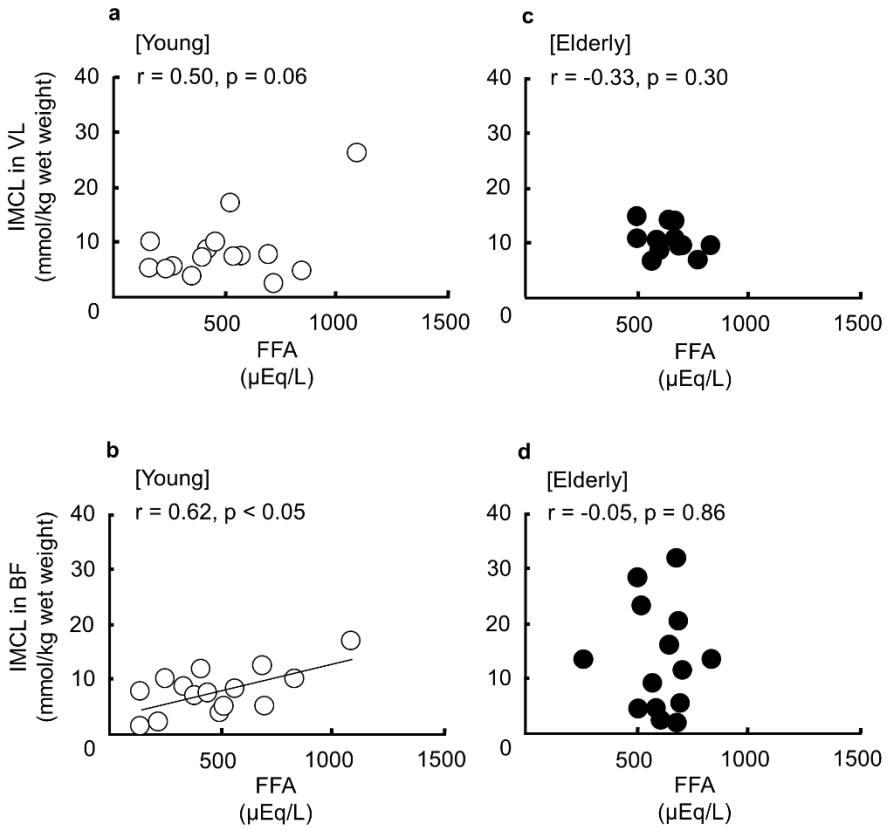

Figure 2 Relationship between FFA and IMCL in VL and BF of the young (a, b) and elderly (c, d) individuals.
BF, long head of biceps femoris; FFA, free fatty acid; IMCL, intramyocellular lipid; VL, vastus lateralis.

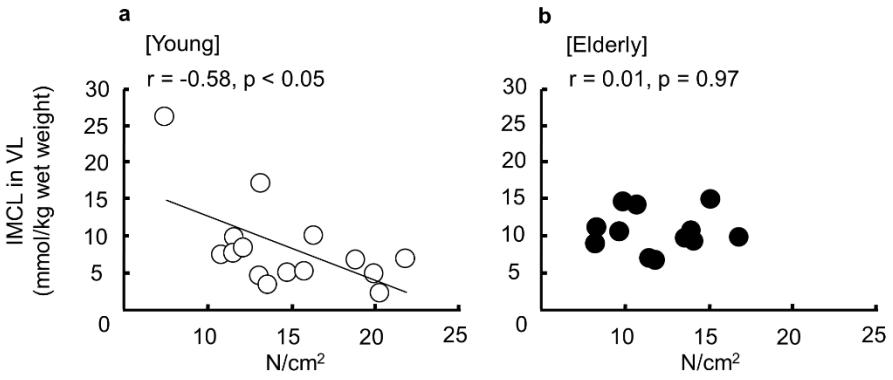

Figure 3 Relationship between IMCL in VL and specific force in young (a) and elderly (b) individuals.
IMCL, intramyocellular lipid; VL, vastus lateralis.