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ORIGINAL ARTICLE

Effect of electromyostimulation training on intramuscular fat accumulation determined by ultrasonography in older adults

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Abstract

Purpose Electromyostimulation (EMS) induces a short-term change in muscle metabolism, and EMS training induces long-term improvements of muscle atrophy and function. However, the effects of EMS training on intramuscular fat in older adults are still poorly known. The purpose of this study was to examine whether the intramuscular fat index and biochemical parameters change with EMS training of the quadriceps femoris muscles in older adults.

Methods Nineteen non-obese older men and women performed EMS training of the quadriceps femoris for 12 weeks (3 times/week; single session for 30 min). The intramuscular fat content index was estimated by echo intensity of the vastus lateralis and rectus femoris muscles on ultrasonography, and muscle thickness was also measured. Muscle strength was assessed as the maximal voluntary contraction during isometric knee extension. Echo intensity, muscle thickness, and muscle strength were measured before and after EMS training. A rested/fasting blood samples were collected before and after EMS training for measuring plasma glucose, insulin, free fatty acid, triglyceride, and interleukin-6 concentrations. To examine the acute effect of a single-EMS session on biochemical parameters, blood samples were taken before and after the EMS session.

Results EMS training did not significantly change echo intensity in muscles, muscle thickness, muscle strength, or biochemical parameters. Regarding the acute effect on blood lipid concentrations, a single-EMS session increased free fatty acid and glucose concentrations.

Conclusion EMS sessions had an acute effect of increasing free fatty acid and glucose concentrations, but EMS training intervention did not improve intramuscular fat content.

Keywords Ectopic adipose tissue · Lipid metabolism · Aging · Skeletal muscle

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Abbreviations

EMS	Electromyostimulation
HOMA-IR	Homeostasis model assessment of insulin resistance
MET h	Metabolic equivalent × hours
MVC	Muscle voluntary contraction
VO_2max	Maximum oxygen uptake

Introduction

Primary aging causes changes in the quality and quantity of skeletal muscles (Cartee et al. 2016). The quality of skeletal muscle, such as metabolic function (i.e., mitochondrial dysfunction induces intramyocellular lipid accumulation) (Crane et al. 2010) and specific tension (i.e., voluntary muscle strength per cross-sectional area) (Akima et al. 2001a), decreases with aging. Regarding the quantity of skeletal muscle with aging, muscle size decreases, and intramuscular fat content increases (Lexell et al. 1988). These structural changes with aging are potentially associated with lifestyle, environmental, or various disease (e.g., metabolic syndrome) factors in the long term (Lexell 1995; Kohrt and Holloszy 1995; Cartee et al. 2016). Middle-aged and older adults with low levels of physical activity are mentioned as being likely to develop metabolic syndrome, such as type 2 diabetes mellitus (Kudo et al. 2021; Wang et al. 2018).

It is well known that the morphological changes with aging differ among the skeletal muscles. For example, skeletal muscle size decreases with aging, prominently in the quadriceps femoris (Lexell 1995; Lexell et al. 1988). Moreover, there is less accumulation of intramuscular fat content in the quadriceps femoris than in the hamstrings of older adults (Hioki et al. 2020). This indicates that age-related reductions in muscle mass or intramuscular fat content accumulation differ between muscles. The morphology of the skeletal muscles has been evaluated using non-invasive procedures, such as ultrasonography and magnetic resonance imaging. Intramuscular fat is ectopic adipose tissue, and extensive intramuscular fat accumulation with aging increases insulin resistance and the risk of developing type 2 diabetes mellitus (Ryan et al. 2011, 2002). Although several studies (Tsintzas et al. 2017; Chee et al. 2016; Hioki et al. 2021) of the enhancement of lipid metabolism in muscles have been conducted, it is difficult to enhance “metabolic flexibility” (i.e., muscle oxidative capacity by stimulation/intervention) (Loher et al. 2016) in older adults, because aging induces less metabolic flexibility.

Electromyostimulation (EMS) is commonly used in clinical settings to improve muscular functions in not only older adults, but also patients with spinal cord injury, chronic obstructive pulmonary disease, and chronic heart failure (Sillen et al. 2013; Gorgey et al. 2015). The advantage of EMS is that the device is portable and easy to operate. Thus, people with lower limb dysfunction and a risk of heart failure with the above diseases can also perform EMS training by themselves in their homes to decrease intramuscular fat content and increase muscle size (Sillen et al. 2013; Gorgey et al. 2015). Moreover, EMS is used in training for healthy athletes or injured athletes, which provides the beneficial effect of enhanced performance (Maffiuletti et al. 2002; Maffiuletti 2010). Improvement of muscle torque and functional performance in older adults is observed, and it is also observed to increase muscle fiber type II size and upregulation of insulin-like growth factor 1–1 and modulation of MuRF-1, a muscle-specific atrophy-related gene in the EMS training (Kern et al. 2014). Especially in less active elderly persons, neuromuscular electrical stimulation could lead to better gait and balance performance (Langeard et al. 2017). Therefore, EMS might be able to enhance metabolism in older adults by repetitive and mechanical muscle contraction. However, the effects of EMS training on intramuscular fat in older adults are poorly known.

Lifestyle intervention strategies in physically activity, such as aerobic or anaerobic exercise, improve age-related muscle atrophy and muscle metabolism (Cartee et al. 2016; Canepari et al. 2005; Verdijk et al. 2009; Caserotti et al. 2008; Aagaard et al. 2010). Therefore, type 2 diabetes mellitus may be avoided in older adults. The physiological responses are determined by exercise time and intensity (Garber et al. 2011). With increases of exercise intensity, the relative contributions of plasma glucose and muscle glycogen are increased (Egan and Zierath 2013; Romijn et al. 1993). In contrast, oxidation of lipid sources (mostly plasma free fatty acids) accounts for most of the energy provision during light intensity exercise (25% of maximum oxygen uptake [VO_2max]). Fatty acids are a major oxidative fuel not only during exercise, but also at rest (Jensen 2003). The lipid (plasma free fatty acids and intramyocellular triglycerides) oxidation rate increases up to 60–70% VO_2max, after which it decreases as intensity increases. The concomitant mobilization of free fatty acids from adipose tissue results in an increase in free fatty acid concentration immediately after exercise (Jensen 2003). Thus, it is considered that EMS sessions have acute and long-term training effects on plasma lipid and glucose concentrations in older adults.

The purposes of this study were to examine: (1) the acute effects of a single-EMS session on the biochemical profile; and the (2) chronic effects of EMS training for 12 weeks on the biochemical profile and intramuscular fat of the quadriceps femoris in older adults. The hypotheses were that a single session of EMS would increase free fatty acid concentration, EMS training would decrease intramuscular fat, increase muscle thickness, and muscle strength, and that it would also decrease free fatty acids concentration in older adult men and women.

Methods

Participants

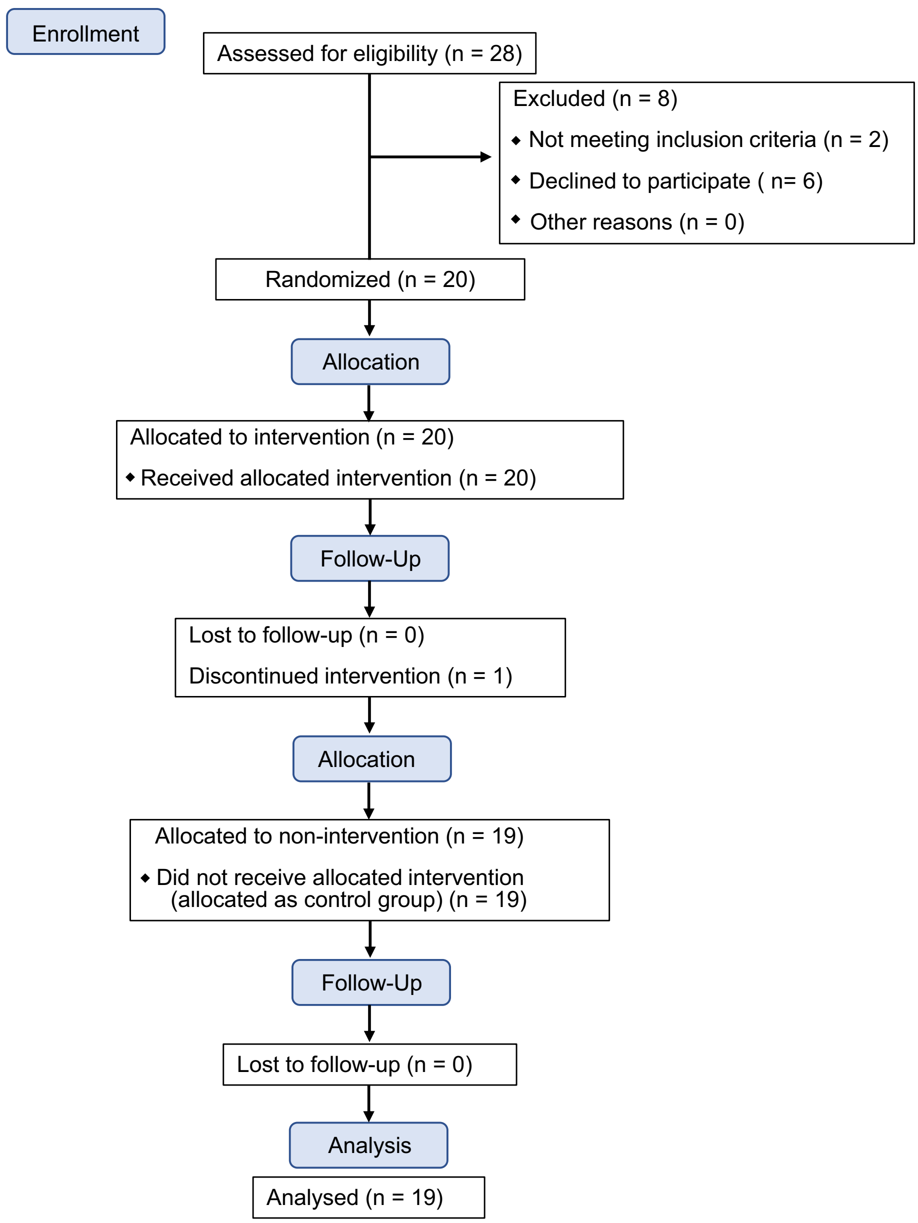
Nineteen physically active, non-obese older adults (10 men, 9 women) participated in this study. All participants were living independently. Participants with a clinical history of heart disease (myocardial infarction, angina pectoris, cardiac insufficiency), cerebrovascular disease (cerebral infarction, hemorrhage), extreme hypertension (systolic blood pressure ≥ 180 mmHg; diastolic blood pressure ≥ 110 mmHg), or neuromuscular disorders were excluded. Moreover, none of the participants had any history of limb surgery. All participants provided written, informed consent to participate in the study. This study was approved by the Ethics Committee of Teikyo Heisei University and registered with the University hospital Medical Information Network (UMIN000043041). This study conformed to the standards set forth by the Declaration of Helsinki and adhered to CONSORT guidelines (Fig. 1).

Study protocol

The flowchart of the study procedures and participants is shown in Fig. 1. This study recruited participants in the age range of 60 to 80 years at four local community centers. When recruited, the exclusion criteria were shown to potential participants, and participant eligibility was assessed using a questionnaire ($n=28$) that resulted in the exclusion of a total of 8 participants for the following reasons: did not meet inclusion criteria ($n=2$) or refused to participate ($n=6$). Moreover, one participant was discontinued from the intervention (at the first body composition measurement). Finally, a total of 19 participants (10 men, 9 women), ranging in age from 61 to 78 years, were included in the study.

The signal EMS session and study protocol are showed Fig. 2A, B, respectively. The study was performed according to the protocol in the morning. However, some participants could not follow the order of the specified by the protocol due to vaccination, the declaration of a state of emergency in Japan due to COVID-19, physical deconditioning, their own convenience, or other reasons. This is shown in the supplementary information, Supplementary Material-1. This study was not blinded; therefore, all evaluated data were assigned serial numbers to prevent individual identification and then subsequently analyzed.

Fig. 1 Flow diagram of the study



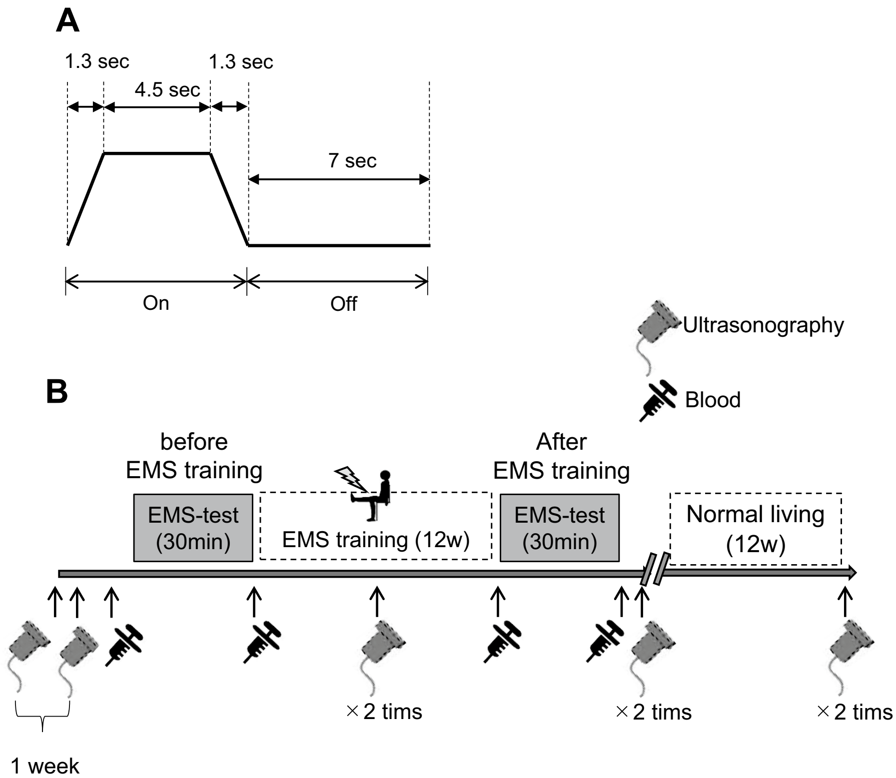


Fig. 2 Contraction/relaxation durations of the EMS session (**A**) and the experimental protocol of EMS training (**B**) EMS, electromyostimulation training

Anthropometric measurements

Body composition was measured before EMS training, in the middle of the EMS training period (at 6 weeks), and 12 weeks after EMS training, another 12 weeks later (at 24 weeks). The participants were advised to take relaxed and natural breaths to minimize the inward pull of the abdominal contents during measurement, and the actual waist circumference of the participants was measured. Hip circumference was taken as the greatest circumference of the pelvis.

EMS training

Transcutaneous EMS (ESPURGE, Ito Co., Ltd. Saitama, Japan) was performed on the vastus lateralis and vastus medialis muscles. Voltage was delivered via four (two, vastus lateralis; two, vastus medialis) 5×9 cm^2 electrodes (Axelgaard Mfg. Co., Ltd. USA) applied to the skin. Two electrodes were placed, one on vastus lateralis at approximately 5 cm distal to the greater trochanter and one approximately 5 cm proximal to the superior aspect of the knee joint. Two electrodes were placed on vastus medialis at the medial distal thigh (approximately 30% of the length of the thigh). All participants performed EMS training of the quadriceps femoris at home for 12 weeks (3 times/week). Before beginning the study protocol, they were familiarized with the operation of the EMS device in the laboratory at least 3 times. In these sessions and the measurement after EMS training, EMS-induced isometric knee extension force was recorded by a custom-made dynamometer (Takei Scientific Instruments Co. Ltd., Tokyo, Japan). A single-EMS session consisted of 30-Hz, biphasic rectangular pulses with a duration of 300 µs, contraction/relaxation durations of 7.1 s on–7.0 s off, for 30 min (Fig. 2A). EMS intensity was set to observe muscle contraction of the quadriceps femoris visually, without causing the patient discomfort. A constant stimulation current was used throughout the EMS training.

Biochemical profile

Blood samples were collected in EDTA-2 k-containing tubes and separating agent-containing tubes with a kit from SRL, Inc. (Tokyo, Japan) after an overnight fast. Biochemical parameters, such as fasting glucose, insulin, free fatty acid, triglyceride, and interleukin-6 concentrations, were measured in a blood sample. Serum free fatty acid and triglyceride concentrations were measured using enzymatic methods. Plasma concentrations of glucose were measured using the hexokinase method, and serum concentrations of insulin and interleukin-6 were measured using chemiluminescentenzyme immunoassay. To determine the acute effect of a single-EMS session, biochemical tests were performed before and after an EMS session. The chronic effect of EMS training was compared between biochemical tests before a single-EMS session measured before and after an EMS training at rest. To evaluate insulin resistance, the homeostasis model assessment of insulin resistance (HOMA-IR) was used (Matthews et al. 1985).

Ultrasonography

Before EMS training, ultrasonography measurement was done twice with an interval of approximately one week to test reliability between measurements. In the middle of the EMS training period and 12 weeks and 24 weeks after the EMS training, ultrasonography measurement was performed twice at each scan location. Measurement and analysis were performed according to the previous study (Hioki et al. 2020). All participants refrained from participating in intense sports causing fatigue to remain for 2 days before ultrasonography measurement.

B-mode ultrasonography scanning was performed using a LOGUQ *e* V2 (GE Healthcare, Japan) by a single investigator (MH). System parameters were set as follows: frequency, 8.0 MHz; gain, 80 dB; and depth, 7 cm. All scans were made in the transverse plane with a linear transducer. Participants were measured in the dorsal position with the knee fully extended and relaxed. Ultrasonographic images of the lateral (vastus lateralis and vastus intermedius of the lateral side) and anterior (rectus femoris and vastus intermedius of the anterior side) sites were obtained at the mid-thigh between the greater trochanter and the lateral condyle of the femur. Five images were collected and stored in the ultrasonographic device in DICOM format for future analysis. All ultrasonography images were analyzed using Image J software (version 1.51; National Institutes of Health, Bethesda, MD).

Intramuscular fat measurement by echo intensity

Intramuscular fat content was estimated as an index based on ultrasonography echo intensity, similar to previous studies (Hioki et al. 2020, 2021). A region of interest was selected in the image of each vastus lateralis and rectus femoris, including as much of the muscle as possible, and bone and surrounding fascia were excluded. To decrease noise in the region of interest, a smoothing function was applied. The mean echo intensity of the region of interest was calculated (8-bit resolution, resulting in a value between 0 (black) and 255 (white). Mean echo intensity within the region of interest in five images was measured for each vastus lateralis and rectus femoris, and five images with the highest and lowest echo intensity values were excluded to minimize variations resulting from technical errors. The echo intensity of the three remaining images was averaged for future analysis.

Muscle and subcutaneous thicknesses

Muscle and subcutaneous tissue thicknesses were measured with electronic calipers placed at the middle of the ultrasound image using Image J. Muscle thickness of the lateral (vastus lateralis and vastus intermedius of the lateral) and anterior (rectus femoris and vastus intermedius of the anterior) sites was measured between the superficial and ventral muscle fascia, respectively. Subcutaneous tissue thickness was measured between the uppermost part of the skin and the superficial fascia of the muscle at the lateral and anterior sites, respectively. Lateral (vastus lateralis and vastus intermedius of the lateral) and anterior (rectus femoris and vastus intermedius of the anterior) muscle thicknesses were estimated. Three images were scanned for each lateral and anterior site of the thigh, and these images were averaged for future analysis.

Muscle strength

All participants were familiarized with muscle voluntary contraction (MVC) at the laboratory at least 1 week before the pre-MVC testing. MVC during isometric knee extension was measured at the pre-EMS and post-EMS training sessions using a custom-made dynamometer (Takei Scientific Instruments Co. Ltd.), as previously described (Hioki et al. 2021). The hip and thigh were strapped to the dynamometer, and the knee joint was flexed at 90° (0° = full extended). MVC tests of the right leg were measured three or four times at approximately 2 to 3-min intervals. The maximal attempt of two tests of three or four tests that yielded the highest force was recorded. Isometric knee extension force was expressed as an absolute value (Nm). During the EMS, the EMS-induced isometric knee extension force was normalized to the MVC muscle strength.

Physical activity levels and dietary habits

Physical activity levels were estimated from the records of the three-dimensional ambulatory accelerometer for 10 days (Lifecorder; Suzuken Co., Nagoya, Japan). Physical activity levels are expressed as time and metabolic equivalent × hours, as in previous studies (Kumahara et al. 2004; Hioki et al. 2019).

Habitual dietary intake was estimated using a food frequency questionnaire, Ver. 2.0, by a nutritionist (HT). The food frequency questionnaire included 29 food and beverage items, and cooking methods of 10 series. The questionnaire asked about the average intake and frequency of consumption of each food. Five categories were used (almost always, often, sometimes, rarely, or never) to describe consumption frequency.

Statistical analysis

Blood biochemical parameter data were compared using two-way analysis of variance (ANOVA) with EMS training and time between pre-EMS test and post-EMS test in the pre- and post-EMS training periods. The effects of EMS training on echo intensity or muscle and subcutaneous thicknesses and body profiles were assessed by one-way ANOVA. When a significant time effect was obtained, Bonferroni significant difference post hoc analysis was used to evaluate changes between individual time points.

Using the paired Student's *t*-test, the chronic effects of EMS training on muscle strength and habitual dietary intake were assessed between the points before and after EMS training.

Ultrasonography measurements were performed twice at each scan location. The mean coefficient of variation was calculated before EMS training, in the middle of the EMS training period (at 6 weeks), and 12 weeks after EMS training, and another 12 weeks following that (at 24 weeks).

The effect size of EMS training was estimated using Cohen's d as trivial (0–0.19), small (0.2–0.49), medium (0.5–0.79), or large (> 0.8) (Cohen 1988). All statistical analyses were performed using SPSS version 28.0 software (SPSS Inc., Chicago, IL). Data are presented as means ± SD. $P < 0.05$ was used to denote significance.

Results

Table 1 summarizes the participants' characteristics and subcutaneous and muscle thicknesses before EMS training, in the middle of the EMS training period (at 6 weeks), 12 weeks after EMS training, and another 12 weeks later (at 24 weeks). Main effect on height was obtained by ANOVA ($p < 0.001$), and post hoc test showed a significantly decrease at 24 weeks compared with that before EMS training ($p = 0.03$).

Table 2 show the acute effects of a single-EMS session before and 12 weeks after EMS training. A single-EMS session increased glucose and free fatty acid concentrations before EMS training. In contrast, a single-EMS session increased only the glucose concentration after EMS training. No significant changes in insulin, triglyceride,

Table 1 Participant characteristics

	Before EMS training		After EMS training			
	pre	6w	12w	24w	*p*	*d*
Age (year)	71.5 ± 5.4					
Blood pressure						
Systolic (mmHg)	134.4 ± 16.2	132.3 ± 15.5	130.7 ± 15.8	129.3 ± 14.6	0.41	0.27
Diastolic (mmHg)	77.6 ± 10.7	76.5 ± 7.5	75.4 ± 11.7	74.2 ± 8.4	0.34	0.27
Body composition						
Height (cm)	160.5 ± 7.8	160.3 ± 7.7	160.3 ± 7.7	160.1 ± 7.8	0.00	0.42
Weight (kg)	59.3 ± 10.5	59.3 ± 10.8	59.3 ± 10.7	59.4 ± 10.6	0.74	0.01
BMI (kg/m^2)	22.9 ± 2.8	22.9 ± 3.0	23.0 ± 3.0	23.1 ± 3.0	0.27	0.16
Waist circumference (cm)	85.9 ± 7.6	84.9 ± 7.6	85.2 ± 8.3	85.1 ± 7.7	0.24	0.28
Hip circumference (cm)	93.0 ± 5.8	93.9 ± 9.4	92.6 ± 6.3	92.6 ± 6.2	0.34	0.19
Waist-to-hip ratio	0.9 ± 0.0	0.9 ± 0.1	0.9 ± 0.0	0.9 ± 0.0	0.31	0.08
Subcutaneous thickness						
Lateral site	0.7 ± 0.4	0.7 ± 0.4	0.7 ± 0.4	0.7 ± 0.4	0.04	0.43
Anterior site	0.8 ± 0.4	0.8 ± 0.4	0.8 ± 0.4	0.8 ± 0.4	0.19	0.09
Muscle thickness						
Vastus lateralis	2.0 ± 0.3	2.0 ± 0.3	2.0 ± 0.3	2.0 ± 0.3	0.18	0.33
Vastus intermedius of the lateral sites	1.6 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	0.13	0.25
Vastus lateralis + vastus intermedius of the lateral site	3.5 ± 0.7	3.5 ± 0.7	3.5 ± 0.7	3.6 ± 0.7	0.60	0.04
Rectus femoris	1.2 ± 0.2	1.2 ± 0.3	1.2 ± 0.2	1.2 ± 0.3	0.77	0.00
Vastus intermedius of the anterior sites	1.1 ± 0.3	1.1 ± 0.4	1.1 ± 0.3	1.1 ± 0.4	0.02	0.28
Rectus femoris + vastus intermedius of the anterior site	2.3 ± 0.5	2.3 ± 0.5	2.3 ± 0.5	2.3 ± 0.5	0.17	0.15

$n = 19$ (M/F: 10/9). Value are means ± SD. *BMI* body mass index. Cohen's *d* shows only the chronic effects of EMS training for 12 weeks. Because Cohen's *d* is similar between pre and 6th weeks, between 6th and 12 weeks, between 12 and 24 weeks after EMS training

Table 2 Acute effects of a single-EMS session on biochemical parameters

	Before EMS training		After EMS training		EMS effect	Time effect	Interaction	*d*
	Pre-test	Post-test	Pre-test	Post-test				
Glucose (mg/dL)	96.4 ± 11.7	98.8 ± 13.1	95.6 ± 8.9	99.6 ± 10.9	1.00	0.00	0.06	1.04
Insulin (μIU/mL)	5.2 ± 2.3	5.0 ± 2.0	5.3 ± 2.0	5.7 ± 2.8	0.19	0.77	0.22	0.26
Free fatty acid (μEq/L)	523.3 ± 187.0	606.9 ± 185.2	503.5 ± 223.0	554.8 ± 190.8	0.36	0.00	0.41	0.42
Triglyceride (mg/dL)	111.6 ± 47.6	112.3 ± 46.9	112.7 ± 55.2	114.1 ± 55.3	0.86	0.10	0.58	0.37
Interleukin-6 (pg/mL)	1.2 ± 0.8	1.2 ± 0.7	1.3 ± 0.5	1.3 ± 0.6	0.45	0.33	0.75	0.15
HOMA-IR	1.2 ± 0.5	1.2 ± 0.4	1.2 ± 0.5	1.4 ± 0.7	0.14	0.37	0.12	0.41

n = 19. Value are mean ± SD. *HOMA-IR* homoeostasis model assessment index, Cohen's d shows only the acute effects of a single-EMS session after EMS training

Table 3 Chronic effects of EMS training on biochemical parameters, muscle profiles, and habitual dietary intake

	Before EMS training	After EMS training 12 weeks	*p*	*d*
Biochemistry profiles				
Glucose (mg/dL)	96.4 ± 11.7	95.6 ± 8.9	0.53	0.14
Insulin (μIU/mL)	5.2 ± 2.3	5.3 ± 2.0	0.81	0.06
Free fatty acid (μEq/L)	523.3 ± 187.0	503.5 ± 223.0	0.63	0.11
Triglyceride (mg/dL)	111.6 ± 47.6	112.7 ± 55.2	0.89	0.03
Interleukin-6 (pg/mL)	1.2 ± 0.8	1.3 ± 0.5	0.41	0.19
HOMA-IR	1.2 ± 0.5	1.2 ± 0.5	0.86	0.04
Habitual dietary intakes				
Energy (kcal/body weight)	38.2 ± 7.3	39.7 ± 9.2	0.26	0.26
Carbohydrates (g/body weight)	4.6 ± 0.9	4.8 ± 1.3	0.45	0.17
Protein (g/body weight)	1.4 ± 0.3	1.5 ± 0.4	0.24	0.27
Fat (g/body weight)	1.4 ± 0.4	1.5 ± 0.5	0.62	0.11
Muscle profile				
Muscle voluntary contraction (Nm)	133.0 ± 43.4	134.1 ± 42.7	0.69	0.09

n = 19. Value are mean ± SD. Results showed pre-data of the single-EMS session performed before and after EMS training. *HOMA-IR* homoeostasis model assessment index

interleukin-6 concentrations, or HOMA-IR were seen after a single-EMS session, both before and after EMS training.

Table 3 show the chronic effects of EMS training for 12 weeks on the biochemical profile, habitual dietary intake, and muscle strength. EMS training did not cause significant changes in biochemical parameters and habitual dietary intake. The peak force of isometric knee extension before and after EMS training was 133.0 ± 43.4 and 134.1 ± 42.7 Nm, respectively. The EMS-induced peak force before and after EMS training corresponded to 5.2% ± 4.1% (range 0.6–16.7%) and 6.9% ± 4.0% (range 0.7–14.1%) of MVC, respectively.

The physical activity levels of participants are shown in Table 4.

Figure 3 show the effects of EMS training on echo intensity in vastus lateralis and rectus femoris. No significant changes in echo intensity of the vastus lateralis and rectus femoris were seen after EMS training.

Table 4 Physical activity levels

Physical activity	min	MET h
Light	58.1 ± 20.9	2.1 ± 0.8
Moderate	20.6 ± 19.0	1.4 ± 1.4
Vigorous	2.4 ± 7.3	0.3 ± 0.8
Total	81.1 ± 32.2	3.76 ± 2.98
Number of steps	7530.8 ± 3583.6	–

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

Echo intensity was measured by ultrasonography twice before EMS training, in the middle of the EMS training period (at 6 weeks), 12 weeks after EMS training, and again another 12 weeks later (at 24 weeks); echo intensity measurement before EMS training was performed twice with an interval of approximately one week. The mean coefficients of variation of the first and second measurements were

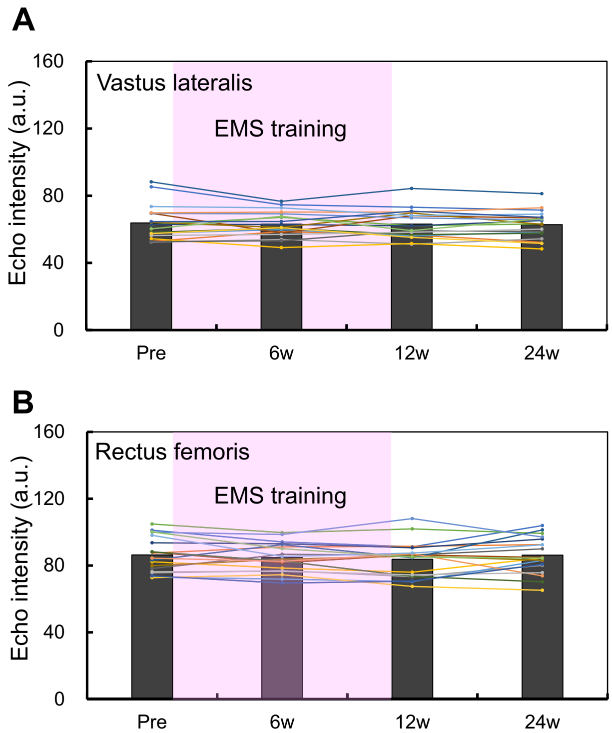


Fig. 3 Effect of EMS training on echo intensity in the vastus lateralis (**A**) and rectus femoris (**B**) EMS, electromyostimulation training

3.2% ± 3.0% in the vastus lateralis and 5.3% ± 4.0% in the rectus femoris (before training), 4.0% ± 2.4% in the vastus lateralis and 2.3% ± 1.4% in the rectus femoris (6 weeks), 3.6% ± 2.4% in the vastus lateralis and 2.9% ± 2.3% in the rectus femoris (12 weeks), and 3.3% ± 2.4% in the vastus lateralis and 4.6% ± 3.7% in the rectus femoris (24 weeks after EMS training), respectively.

Cohen's *d* showed a chronic effect of EMS training for 12 weeks on participants' characteristics and subcutaneous and muscle thicknesses (Table 1). Cohen's *d* showed the acute effects of a single-EMS session after EMS training on biochemical parameters (Table 2), as well as chronic effects of EMS training on biochemical parameters and muscle strength (Table 3). Cohen's *d* showed chronic effects of EMS training for 12 weeks on echo intensity in the vastus lateralis ($d = 0.16$, $p = 0.81$) and rectus femoris ($d = 0.38$, $p = 0.32$).

Supplementary Materials 2 and 3 compare the participants' characteristics, physical activity levels, and dietary habit characteristics between men and women, respectively.

Discussion

This study examined the acute effects of a single-EMS session on biochemical parameters and the chronic effects of EMS training for 12 weeks on biochemical parameters and echo intensity of the quadriceps femoris in older adults. A single-EMS session increased fasting free fatty acid and glucose concentrations, but not triglyceride, insulin, interleukin-6, and HOMA-IR. EMS training did not cause significant changes in echo intensity-estimated intramuscular fat, muscle thickness, muscle strength, or resting/fasting any biochemical parameters (free fatty acids, triglycerides, glucose, insulin, interleukin-6, and HOMA-IR). These results suggest that a single-EMS session has acute effects on increasing serum/plasma lipid and glycolytic metabolism, and they suggest that EMS training for 12 weeks did not improve intramuscular fat content of the quadriceps femoris or biochemical parameters in older adult men and women.

Acute effects of an EMS session on biochemical parameters

The magnitude of the increase in muscle lipid and glucose uptake is affected by exercise duration and intensity (Romijn et al. 1993; Egan and Zierath 2013). Egan and Zierath (2013) demonstrated that the absolute power output determines the rate of adenosine triphosphate demand and energy expenditure, whereas the relative exercise intensity affects the relative contributions of carbohydrate and lipid sources and circulating (extramuscular) and intramuscular fuel stores to energy provision. Up to 30% maximum oxygen uptake (VO_{2max}), oxidation of lipid sources (mostly plasma free fatty acids) accounts for most of the energy provision. The lipid oxidation rate increases up to 60–70% VO_{2max}, after which it declines with increases in intensity. As exercise intensity increases, the absolute carbohydrate oxidation rate (glycolysis system) and its relative contribution to energy provision increase. To enable provision of plasma free fatty acids to the contracting muscle, adipose tissue lipolysis is substantially increased (Jensen 2003). Therefore, plasma free fatty acid concentrations increase during exercise, and that increase remains for some time. The present results suggest that the EMS protocol increases free fatty acid concentrations, namely that an exercise effect was provided.

In the current study, a single-EMS session increased the plasma glucose level. This finding contradicts those of our previous study (Hioki et al. 2021), in which a single-EMS session decreased the plasma glucose concentration. In our previous study (Hioki et al. 2021), only two electrodes were placed on the vastus lateralis, and the mean EMS-induced knee extension (only vastus lateralis) force corresponded to 17.4% MVC of the entire quadriceps muscle group (i.e., vastus lateralis, vastus intermedius, vastus medialis, and rectus femoris). Since the vastus lateralis volume accounts for only 30% of the quadriceps femoris muscles (Akima et al. 2001b). Therefore, it is that the EMS intensity protocol was high-intensity for the vastus lateralis in our previous study. Based on the above conclusion in the present study, care was taken to avoid an EMS intensity that was too high because the aim was to enhance lipid metabolism. Hence two electrodes were placed on each vastus lateralis and vastus medialis and EMS-induced knee extension force before and after EMS training corresponded to a mean ± SD of 5.2% ± 4.1% (range 1.0–16.7%) and 6.9% ± 4.0% (range 0.7–14.1%) of MVC, respectively. Contradicting our previous result, plasma glucose increased after a single-EMS session in the present study. According to Suh et al. (Suh et al. 2007), during exercise, the blood glucose concentration can be maintained or increased by release of glucose from the liver and kidneys into the blood, as well as by mobilization of other fuels that may serve as alternatives.

Chronic effects of EMS training on muscle thickness and muscle strength

The EMS training for 12 weeks did not significantly change muscle thickness and muscle strength. It is well known that EMS is effective, with increases in the muscle fiber type II proportion in adults (age range 17–30 years) (Theriault et al. 1996) and with increases in fiber type I and II sizes in sedentary adults (mean age 26 years) and in active adults (mean age 25 years) (Gondin et al. 2011) determined using muscle biopsies. Increases in the muscle fiber type II proportion and decreases in the fiber type I proportion in sedentary male young adults (mean age 22 years) have also been reported (Perez et al. 2002). Moreover, a previous study (Caggiano et al. 1994) demonstrated a 9% average increase in MVC (torque) levels in older male adults. EMS training also changes the myosin heavy chain, with decreases in myosin heavy chain IId/x (22–28%) and increases in myosin heavy chain I (30–34%), and such changes in muscle morphology are accompanied by increased muscle strength (Gondin et al. 2011). According to Bickel et al. (Bickel et al. 2005), a single-EMS session activates mRNA related to development or growth in skeletal muscle (i.e., insulin-like growth factor binding protein-4, MyoD, myogenin, cyclin D1, and p21-Waf1) in young men and women. Activation of these mechanisms precedes skeletal muscle growth, resulting in the accretion of the proteins necessary to support an increase in myofiber size, which is sufficient to stimulate molecular-level responses. However, to increase muscle size, i.e., muscle hypertrophy, higher EMS intensity (maximum tolerance level) is needed (Gondin et al. 2011). Therefore, such changes in muscle thickness and strength might not be observed in older adults in the present study.

Chronic effects of EMS training on intramuscular fat

In the current study, EMS training did not significantly change echo intensity as an index of intramuscular fat. So far, many studies have reported that EMS training changed oxidative enzymes, i.e., 3-hydroxylacyl-CoA dehydrogenase (Gauthier et al. 1992; Theriault et al. 1994) and enoyl CoA hydrates (Gondin et al. 2011), which are key enzymes of β-oxidation of fatty acids. It is clear that EMS affects lipid metabolism in muscle cells. However, in those previous studies, EMS intensity was comparable at about 50% VO_2max when ergocycle exercise was performed for 30 min (Gauthier et al. 1992), and muscle force evoked was > 50% MVC during EMS. The EMS intensities of previous studies were likely higher than the EMS intensity in the present study. Muscle hypertrophy was accompanied with a decrease in intramuscular fat in patients with spinal cord injury, suggesting that muscle hypertrophy could potentially negate the deleterious metabolic effects of intramuscular fat (Gorgey and Shepherd 2010; Gorgey et al. 2012). In contrast, a recent finding showed that increases in intramuscular fat with aging are independent of muscle. The effectiveness of EMS may differ between muscle in older adults and muscle in patients with spinal cord injury. The aim of the present study was to clarify whether EMS as home exercise as part of daily living in older adults can be used to decrease intramuscular fat content. The level of EMS intensity was set so that participants would not feel distress and could continue safe EMS training. Though it is reasonable to expect that EMS training of long duration and more sessions would enhance lipid metabolism, the present results suggest that EMS training for 12 weeks did not improve intramuscular fat content of the quadriceps femoris or muscle size.

Chronic effects of EMS training on biochemical parameters

We speculated that EMS approach on muscle (EMS-induced mechanical muscle contraction) enhances lipid metabolism in muscle with whole-body lipid metabolism in the older adults. Energy in the body is primarily stored as triglyceride in adipose tissue. Free fatty acids are induced rapid mobilization into circulation, through triglyceride lipolysis and free fatty acid export from adipose tissue (Abdollahi et al. 2022). Adams et al. (Adams et al. 1993) reported map the pattern of muscle contractile activity evoked by EMS and revealed that EMS evoked higher muscle contractile activity than voluntary muscle contractile activity. These results indicate that EMS-induced muscle contraction uses more energy substrate such as free fatty acid than voluntary muscle contraction. Earlier, we hypothesized that EMS training may decrease fasting/rested free fatty acid concentrations. In the present study, lipid metabolism is enhanced by a single-EMS session. The present study results showed increases in free fatty acid concentrations after a single-EMS session. Such enhancement of lipid metabolism was repeated for 12 weeks. However, the present results showed no significant fasting/rested free fatty acid (before 523.3 ± 187.0 μEq/L; after 503.5 ± 223.0 μEq/L) or triglyceride (before 111.6 ± 47.6 mg/dL; after 112.7 ± 55.2 mg/dL) concentration changes. Physical activity, exercise, and free fatty acid influence on intramyocellular lipid content or intramyocellular metabolism (Boesch et al. 2006). Previously, we have researched correlation between fasting/rested serum free fatty acid concentration and intramyocellular lipid content (Hioki et al. 2016) and correlation between physical activity levels and intramyocellular lipid content (Hioki et al. 2019). In the results, fasting/rested serum free fatty acid concentration significantly correlated with intramyocellular lipid content in the young adults, or daily physical activity level significantly and inversely correlated with intramyocellular lipid content in the young adult. However, in the older adults, such correlation did not observe both researches. These results suggest that fasting/rested serum free fatty acid concentration, and physical activity levels relate to lipid metabolism in muscle, and age-related changes in morphology, function, or metabolic factors influence intramyocellular metabolism. Namely, lipid metabolism differs between older and young adults. Inflexibility lipid metabolism in muscle may directly relate to intramuscular fat accumulation. Our results suggest that mechanical muscle contraction of EMS not induce decrease in fasting/rested free fatty acid concentration in the older adults.

Our results are congruent with the results for obese young adults (mean age 30 years; mean body mass index 32 kg/m^2; $n = 5$) that showed that EMS intervention did not change triglyceride concentrations (Galvan et al. 2022). EMS training also did not decrease glucose concentrations in the current study. The present result does not concur with that of Galvan et al. (2022) who reported improvement of glucose tolerance after EMS in obese young adults. EMS induces muscle contraction; it effectively increases glucose uptake via an insulin-independent mechanism in young male adults (Hamada et al. 2003). In contrast, glucose concentrations did not change after whole-body EMS (single session 20 min; 6 months) in women ≥ 70 years of age with sarcopenic obesity (Wittmann et al. 2016). Despite whole-body EMS, it seems that no significant effect was observed. From a review of previous studies, we can suggest that not only EMS, but also exercise effects differ between older and young adults. It is a fact that aging induces changes in skeletal muscle morphology, function, and metabolism (Lexell 1995; Cartee et al. 2016; Hioki et al. 2016, 2020). Such age-related changes in quality and quantity of muscle might affect changes in free fatty acid, triglyceride, and glucose concentrations with EMS training. In contrast, point to notice, our participants were no-obesity (before EMS training, mean body mass index 22.9 kg/m^2) in the study. Mean fasting free fatty acid, triglyceride, and glucose concentrations were also normal at rest. Therefore, our results might be different from obese young and older adults (Galvan et al. 2022; Wittmann et al. 2016).

Limitations

According to previous studies, EMS training needs to be performed for 2–8 h/day, 1–2 sessions/day, for 6–7 days/week to improve oxidative enzyme function (Gauthier et al. 1992; Perez et al. 2002; Theriault et al. 1994, 1996; Nuhr et al. 2003). Thigh composition may affect the amplitude of the current needed to evoke dynamic leg extension via neuromuscular electrical stimulation (Gorgey et al. 2015). This indicates that higher amplitude intensity might be needed to improve atrophied muscle and intramuscular fat accumulation in patients with spinal cord injury. Realistically, the level of EMS intensity could not be further increased, and having more session time per day was also difficult. Therefore, the disagreements in results could have been related to differences in protocols, principally the duration of the EMS session, the duration of training, and stimulation intensity.

Conclusion

The results showed that: (1) the acute effects of a single-EMS session increased free fatty acid and glucose concentration at fast; (2) EMS training did not significantly change echo intensity-estimated intramuscular fat, muscle thickness, muscle strength, or any biochemical parameters at fast and rest. These results suggest that a single-EMS session enhances serum/plasma lipid and glycolytic metabolism, but EMS training for 12 weeks did not improve intramuscular fat content of the quadriceps femoris or biochemical parameters in older adult men and women. Excessive intramuscular fat accumulation may interfere with insulin signaling and increases in serum free fatty acid and triglyceride concentrations are also risk factors for metabolic syndrome. A strategy to enhance lipid metabolism in muscle containing an abundance of ectopic adipose tissue in older adults is needed. Though exercise would be better, e.g., physical activity in daily living, running, walking, or resistance training, such exercises are difficult for some older adults. EMS training may contribute as an alternative for these exercises in older adults.

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Author contribution MH was involved in conceptualization and design of the experimental protocol. MH, HT, and MI were responsible for investigation. Data collection, analysis, and interpretation were performed by all authors. MH and AS drafted the article. All authors critically revised the article and approved its final version.

Declarations

Conflict of interest This project was supported by a JSPS KAKENHI Grant-in-Aid for Early-Career Scientists (#18K17764) to MH. The authors declare that they have no conflicts of interest.

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