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ORIGINAL ARTICLE

Relationship between physical activity and intramyocellular lipid content is different between young and older adults

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Abstract

Purpose Intramyocellular lipid (IMCL) is influenced by physical exercise; however, whether the habitual level of physical activity affects resting IMCL content remains unclear. The purpose of this study was to determine the relationship between physical activity levels and resting IMCL content in young and older adults.

Methods In total, 15 nonobese young adults (21.0 ± 0.0 years) and 15 older adults (70.7 ± 3.8 years) were recruited. Time spent performing physical activities for 10 days was assessed using a three-dimensional ambulatory accelerometer, 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. The IMCL content in the vastus lateralis was determined using ^{1}H-magnetic resonance spectroscopy after overnight fasting.

Results No significant differences in IMCL content were observed between young and older adults. Vigorous intensity physical activity (time and MET h) was significantly lower in older than young adults ($p < 0.01$); this difference was not observed for light and moderate intensity physical activity. Light intensity physical activity (time and MET h) was significantly and inversely correlated with IMCL content in young adults ($r = -0.59$ and $r = -0.58$; both $p < 0.05$), but not in older adults.

Conclusions These results suggest that daily light intensity physical activity reduces resting IMCL content in young adults, whereas no significant relationship was seen between daily physical activity and resting IMCL content in older adults.

Keywords Metabolism · Metabolic equivalent · ^{1}H-magnetic resonance spectroscopy · Aging · Skeletal muscle · Daily living

Abbreviations

EMCL	Extramyocellular lipid
FFA	Free fatty acid
^{1}H-MRS	Magnetic resonance spectroscopy
IMCL	Intramyocellular lipid
IMTG	Intramyocellular triglyceride
MET	Metabolic equivalent
MET h	Metabolic equivalent × hours
$VO_{2\,max}$	Maximum oxygen uptake
VL	Vastus lateralis

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Introduction

Lipids, which are hydrophobic molecules, play an important physiologic role by supplying energy substrates during physical activity. During light to vigorous intensity physical activity, plasma free fatty acid (FFA) is the energy source responsible for approximately 20–80% of the energy expended to produce adenosine triphosphate in mitochondria (Egan and Zierath 2013; Romijn et al. 1993). Excessive FFA is re-esterified in myocytes and stored as intramyocellular lipid (IMCL) for future use as a metabolic fuel (Coen and Goodpaster 2012; Jensen et al. 2001). ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) and muscle tissue samples obtained using needle biopsy have shown that IMCL decreases after aerobic endurance exercise (Bucher et al. 2014; Egger et al. 2013; Van Proeyen et al. 2011; White et al. 2003a, b) and resistance exercise (Koopman et al. 2006; Shepherd et al. 2014) in healthy participants. This evidence clearly shows that IMCL is used as a fuel during endurance and resistance types of exercise.

Exercise time and intensity are important determinants of the physiologic responses to exercise training (Garber et al. 2011). Plasma FFA oxidation accounts for almost all fat oxidation that occurs during light intensity exercise (25% of maximum oxygen uptake [$VO_{2\,max}$]), whereas intramyocellular triglycerides (IMTGs) are an important fuel during prolonged (> 90 min) moderate intensity exercise and provide 25% of total energy; however, they tend to contribute less during low and high intensity exercise (Egan and Zierath 2013). Sedentary and light intensity physical activities comprise a relatively large portion (97%) of the daily activities performed by men and women aged 20–79 years compared with moderate to vigorous intensity physical activity intensity (3%) (Colley et al. 2011). Therefore, IMCL oxidation depends on the time and intensity of physical activities in daily living, i.e., metabolic equivalents (METs) × hours (MET h).

Aging induces an imbalance between muscle lipid delivery and oxidation (Chee et al. 2016). According to Sial et al. (1996), when young and older adults performed similar, relatively intense, moderate exercise [50% $VO_{2\,max}$], FFA oxidation was lower and carbohydrate oxidation was higher in older adults. Moreover, despite similar physical activity patterns in older and young adults, IMCL content was higher in older adults (Crane et al. 2010). These results suggest that aging may affect the relationship between physical activity levels and IMCL content. Our previous study (Hioki et al. 2016) demonstrated that fasting FFA correlated with IMCL in young but not older adults. These results suggest that 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. By contrast, physically active older adults have a high lipid oxidation capacity and IMCL uptake (Rouffet et al. 2013). Increased adiposity and lower habitual levels of physical activity also affect lipid oxidation in older individuals (Boon et al. 2007). Therefore, physical activity levels might decrease IMCL content significantly in older adults; however, this process remains poorly understood.

The present study therefore investigated the relationship between physical activity and resting IMCL content in young and older adults. Physical activity level was expressed as time and MET h. We hypothesized that physical activity (time and MET h) would correlate with resting IMCL content in young but not older adults. IMCL accumulation is a major factor in inducing skeletal muscle insulin resistance (Goodpaster and Sparks 2017). Therefore, elucidating differences in the relationship between physical activity and IMCL content in older and young adults is an important step in understanding the causes of abnormal IMCL accumulation with aging, which may promote better health in adults.

Methods

Participants

Fifteen young (eight men, seven women; all 21 years of age) and 15 older adults (seven men, eight women; age range, 65–78 years) who were living independently participated in the present study. Athletes with good endurance or resistance training (marathon runners or sprinters) who were participating in competitive sports were excluded from the study. The older adults exercised once a week in local sports clubs designed specifically for older people. Participants with a history of heart disease (e.g., myocardial infarction, angina pectoris, cardiac insufficiency), cerebrovascular disease (e.g., cerebral infarction, hemorrhage), or extreme hypertension (e.g., systolic blood pressure ≥ 180 mmHg, diastolic blood pressure ≥ 110 mmHg) were excluded. The clinical histories of the older adults were assessed using questionnaires. Among four participants with type 2 diabetes, one was receiving an α-glucosidase inhibitor and thiazolidinedione, one a dipeptidyl peptidase inhibitor, and two were medicated with metformin. The menstrual cycle histories of the young women were determined using questionnaires. ^{1}H-MRS was performed during the follicular phase of their menstrual cycle.

All participants provided written informed consent to participate in this study, which was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the Graduate School of Medicine, Nagoya University.

Parts of the data have been previously reported (Akima et al. 2015, 2016; Hioki et al. 2016; Yoshiko et al. 2017).

Study design

The study protocol consisted of measuring body composition and assessing IMCL by ^{1}H-MRS in the morning in all participants. ^{1}H-MRS was performed after 10 h of overnight fasting. The spontaneous 10 days of physical activity and habitual dietary intake were assessed. The participants continued life as usual, but refrained from eating high-fat foods, participating in sports, and consuming more than one alcoholic drink per day for 2 days before the ^{1}H-MRS measurements. Dietary intake during the 3 days before ^{1}H-MRS assessment and habitual dietary intake were calculated by a nutritionist.

Physical activity

The physical activity time during these 10 days was determined from the records of the three-dimensional ambulatory accelerometer (Lifecorder, Suzuken Co., Ltd, Nagoya, Japan). According to technical details provided by Kumahara et al. (2004) and the manufacturer, a 32-Hz sampling acceleration assesses values ranging from 0.06 to 1.94 g (1.00 g is equal to the acceleration of free fall). Physical activity levels and METs while walking are strongly correlated when measured using this accelerometer ($r^2 = 0.93$; $p < 0.01$) (Kumahara et al. 2004). The accelerometric signal is filtered by an analog bandpass filter and digitized. A maximum pulse recorded over 4 s is taken as the accelerometric value. Physical activity was categorized into 11 activity levels [level 0.0 (slight movement corresponding to < 0.06 g), level 0.5, and levels 1–9 of activity] based on the pattern of the accelerometric signal. A level 0.5 (slight movement corresponding to > 0.06 g) assumes that the participant is standing up (or sitting down) and maintaining that state. Physical activity levels were converted by an algorithm to calculate the energy expended (kcal) when the sensor detected three acceleration pulses or acceleration for > 4 consecutives, and the activity levels were categorized into levels 1–9. Movements categorized as level 0 and 0.5 were excluded. These acceleration pulses (i.e., energy expenditures) were counted every 4 s and used to calculate activity levels using body weight and a modulus for each activity level. See Kumahara et al. (2004) for additional details. The movements counted every 4 s and the data were converted to physical activity time. The physical activity intensity levels 1–9 were defined as follows: level 1 (1.8 METs), level 2 (2.3 METs), level 3 (2.9 METs), level 4 (3.6 METs), level 5 (4.3 METs), level 6 (5.2 METs), level 7 (6.1 METs), level 8 (7.1 METs), and level 9 (> 8.3 METs). These physical activity levels were broadly classified as light (< 3.0 METs), moderate (3.0–6.0 METs), and vigorous intensity (> 6.0 METs) (Kumahara et al. 2004).

The accelerometer was worn on either the left or right side of the belt at waist level during physical activity, but was not worn while bathing or sleeping. We instructed that the accelerometer was to be kept horizontal throughout the 14 days of continuous wear, after which, the device was retrieved. Data were transferred from the accelerometer to a computer using Excel software (Microsoft Japan Co., Ltd, Tokyo, Japan). The data for times that participants forgot to wear the accelerometer were excluded. This investigation analyzed 10 days starting from the first or second day in the morning, and the data were averaged for the 10-day period.

^{1}H-MRS

^{1}H-MRS was performed using a 3.0 T MAGNETOM Verio (Siemens Healthcare GmbH, Eschborn, Germany) with a 4-channel flex coil (366 × 174 mm). Voxels of 11 × 11 × 20 mm were placed in the vastus lateralis (VL) at the middle thigh between the greater trochanter and lateral condyle of the femur, and visible adipose tissue, connective tissue, and vessels were avoided. We acquired ^{1}H-MRS spectra from a volume of interest using a point-resolved spectroscopy sequence and 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 processed using LCModel version 6.2-4A (Stephen Provencher, Inc., Oakville, Ontario, Canada) (Provencher 1993). Data were transferred from the magnetic resonance scanner to a Linux computer, and metabolite quantification was performed using eddy current correction and water scaling. The water concentration was assumed to be 42.4 mmol per kg wet weight based on a mean water content of 77% in adult muscle tissue (Sjogaard and Saltin 1982). The concentrations of $IMCL_{CH2}$ and extramyocellular lipid ($EMCL_{CH2}$) were computed as mmol per liter of muscle tissue (mM), and collected for the T1 and T2 relaxation effects of the water reference using LCModel's control parameter atth2o, which were determined using the following equation: ext (− TE/T2) [1 − ext (− TR/T1)] (Drost et al. 2002), assuming relaxation times T1 = 369 ms, T2 = 89.4 ms and T1 = 369 ms, T2 = 77.6 ms for the $IMCL_{CH2}$ and $EMCL_{CH2}$, respectively (Krssak et al. 2004). The concentration of total lipid content was computed by the summation of $IMCL_{CH2}$ and $EMCL_{CH2}$ concentrations and divided by a factor of 31. The value 31 follows from the assumption that the average number of methylene

protons is 62 per triglyceride molecule (equivalent to 31 CH_2 groups) (Boesch et al. 1999; Szczepaniak et al. 1999; Weis et al. 2009). This value was divided by the tissue density of 1.05 kg mL^{-1} for normal skeletal muscle tissue (Szczepaniak et al. 1999) to convert mM to millimoles per kg wet weight (Weis et al. 2009).

First, we acquired ^{1}H-MRS spectra in young ($n = 15$) and older ($n = 15$) adults. Second, all ^{1}H-MRS data ($n = 30$) were processed using LCModel version 6.2-4A (Stephen Provencher, Inc., LCModel and LCMgui user's manual). Before looking at the IMCL concentrations, we checked the estimated standard deviations (SDs) (Cramér–Rao lower bounds) expressed in percent of the estimated concentrations; %SD < 20% has often been used as a very rough criterion to estimate acceptable reliability. In the present study, IMCL data in three of the 15 older adults showed %SD > 20%. Therefore, we excluded the data of these older adults from analysis and acquired IMCL data from the VL for all 15 of the young participants and 12 of the older participants.

Dietary intake during the 3 days before ^{1}H-MRS assessment and dietary habits

A nutritionist determined dietary intake during the 3 days before ^{1}H-MRS assessments and the habitual dietary intake of the participants. Dietary intake during the 3 days before ^{1}H-MRS assessments was calculated from diaries and photos. Habitual dietary intake was estimated using a food frequency questionnaire (Perez Rodrigo et al. 2015), which included 39 food and beverage items. The questionnaire asked about the 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). Dietary assessment indicated the energy (kcal body weight^{-1}), carbohydrate (g body weight^{-1}), protein (g body weight^{-1}), and fat (g body weight^{-1}) intakes for the 3 days before ^{1}H-MRS as well as dietary habits.

Statistical analysis

Body composition, IMCL content, and dietary intake are shown as the means and SD, and physical activity (time and MET h) is shown as the mean and standard error. Differences between the young and older adults were evaluated using the two-tailed Student's *t* test. Pearson's correlation coefficient (*r*) was used to assess the correlation between physical activity (time and MET h) and IMCL content. All analyses were performed using SPSS (version 24.0; SPSS Inc., Chicago, IL, USA). The level of significance was set at $p < 0.05$.

Results

Characteristics of the study participants

The characteristics of the participants are provided in Table 1. Older adults were significantly smaller than the young adults, but weight and body mass index were not significantly different. The IMCL content did not significantly differ between the young and older adults. Regarding dietary intake during the 3 days before ^{1}H-MRS assessments, protein intake was significantly higher in older adults, but energy, carbohydrate, and fat intake did not significantly differ. Regarding habitual dietary intake, protein intake was significantly greater in older adults, but energy, carbohydrate, and fat intake did not significantly differ.

Physical activity level

Table 2 compares the time spent performing physical activities and MET h between young and older adults. Physical activity (time and MET h) did not significantly differ ($p = 0.43$ and $p = 0.98$, respectively). Time spent performing

Table 1 Participants' characteristics

Characteristic	Young	Older
No. of participants (men/women)	15 (8/7)	15 (7/8)
Physical characteristics		
Age (years)	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
Skeletal muscle lipid		
IMCL (mmol kg^{-1} wet weight)[a]	8.5 ± 6.1	10.4 ± 2.7
Dietary intake during the 3 days before ^{1}H-MRS		
Energy (kcal body weight^{-1})	28.7 ± 6.7	33.0 ± 5.1
Carbohydrates (g body weight^{-1})	4.2 ± 1.1	4.6 ± 0.8
Protein (g body weight^{-1})	1.0 ± 0.2	1.4 ± 0.3***
Fat (g body weight^{-1})	0.8 ± 0.2	0.9 ± 0.2
Dietary habits		
Energy (kcal body weight^{-1})	29.0 ± 9.7	33.0 ± 5.1
Carbohydrates (g body weight^{-1})	3.9 ± 1.3	4.4 ± 0.8
Protein (g body weight^{-1})	1.0 ± 0.3	1.3 ± 0.3*
Fat (g body weight^{-1})	1.0 ± 0.4	1.0 ± 0.3

Value are means ± standard deviation

All participants were the same as those previously reported by Akima et al. (2015, 2016), Hioki et al. (2016) and Yoshiko et al. (2017)

BMI body mass index, *^{1}H-MRS* magnetic resonance spectroscopy, *IMCL* intramyocellular lipid

[a]IMCL data include 12 older participants (men, $n = 5$; women, $n = 7$)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs. young adults

Table 2 Comparison of physical activity time between young and older

	Young		Older	
Level	Min	MET h	Min	MET h
1	14.8 ± 1.6	0.44 ± 0.05	18.4 ± 2.1	0.55 ± 0.06
2	28.6 ± 3.1	1.10 ± 0.12	37.9 ± 3.8	1.45 ± 0.15
3	13.5 ± 1.7	0.65 ± 0.08	16.8 ± 1.7	0.81 ± 0.08
Light	56.9 ± 5.6	2.19 ± 0.22	73.1 ± 6.6	2.82 ± 0.25
4	13.7 ± 1.5	0.82 ± 0.09	14.3 ± 1.7	0.86 ± 0.10
5	11.5 ± 2.2	0.83 ± 0.15	9.5 ± 2.6	0.68 ± 0.18
6	5.6 ± 1.2	0.48 ± 0.11	3.0 ± 1.0	0.26 ± 0.08
Moderate	30.8 ± 3.6	2.13 ± 0.25	26.8 ± 4.6	1.80 ± 0.33
7	1.6 ± 0.3	0.16 ± 0.03	0.7 ± 0.2*	0.07 ± 0.02*
8	1.6 ± 0.4	0.19 ± 0.05	0.3 ± 0.1*	0.04 ± 0.02*
9	0.6 ± 0.2	0.08 ± 0.03	0.1 ± 0.0*	0.01 ± 0.00*
Vigorous	3.7 ± 0.7	0.43 ± 0.08	1.1 ± 0.3**	0.12 ± 0.04**
Total	91.4 ± 7.7	4.75 ± 0.41	101.0 ± 9.4	4.73 ± 0.48
Number of steps		9097.4 ± 743.0		9574.6 ± 891.9

Values are means ± SE. Physical activity intensity was categorized as light (levels 1–3), moderate (levels 4–6), and vigorous (levels 7–9) intensity. Study population consisted of 15 young and 15 older adults

*$p < 0.05$, **$p < 0.01$ vs. young adults

physical activities at levels 7–9 and at vigorous intensity was significantly shorter in older than in young adults, but time spent performing physical activities at levels 1–6 and at light and moderate intensities did not significantly differ. Physical activities (MET h) performed at levels 7–9 and at vigorous intensity were significantly lower in older than in young adults, but physical activity did not significantly differ at levels 1–6 or at light or moderate intensity.

Association between physical activity and IMCL content

The most commonly identified metabolite peaks included EMCL at 1.5 ppm, IMCL at 1.3 ppm, total creatine at 3.0 ppm, and trimethylamines at 3.2 ppm (Fig. 1).

Physical activity (total time) significantly and inversely correlated with IMCL content in young adults ($r = -0.66$, $p = 0.007$), but not in older adults ($r = -0.14$, $p = 0.66$). Physical activity (total MET h) also significantly and inversely correlated with IMCL content in young adults ($r = -0.64$, $p = 0.01$), but not in older adults ($r = -0.18$, $p = 0.57$).

Figure 2 shows that the relationship between time spent performing physical activities, physical activity, and IMCL content in young adults. Time spent performing light intensity physical activities significantly and inversely correlated with IMCL content ($r = -0.59$, $p = 0.02$), but no significant correlation was observed with moderate or vigorous intensity in young adults. Light intensity physical activity (MET h) significantly and inversely correlated with IMCL content ($r = -0.58$, $p = 0.02$), but no significant correlation was observed with moderate and vigorous intensity in young adults.

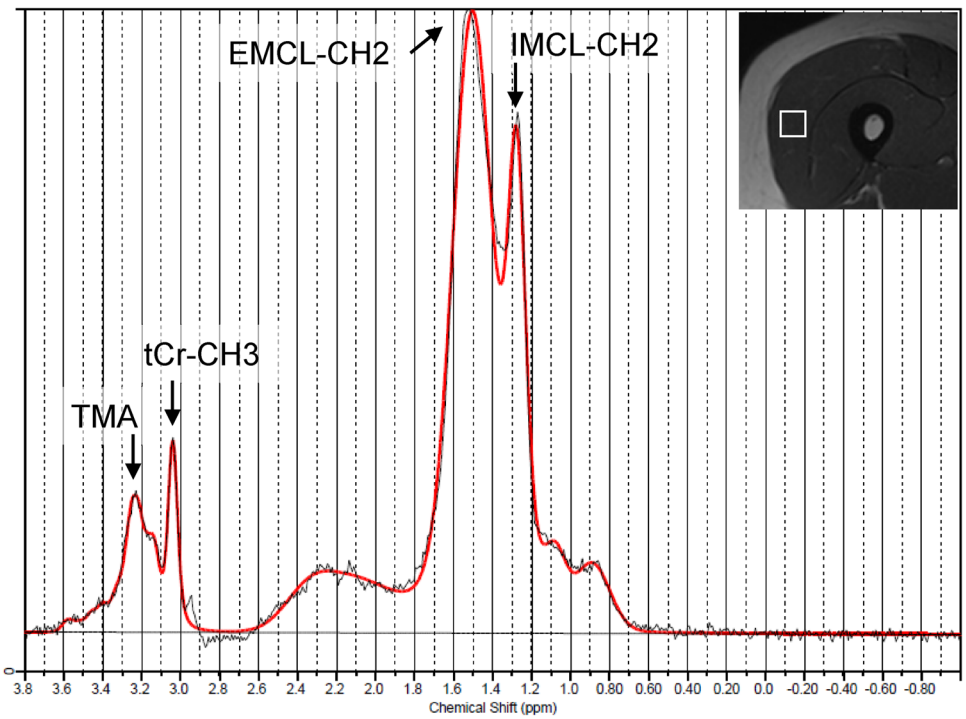

Fig. 1 Representative spectrum from a signal voxel in a 22-year-old woman. The red line is the spectrum fitted by the LCModel. Inset T1-weighted magnetic resonance imaging shows ^{1}H-MRS voxel locations. Peaks from extramyocellular lipid (EMCL), intramyocellular lipid (IMCL), total creatine (tCr), and trimethylamines (TMA) are identified

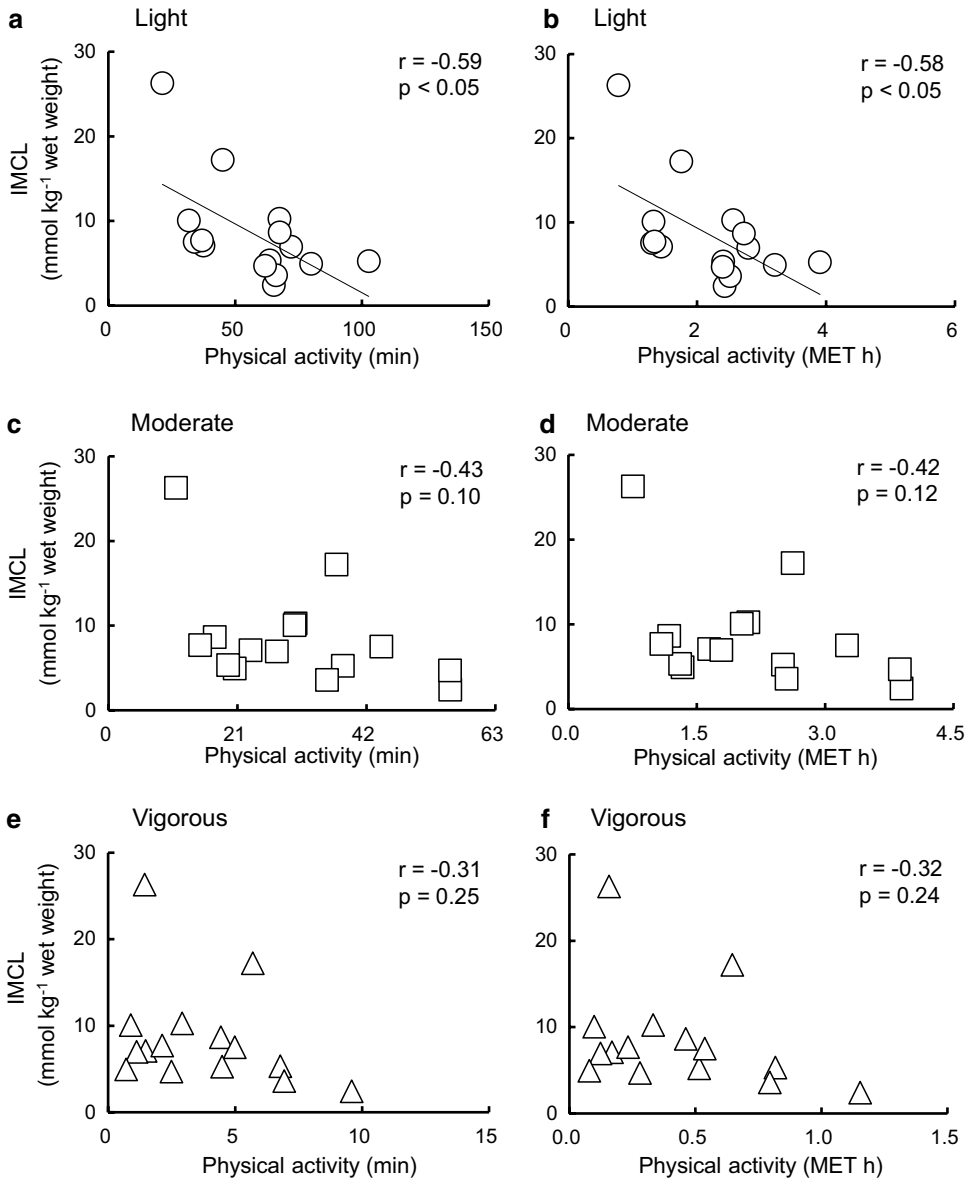

Fig. 2 Relationship between physical activity levels and intramyocellular lipid (IMCL) content for individual activity levels in young adults ($n = 15$). Physical activity is defined by time (**a, c, e**) or metabolic equivalent × hours (MET h) (**b, d, f**)

Figure 3 shows that the relationship between time spent performing physical activities, physical activity (MET h), and IMCL content in older adults. Light, moderate, and vigorous intensity physical activities were not correlated with IMCL content in older adults.

Discussion

We compared the relationship between physical activity (time and MET h) and IMCL content at light, moderate, and vigorous intensities in young and older adults. Light intensity physical activity (time and MET h) was significantly and inversely correlated with IMCL content in young adults, but no such correlation was found in older adults. Our results suggest that daily light intensity physical activity is related to resting IMCL content in young adults, whereas no significant relationship was seen between daily physical activity and resting IMCL in older adults.

We also found no significant differences in the time or quantity of physical activity performed by older and young adults. However, regarding physical intensity, vigorous intensity was significantly greater in young compared with older adults, but no significant difference was seen in light and moderate intensities. According to the Canadian Health Measures Survey (Colley et al. 2011), light, moderate, and vigorous intensity levels in older adults were lower compared with young adults, and the difference was greatest for vigorous intensity. Our results agree with those of the Canadian Health Measures Survey. According to Krems et al. (2004), light and moderate intensity physical activities (e.g., walking or gardening) are higher in older than in young

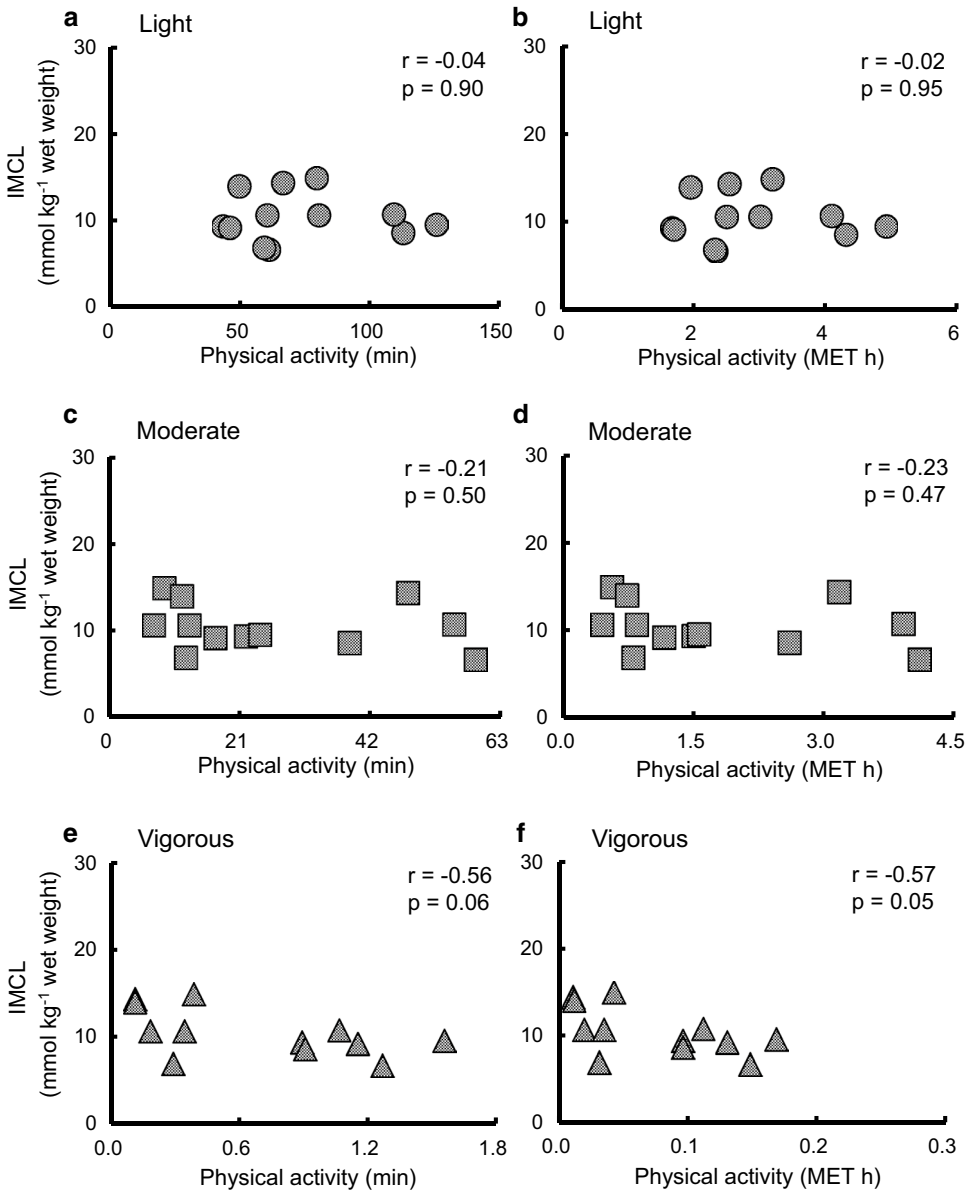

Fig. 3 Relationship between physical activity levels and intramyocellular lipid (IMCL) content for individual activity levels in older adults ($n = 12$). Physical activity is defined by time (**a, c, e**) or MET h (**b, d, f**)

adults; by contrast, time spent performing vigorous intensity physical activities (e.g., any sport activity) is shorter in older adults. Aging decreases muscle mass and increases muscle dysfunction (Akima et al. 2015; Hioki et al. 2016), and such changes might decrease the performance of vigorous intensity physical activity in daily living.

A previous study of healthy young individuals demonstrated the significant utilization of IMCL and its subsequent resynthetization during recovery from both endurance and resistance exercise (Loher et al. 2016). By contrast, whether a habitual level of physical activity affects resting IMCL content has not been investigated. We found that both total time spent performing physical activities and MET h correlated with IMCL content in young adults, which is consistent with the results of previous studies on the influence of physical exercise on IMCL content (Boesch et al. 2006; Loher et al. 2016). Moreover, we investigated the relationship between three intensity levels of physical activity (time and MET h) and IMCL content. Time spent performing light intensity physical activity was inversely correlated with IMCL content in young adults and showed a tendency toward a relationship between IMCL and moderate and vigorous intensity physical activity; however, this finding was not significant. Exercise training causes a shift in the subcellular localization of IMCL, such that lipid droplets are more closely associated with mitochondria (Crane et al. 2010). Previous findings indicate that IMCL close to mitochondria might result in effective oxidative metabolism. IMCL close to mitochondria was frequently seen in young in comparison with older adults on electron microscopy (Crane et al. 2010;

Lee et al. 2010). Therefore, our results suggest that daily light intensity physical activity is related to resting IMCL content in young adults.

In the present study, the correlation coefficients (r) between physical activity and IMCL content were − 0.58 (time) and − 0.59 (MET h), respectively, and the coefficients of determination (r^2) were 0.34 (time) and 0.35 (MET h), respectively, in young adults. Our results indicate that approximately 65% of the variance in IMCL content may be explained by other factors. According to Boesch et al. (2006), IMCL levels are influenced by many parameters, including fasting/diet, FFA concentration, muscle group, aerobic capacity, training, sex, and obesity. Therefore, IMCL content may be related to not only physical activity, but also other such factors.

Whole-body energy expenditure depends on exercise intensity and time (Romijn et al. 1993). Previous studies have found that IMCL content decreases in healthy participants after ≥ 45 min of physical exercise performed at 50–90% VO_{2max} (Loher et al. 2016). Previous results indicate that moderate to high intensity physical exercise is required to induce a decrease in IMCL content. By contrast, total caloric expenditure was similar for the light and long duration exercise and moderate and short duration physical exercise levels; nevertheless, more systemic fat was utilized as a substrate during lower (33% VO_{2max}, 90 min) compared with moderate intensity exercise (66% VO_{2max}, 45 min) in healthy young men (Thompson et al. 1998). Previous results suggest that low intensity and long duration exercise results in greater total fat oxidation than moderate intensity exercise. In addition, similar plot patterns were observed between the amount of physical activity and IMCL content and between time spent performing physical activities and IMCL content at all three intensity levels in young adults. Therefore, IMCL oxidation might depend more on time spent performing physical activities in daily living than on intensity.

We did not find any significant correlations between any categorized intensity based on physical activity level (time and MET h) and IMCL content in older adults. In accordance with previous studies, the age-related decrease in lipid oxidation is induced by the decrease in skeletal muscle mitochondrial content and several oxidative enzymatic activities (Crane et al. 2010; Petersen et al. 2003; Trounce et al. 1989). Recently, Tsintzas et al. (2017) reported the higher expression of skeletal muscle genes involved in fatty acid synthesis (fatty acid synthase and peroxisome proliferator-activated receptor gamma) and of genes related to cell stress and inflammation (interleukin-6), and the lower expression of adipose triglyceride lipase (a key enzyme involved in IMCL lipolysis), and of genes involved in fat metabolism and the tricarboxylic acid cycle activity (lipoprotein lipase, acetyl-CoA acetyltransferase-1, and succinate-CoA ligase-1) pathway in older adults. Previous results indicate that age-related changes in gene expression might lead to a decline in fat metabolism. Moreover, according to Sial et al. (1996), fat oxidation during exercise was lower in older adults compared with young adults who exercised at either the same absolute or similar relative intensities. FFA release, uptake, and oxidation rates were higher in endurance-trained men (mean age 57 years) compared with sedentary men (mean age 60 years), but IMTG did not seem to be a major substrate during moderate exercise in endurance-trained or sedentary men (Boon et al. 2007). Previous results indicate that IMTG might contribute to reducing energy substrates in the aging process. Aging usually induces a decrease in skeletal muscle quality, e.g., skeletal muscle atrophy, decreased skeletal muscle strength, reduced lipid oxidation, and increased intramuscular fat (Akima et al. 2015; Crane et al. 2010; Hioki et al. 2016). Such changes might influence physical activity levels and IMCL content in older adults.

Our study has some limitations. First, the sample size for IMCL content differed between the young ($n = 15$) and older ($n = 12$) adults. The study instituted the exclusion criterion of %SD < 20% (Cramér–Rao lower bounds) as estimates of acceptable reliability. Although the IMCL data satisfied the exclusion criterion in the young adults, it did not in three of the 15 older adults. Next, the homogeneity of young and older adults was different in regard to IMCL content. Mean (± SD) IMCL content was 8.5 ± 6.1 mmol/kg wet weight in young and 10.4 ± 2.7 mmol/kg wet weight in older adults, and the coefficients of variation (SD divided by the mean) were 0.72 (72%) in young and 0.26 (26%) in older adults. These different coefficients of variation might have influenced the correlation coefficients between IMCL content and physical activity. We suspected two factors to explain these differences. First, the older adults were more homogenous than their younger counterparts. Second, inter-individual variation might be small because of the decrease in IMCL metabolism with aging. Our previous study (Hioki et al. 2016) suggested that age-associated changes in morphology, function, and metabolic factors influence IMCL metabolism. Previous results indicate that aging might reduce IMCL metabolism.

In conclusion, the relationship between physical activity (time and MET h) and IMCL content differs between young and older adults. Our results suggest that daily light intensity physical activity reduces resting IMCL content in young adults, whereas no significant relationship was seen between daily physical activity and resting IMCL content in older adults, suggesting that aging itself causes this effect.

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Author contributions MH designed the study, and wrote the initial draft of the manuscript. HA contributed to analysis and interpretation of data, and assisted in the preparation of the manuscript. All other authors have contributed to data collection and interpretation, and critically reviewed the manuscript. All authors approved the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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