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Effects of 5-aminolevulinic acid with iron supplementation on respiratory responses to graded cycling and interval walking training achievement in older women over 75 yrs

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ABSTRACT

Background: Exercise training above a given intensity is necessary to prevent age-associated physical disability and diseases; however, the physical and psychological barriers posed by deteriorated physical fitness due to aging may hinder older people from performing daily exercise training. Because 5-aminolevulinic acid (ALA), a precursor of heme, reportedly improves mitochondrial function, we examined whether ALA, combined with sodium ferrous citrate (SFC) for enhancement, improved aerobic capacity and voluntary exercise training achievement in older women aged over 75 yrs.

Methods: The study was conducted using a placebo-controlled, double-blind crossover design. Fifteen women aged ~78 yrs. with no exercise habits underwent two trials for 7 days each where they performed interval walking training (IWT), repeating fast and slow speeds of walking for 3 min each, at >70% and at ~40% of peak aerobic capacity for walking, respectively, with ALA+SFC (100 and 115 mg/day, respectively) or placebo supplement intake (CNT), with a 12-day washout period. Before and after each trial, subjects underwent a graded cycling test while having their oxygen consumption rate ($\dot{V}O_2$), carbon dioxide production rate ($\dot{V}CO_2$), and plasma lactate concentration ($[Lac^-]_p$) measured. Furthermore, during the supplement intake period, exercise intensity for IWT was measured by accelerometry.

Results: In ALA+SFC, the increases in $\dot{V}O_2$ and $\dot{V}CO_2$ during the graded cycling test were attenuated (both, $P < 0.01$) with a 13% reduction in $[Lac^-]_p$ ($P = 0.012$) while none of these attenuated responses occurred in CNT (all, $P > 0.46$). Furthermore, energy expenditure and time during fast walking for IWT were 25% ($P = 0.032$) and 21% ($P = 0.022$) higher in ALA+SFC than in CNT.

Conclusion: Thus, ALA+SFC supplementation improved aerobic capacity and thus increased fast-walking training achievement in older women.

1. Introduction

Physical fitness peaks in our twenties; thereafter, it decreases by 5–10% every decade (Åstrand and Rodahl, 1986). After age 75, the rate accelerates, and when fitness has decreased to less than 25% of the peak level, people find it difficult to live independently (Kickbusch, 1997; Warburton et al., 2001a; Warburton et al., 2001b). To prevent age-associated declines in physical fitness, exercise training above a given intensity relative to individual peak aerobic capacity ($\dot{V}O_{2peak}$) has been recommended (American College of Sports Medicine, 2006a; U.S. Department of Health and Human Services, 2008). However, many people aged more than 75 yrs. may feel difficulty in performing the recommended regimen due to elevated physical and psychological barriers brought about by reduced physical fitness (Makizako et al., 2019; Sugiura et al., 1998). One of the underlying causes might be dysfunction of mitochondria. Indeed, mitochondrial function, especially

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complex IV (cytochrome *c* oxidase) activity in electron transport chain (ETC), was reported to decline with aging in human muscles (Muller-Hocker, 1990; Rooyackers et al., 1996; Trounce et al., 1989). To solve this problem, nutritional supplements may be helpful in reducing such barriers.

A candidate for these supplements is 5-aminolevulinic acid (ALA). ALA, a precursor of heme, is incorporated into cytochromes in the mitochondrial ETC in vitro (Ogura, 2011; Ota et al., 2013). Experimentally, it has been reported that the oral ingestion of ALA by mice increased complex IV activity and raised the ATP production rate in liver mitochondria (Ogura et al., 2011). Therefore, ALA supplementation might be effective for older people to increase the oxygen utilization efficiency of mitochondria during exercise.

Recently, Masuki et al. (2016) examined the effects of 5-aminolevurinic acid (ALA) supplementation, combined with sodium ferrous citrate (SFC) for enhancement, on the achievement of interval walking training (IWT), having participants repeat 5 or more sets of fast and slow walking for 3 min each, at more than 70% and at ~40% VO_{2peak}, respectively, for 4 or more days/week. Subjects for that study were middle-aged and older women aged ~65 yrs. who had already performed IWT for more than 12 months before participating in the study, so the effects likely reached plateau levels. The experiment, which was conducted using a randomized, placebo-controlled, double-blind crossover design, found that ALA supplementation decreased oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) rates with a reduced increase in plasma lactate concentration ($[Lac^-]_p$) at a given mechanical exercise intensity during a graded cycling exercise test. In addition, our research group found that the supplementation increased training days and fast walking time during the 7 day-supplement intake period. These results suggest that the ALA supplementation reduced the physical and psychological barriers in middle-aged and older people (Masuki et al., 2016); however, it remains unknown whether the effects would also be observed in older people aged over 75 yrs. who had not performed habitual exercise training before and thus might be expected to feel more difficulty in performing an exercise training regimen above a given intensity due to a rapid decline in physical fitness with aging.

Taking the above-mentioned factors into account, in the present study we examined the effects of the ALA with iron supplementation on the IWT achievement of older women over 75 yrs. using an experimental design similar to that of our previous study (Masuki et al., 2016). We hypothesized that the ALA and SFC combination would improve oxygen utilization efficiency during exercise and increase the IWT achievement. If our hypothesis is correct, the supplementation will be helpful in encouraging older people over 75 yrs. who do not have exercise habits to start training to increase their physical fitness.

2. Methods

2.1. Subjects

This study was approved by the Review Board on Human Experiments, Shinshu University School of Medicine, and it conformed to the standards set by the Declaration of Helsinki. The trial was registered with the University Hospital Medical Information Network (UMIN) in Japan, (trial registration number: UMIN000013211) on February 21, 2014.

We recruited female subjects aged 75–85 yrs. old [78.4 ± 3.1 (SD) yrs] who were visiting Fujimikougen Hospital for treatment of lifestyle-related diseases and other age-associated diseases once a month, or for regular medical examinations. They were all nonsmokers who had no overt cardiopulmonary and orthopedic diseases which would limit the exercise tests and training, and had no iron deficiency anemia which would influence the results. We recruited only female subjects to minimize any confounding effects of gender.

After we explained the study protocol to the 24 responders, 15 subjects provided written informed consent and agreed to participate in this study (Fig. 1). No harmful event occurred during the intervention. By interviewing the 15 women regarding their past and current health status using questionnaires including mini-mental state examination (MMSE), we confirmed that all subjects were nonsmokers without overt history of diseases that would limit the exercise tests and training in the present study. Physical characteristics before the intervention are shown in Table 1, where isometric knee extension (F_{EXT}) and flexion forces (F_{FLX}) on the dominant side of the leg were measured with an isometric force meter (GT330; OG Giken, Okayama, Japan) and hemoglobin concentration [Hb] was obtained from the latest records of their medical examinations. Additionally, in the records, mean corpuscular volume and mean corpuscular hemoglobin concentration were 94.7 ± 4.2 (mean ± SD) fl and 33.1 ± 1.0%, respectively. We confirmed that no subjects showed symptoms of iron deficiency anemia or other symptoms related to iron deficiency. Furthermore, the key measurements before and after each supplement intake period are shown in Table 2.

2.2. Outcomes

The primary outcome was the fast-walking training achievement of IWT, and the secondary outcomes were $\dot{V}O_2$, $\dot{V}CO_2$, and $[Lac^-]_p$ during the graded cycling test.

2.3. Sample size

The sample size for our primary outcome, calculated with JMP software (version 14.2; SAS Institute Japan, Tokyo, Japan), was 14 subjects based on 80% statistical power (1-β), α = 0.05, a difference in fast waking time of 27 min between trials and an SD of 33 min during the supplement intake period, as reported in the previous ALA + SFC supplementation study (Masuki et al., 2016). Similarly, another sample size of 12 subjects was calculated based on a difference in fast waking impulse of 6.3×10^5 N min between trials and an SD of 7.0×10^5 N min during the supplement intake period. In order to account for potential dropout, 15 subjects were included in the present study.

2.4. Randomization

Subjects were randomly assigned to placebo (CNT) − (ALA + SFC) sequence or (ALA + SFC) − CNT sequence by an independent investigator (K.H.) using permuted-block randomization (block size: 4) with an allocation ratio of 1:1. The randomization allocation sequence was generated using a computer.

2.5. Protocol

This study was carried out from August 17, 2013 to December 6, 2015. As shown in Fig. 1, all subjects underwent two trials. Each trial consisted of 9 days each; 7 days (days 1–7) for the supplement intake and 2 days for the graded cycling tests before and after the period (days 0 and 8), followed by more than a 12-day washout period. During the supplement intake period, subjects consumed either the ALA + SFC, 100 and 115 mg/day, respectively, (ALA + SFC trial) or the placebo supplement (CNT trial) for more than 1 h before breakfast and dinner. Before and after each supplement intake period, subjects underwent the graded cycling test while $\dot{V}O_2$, $\dot{V}CO_2$, and $[Lac^-]_p$ responses were measured. Furthermore, during days 1–6 of the supplementation intake period, the training days, intensity, and time were recorded with a tri-axial accelerometer (JD Mate; Kissei Comtec, Matsumoto, Japan) (Morikawa et al., 2011; Nemoto et al., 2007; Yamazaki et al., 2009). To avoid any acute influence of IWT on the graded cycling test, there was no training on day 7.

Because the subjects had not experienced the cycle ergometer test or IWT before the study, they were instructed on how to perform the test

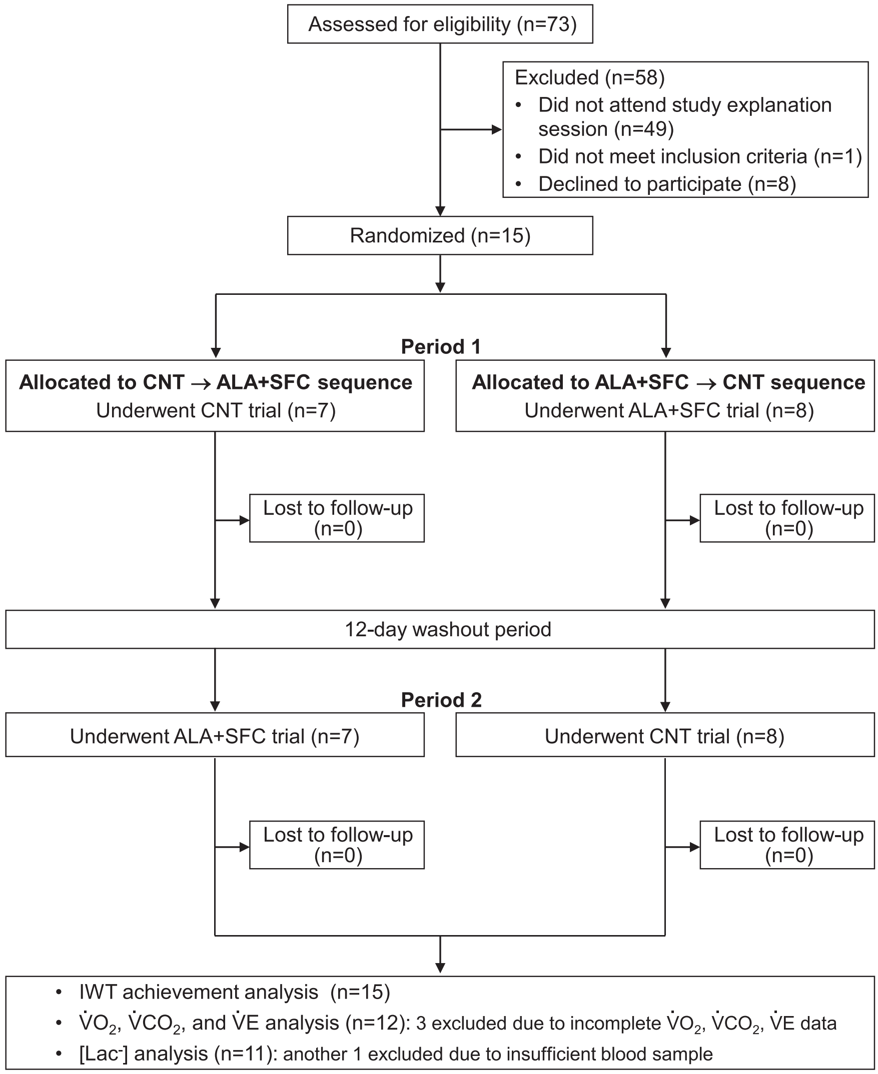

Fig. 1. CONSORT flow diagram for crossover design. CNT, placebo intake; ALA + SFC, 5-aminolevurinic acid + sodium ferrous citrate intake; IWT, interval walking training; $\dot{V}O_2$, oxygen consumption rate; $\dot{V}CO_2$, carbon dioxide production rate; $\dot{V}_E$, ventilation volume; and $[Lac^-]_p$, plasma lactate concentration.

and the training once a day for a total of three days within the week before starting the study.

2.6. Supplements

The composition of supplements (SBI ALApromo, Tokyo) is shown in Table 3. The dose of ALA phosphate (100 mg/day) was the same as in the previous study using relatively young older women (Masuki et al., 2016). SFC, as a source of the iron ion in the supplements, was used to enhance the final step of heme biosynthesis by the ABCB6 transporter and ferrochelatase in mitochondria (Ota et al., 2013). The ALA + SFC and placebo supplements were similar in appearance, and all of the subjects and investigators who performed experiments and analyses were blinded to which trial the subjects actually underwent until all of the analyses were finished.

2.7. Dietary intake

Subjects in both trials were instructed to maintain their dietary habits including medications, except for the supplements, throughout the study while reporting food consumed during the period by answering a questionnaire prepared by a dietician (FFQg version 4.0; Kenpakusha, Tokyo). The results are shown in Table 4. We confirmed that there were no significant differences in the measurements between the CNT and ALA + SFC trials ($P > 0.33$). Moreover, we confirmed that the amount of ALA contained in the diet (Fueki et al., 2010) was negligible compared with that in the supplement (Table 3).

2.8. Graded cycling test

To minimize any inter-individual variation by different levels of food intake on the graded cycling test, we provided standard meals on the day before the test. At 0800 on the morning of the test (days 0 and 8), the subjects reported to the hospital that they were normally hydrated but had not eaten any food for more than 11 h before the experiment, except for the prescribed experimental supplement 2 h before the visit on day 8. After having their anthropological variables measured, the subjects entered a laboratory in the hospital which had a temperature of 23.5 °C and 46.4% relative humidity. Subjects had a Teflon catheter placed in their antecubital vein for blood sampling and then rested quietly in a semi-recumbent position on the cycle ergometer (StrengthErgo240; Mitsubishi Electronic, Tokyo) for 15 min while the measurement devices were applied. After 10 min rest, subjects started cycling at the intensity of 0 W for 3 min, and then the intensity was increased to 10 W for 3 min; after that, the intensity was increased by 10 W every 2 min until the subjects were exhausted, during which time $\dot{V}O_2$, $\dot{V}CO_2$, and ventilation

Table 1
The baseline physical characteristics, current illnesses and medications in the subjects.

Subject #	Age, yr	Height, cm	BMI, kg/m^2	MMSE, points	F_{EXT}, Nm	F_{FLX}, Nm	[Hb], g/dl	Current illnesses	Current medications
1	76	154	24	24	80.1	55.5	13.8	–	–
2	75	142	22	29	69.4	39.8	13.4	HT, DL, OP	Candesartan, Benidipine, Atorvastatin, Raloxifene
3	77	153	23	29	98.3	26.7	14.4	HT, DL, cerebral infarction	Candesartan, Amlodipine, Atorvastatin, Cilostazol
4	81	146	20	28	75.7	42.1	13.7	–	–
5	83	138	24	25	79.3	38.7	12.9	–	–
6	77	150	20	26	100.2	44.4	12.5	HT, DL	Amlodipine, Atorvastatin
7	79	141	23	26	97.5	38.3	13.4	HT	Amlodipine
8	78	148	22	30	114.8	40.4	14.7	OA	Celecoxib
9	78	154	27	26	97.2	35.4	14.3	HT, DL, type 2 diabetes	Cilnidipine, Candesartan, Atorvastatin, Sitagliptin, Metformin
10	81	146	30	30	118.8	44.9	13.8	HT, DL, lumbar spinal canal stenosis	Candesartan, Benidipine, Losartan, Atorvastatin, Limaprost
11	85	154	20	30	116.5	42.4	13.9	HT	Telmisartan, Isopropylantipyrine
12	75	150	22	30	88.8	33.6	13.7	–	–
13	75	155	22	27	64.8	24.5	14.8	OA	Meloxicam, Loxoprofen, Felbinac
14	80	149	20	28	69.9	26.4	13.6	HT	Cilnidipine
15	76	145	22	27	52.7	19.9	14.5	–	–
Means±SD	78 ± 3	148 ± 5	23 ± 3	28 ± 2	88.3 ± 20.1	36.9 ± 9.3	13.8 ± 0.6		

Values are means ± SD for 15 subjects. BMI, body mass index; MMSE, mini-mental state examination; F_{EXT} and F_{FLX}, isometric knee extension and flexion forces, respectively; [Hb], hemoglobin concentration; HT, hypertension; DL, dyslipidemia; OP, osteoporosis; OA, osteoarthritis.

Table 2
The key measurements before and after each supplement intake period.

	CNT		ALA+SFC	
	Before	After	Before	After
Body weight, kg	50.0 ± 1.7	49.9 ± 1.7	50.2 ± 1.8	50.0 ± 1.8 *
# HR_{rest}, beats/min	65 ± 2	63 ± 2	65 ± 2	65 ± 2
SBP_{rest}, mmHg	137 ± 4	135 ± 4	143 ± 5	140 ± 4
DBP_{rest}, mmHg	82 ± 2	81 ± 2	82 ± 2	82 ± 2
# VO_{2peak} for cycling, l/min	0.88 ± 0.03	0.89 ± 0.02	0.89 ± 0.03	0.89 ± 0.03
# HR_{peak}, beats/min	138 ± 4	140 ± 4	139 ± 4	138 ± 4
# WL_{peak}, W	61 ± 2	62 ± 2	61 ± 2	64 ± 2 *
# $Time_{exhst}$, min	16.3 ± 0.5	16.6 ± 0.4	16.3 ± 0.4	16.7 ± 0.4

Values are means ± SE for 15 subjects (#, 12 subjects). CNT, placebo intake trial; ALA + SFC, 5-aminolevulinic acid + sodium ferrous citrate intake trial; HR_{rest}, resting heart rate; SBP_{rest} and DBP_{rest}, resting systolic and diastolic blood pressure, respectively; VO_{2peak}, peak oxygen consumption rate during the graded cycling test; HR_{peak}, peak heart rate at VO_{2peak}; WL_{peak}, peak workload at VO_{2peak}; $Time_{exhst}$, exercise time to exhaustion.*, Compared with before supplement intake, $P < 0.05$.

Table 3
Composition of supplements.

	Placebo supplement (250.00 mg/dose)	ALA + SFC supplement (250.00 mg/dose)
ALA phosphate, mg	0.00	50.00
SFC, mg	0.00	57.36
Pregelatinized starch, mg	247.50	140.14
Silicon dioxide mixture, mg	2.50	2.50

Table 4
Total energy, protein, fat, carbohydrate, ALA, and iron intake per day during the supplement intake period.

	CNT	ALA+SFC
Energy, kcal	1891 ± 40	1926 ± 55
Protein, g	71.3 ± 2.0	71.4 ± 2.2
Fat, g	56.5 ± 2.5	58.3 ± 2.5
Carbohydrate, g	270 ± 6	274 ± 8
ALA, μg	24 ± 2.1	24 ± 1.9
Iron, mg	8.3 ± 0.4	8.1 ± 0.2

Value are means ± SE for 15 subjects.

volume ($\dot{V}_E$) were measured with a respiratory gas analyzer (Metamax3B; Cortex, Leipzig, Germany) (Fig. 2) and heart rate (HR) was measured with an electrocardiogram (Polar RS400; Kemple, Finland). The subjects were allowed to perform cycling exercise in the range of 55–65 revolutions/min while exercise intensity was controlled to the target levels at or above 10 W through a servo-control system by varying the torque for the revolution electrically. The criteria for judging whether exercise intensity reached VO_{2peak} were that 1) subjects were not able to maintain the range of the rhythm, 2) the respiratory quotient increased to over 1.1, and 3) HR reached the age-predicted maximal value. The VO_{2peak} was determined by averaging the highest consecutive values for 45 s at the end of exercise. Peak HR was adopted at VO_{2peak}. In the subjects selected for analyses, the highest workload at which all of them could maintain the rhythm for 1 min in the four graded cycling tests (before and after each supplementation period) was for the first 1 min of 50 W; therefore, the data were presented from rest to 50 W.

2.9. $[Lac^-]_p$

$[Lac^-]_p$ was determined from blood samples taken at rest and at the last min of each intensity during the graded cycling test (JCA-BM8000; JEOL, Tokyo) (Fig. 2).

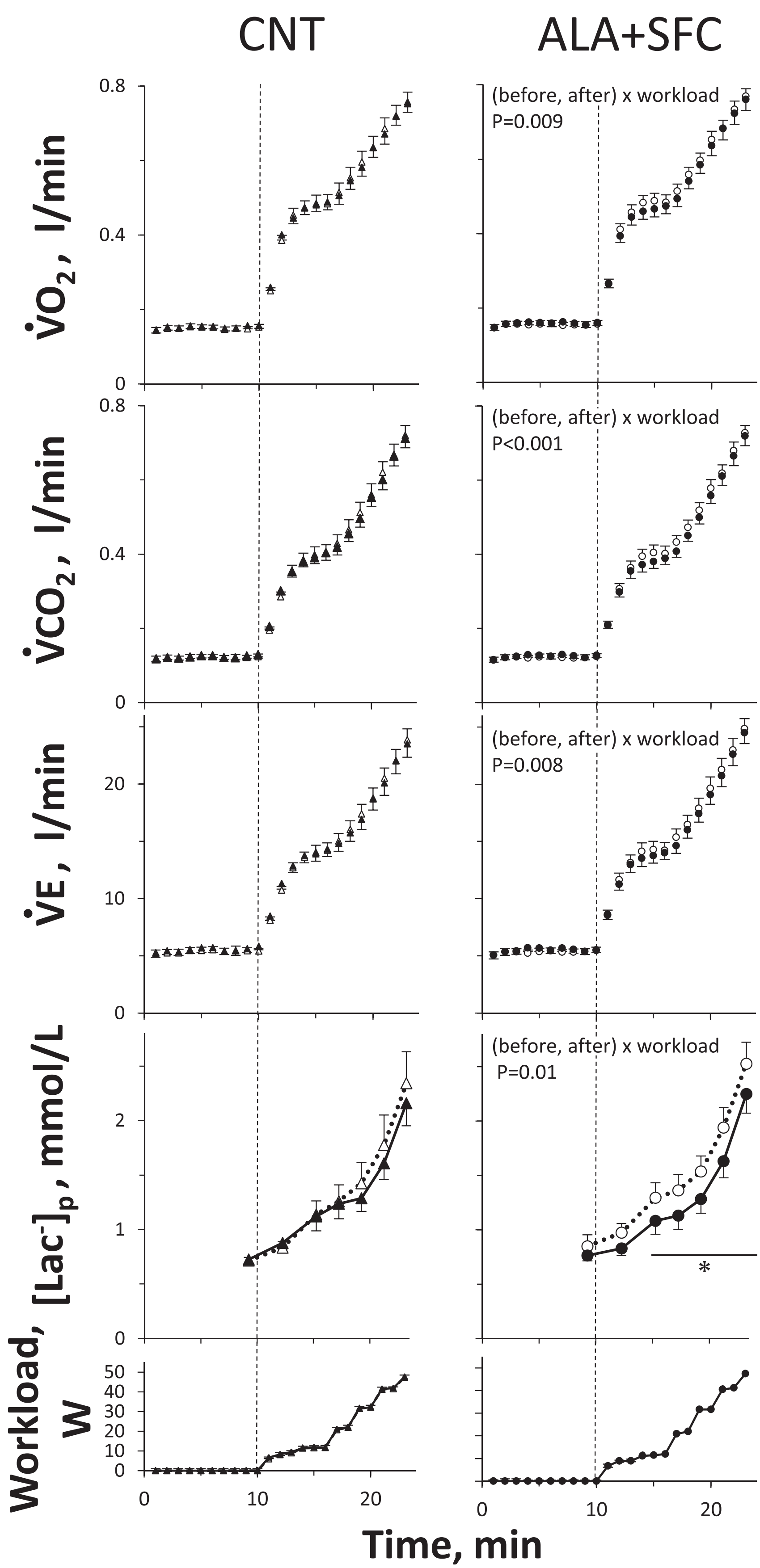

Fig. 2. Oxygen consumption rate ($\dot{V}O_2$), carbon dioxide production rate ($\dot{V}CO_2$), ventilation volume ($\dot{V}_E$), and plasma lactate concentration $[Lac^-]_p$ responses during the graded cycling exercise before and after the placebo (CNT; left) and ALA + SFC supplement intake (right) periods. The average values per minute are presented from rest to the highest workload of 50 W until which all subjects could maintain the rhythm. Open symbols, before the supplement intake period; solid symbols, after the supplement intake period. Values of are means ± SE; $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ for 12 subjects and $[Lac^-]_p$ for 11 subjects. *, $P < 0.05$ vs. before the supplement intake period.

2.10. Training achievement

During the supplement intake period, except for the day before the graded cycling test (days 1–6), subjects were instructed to perform IWT with the goal of repeating 5 sets of fast and slow walking for 3 min each, at more than 70% and ~40% of VO_{2peak}, respectively, per day, every day, during this period while the intensity and duration were recorded with a portable tri-axial accelerometer (JD Mate) (Morikawa et al., 2011; Nemoto et al., 2007; Yamazaki et al., 2009). After the supplement intake period, the measurements were transferred to the server computer at Shinshu University through the Internet (Masuki et al., 2020; Morikawa et al., 2011; Nose et al., 2009). Training intensity was calculated from the product of body weight and average norm of three-dimensional accelerations and presented as the accumulated training impulse (N min) (Iwashita et al., 2003; Yamazaki et al., 2009) for 6 days (Fig. 3).

2.11. Exercise efficiency

In the present study, we could not calculate the exercise efficiency precisely because the period at a given intensity during the graded cycling test was only 2 min, too short to use the steady state assumption (Brooks et al., 1996). However, to compare the results with those from our previous study (Masuki et al., 2016), we attempted to determine the exercise efficiency from the relationship between the average values of $\dot{V}O_2$, $\dot{V}CO_2$ and workload during the last 1 min of each exercise intensity at 10–40 W after converting $\dot{V}O_2$ to an equivalent physical unit of energy using the respiratory quotient, assuming that the value approximately reflected the exercise efficiency during the period (Gaesser and Brooks, 1975). Because the values at 50 W were only for the first 1 min and unlikely to have reached the steady state, we did not use them for the calculation.

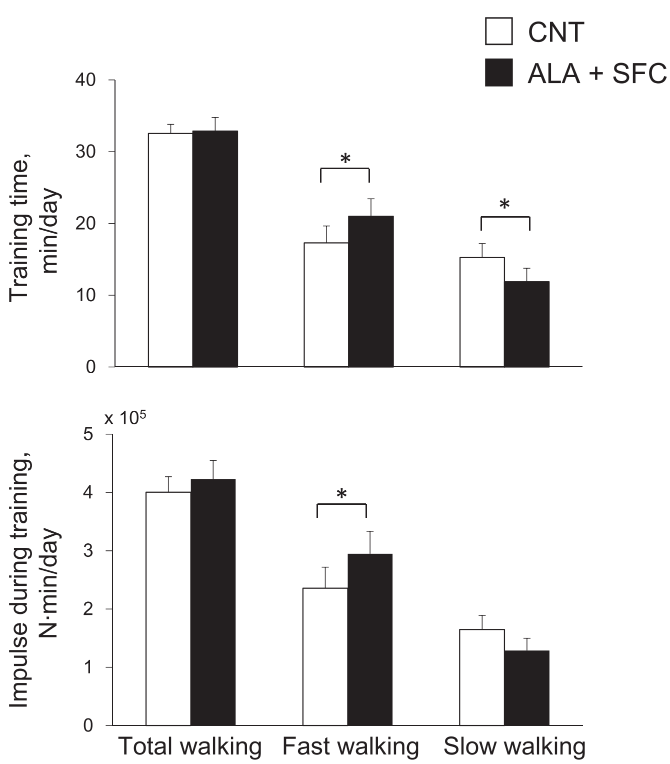

Fig. 3. Training time and training impulse at total, fast, and slow walking during the supplement intake period. Values are means ± SE for 15 subjects. *, $P < 0.05$ between the CNT and ALA + SFC trials.

2.12. Subjects for analyses

As shown in Fig. 1, all subjects underwent two trials and were used for IWT achievement analysis. Also, all subjects underwent the graded cycling tests 4 times, before and after each supplementation period, respectively. However, we failed to measure a portion of the respiratory variables during the graded cycling tests in 3 of 15 subjects. In 2 subjects, the measurements in one trial were unstable compared with those in their other trials because their expiratory gas likely leaked from a space between their respiratory mask and their facial skin surface, and in the third subject the measurements at 50 W were not obtained because she could not maintain the rhythm for pedaling. Moreover, in another one of the 12 remaining subjects, we could not sample a satisfactory volume of blood from the cutaneous vein for the $[Lac^-]_p$ analysis probably because vasoconstriction occurred at the high exercise intensity. Therefore, we analyzed $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}_E$ in 12 subjects and $[Lac^-]_p$ in 11 subjects (Fig. 1).

2.13. Statistics

One-way ANOVA for repeated measures was used to examine any significant differences in physical characteristics before vs. after the supplement intake period (Table 2). Two-way ANOVA for repeated measures was used to examine any significant differences in the variables at every intensity during the graded cycling test before vs. after the supplement intake period in each trial, with a significant interactive effect of [(before vs. after the supplement intake period) × the intensity during the graded exercise test] (Fig. 2). One-way ANOVA for repeated measures was also used to examine any significant differences in training time and training impulse during the supplement intake period (Fig. 3) between the CNT and ALA + SFC trials. The 1-β is presented in the text at $\alpha = 0.05$ when the key variables were significantly different between the CNT and ALA + SFC trials (Cohen, 1988). As a subsequent post hoc test, the Tukey-Kramer test was used to perform any pairwise comparisons between trials.

Because this study was conducted in a two-period crossover design, our primary outcome (training time and impulse for fast walking) was further analyzed to examine three effects: carryover (the effect of the first trial persisting into the second period), period [the effect of stimulation order present in the CNT-(ALA+SFC) sequence group vs. the (ALA+SFC)-CNT sequence group], and supplement effects. These three effects were determined using the method reported by Chow and Liu (1992). The null hypothesis was rejected when $P < 0.05$. Values are expressed as the means ± SE, unless otherwise indicated.

3. Results

3.1. The key measurements before and after the supplement intake period

As shown in Table 2, HR and blood pressure at rest, VO_{2peak} and peak HR remained unchanged after the supplement intake period in both trials (all, $P > 0.12$). On the other hand, peak workload increased significantly after the supplement intake period in the ALA + SFC trial ($P = 0.029$) while not in the CNT trial ($P > 0.07$) but with marginal increase in the time to exhaustion in the ALA + SFC trial ($P = 0.09$).

3.2. $\dot{V}O_2$, $\dot{V}CO_2$, and $[Lac^-]_p$ during the graded cycling test

As shown in Fig. 2, although $\dot{V}O_2$, $\dot{V}CO_2$ $\dot{V}_E$, and $[Lac^-]_p$ increased similarly as the workload increased during the graded cycling test before the supplement intake period in both trials, their increases were significantly attenuated in the ALA + SFC trial after the period while not in the CNT trial with significant interactive effects of [(before vs after the supplement intake period) × workload] ($P = 0.009$, 0.0002, 0.008, and 0.01; 1-β = 0.998, 1.000, 0.998, and 0.781, respectively).

The average gross exercise efficiency at 10-40 W during the graded cycling test was 11.6 ± 0.4% in the ALA + SFC trial before the supplement intake period, similar to 11.9 ± 0.5% in the CNT ($P > 0.1$). After the supplement intake period, the efficiency significantly increased to 11.9 ± 0.4% in the ALA + SFC trial ($P = 0.049$) while remaining unchanged as 11.9 ± 0.4% in the CNT trial ($P > 0.9$) but with no significant difference in the change between the trials ($P > 0.2$). Similarly, the average net exercise efficiency was 15.6 ± 0.6% in the ALA + SFC trial before the supplement intake period, similar to 16.0 ± 0.7% in the CNT ($P > 0.2$). After the supplement intake period, the efficiency significantly increased to 16.3 ± 0.7% in the ALA + SFC trial ($P = 0.006$) while remaining unchanged as 16.4 ± 0.9% in the CNT trial ($P > 0.5$) but with no significant difference in the change between the trials ($P > 0.5$).

3.3. Training achievement during the supplement intake period

As shown in Fig. 3, the training times per day were 32.9 ± 1.9 min in the ALA + SCF trial, not significantly different from 32.5 ± 1.3 min in the CNT trial during the supplement intake period ($P > 0.8$). However, we found that the fast walking time during training per day was 21.0 ± 2.9 min in the ALA + SCF trial, significantly higher than 17.3 ± 2.4 min in the CNT trial ($P = 0.022$, $1\text{-}\beta = 0.699$). We also found that the impulse during training per day was $(2.9 \pm 0.4) \times 10^5$ N min for fast walking in the ALA + SCF trial, significantly higher than $(2.4 \pm 0.4) \times 10^5$ N min in the CNT trial ($P = 0.032$, $1\text{-}\beta = 0.636$).

Training time and impulse for fast walking were further examined by comparing the CNT – (ALA+SFC) sequence group (n = 7) vs the (ALA+SFC) − CNT sequence group (n = 8) (Chow and Liu, 1992; Karst et al., 2003). For the CNT – (ALA+SFC) sequence, the fast walking time during training per day was 20 ± 3 in period 1 and 22 ± 4 min/day in period 2, and the difference over time (period 1–2) was −2 ± 2 min/day. For the (ALA+SFC) – CNT sequence, the fast walking time during training per day was 20 ± 3 in period 1 and 15 ± 3 min/day in period 2, and the difference over time (period 1–2) was 5 ± 2 min/day, which was significantly different from that observed in the CNT – (ALA+SFC) sequence ($P = 0.027$), thus indicating a significant supplement effect. Similarly, a significant supplement effect was observed in the fast walking impulse ($P = 0.040$). No carryover or period effects were observed in training time and impulse for fast walking ($P > 0.3$).

4. Discussion

The major findings in the present studies are that 1) the increases in $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$, and $[Lac^-]_p$ during the graded cycling test were significantly suppressed in the ALA + SFC trial with significantly increased exercise efficiencies, while none of these changes occurred in the CNT trial; and 2) training time and impulse for fast walking of IWT significantly increased in the ALA + SFC trial compared with in the CNT trial in older women over 75 yrs. who had not performed IWT before.

In Japan, older people aged over 75 yrs. comprised 1.3% of the total population in 1950. By 2016, the figure had increased to 13%, and it is predicted to continue to increase to 26% by 2055 (Cabinet Office of Japan, 2017). One of the serious problems of the rapid increase in the older generation is the high prevalence of age-associated physical disability and diseases, including impaired cognitive function (Ministry of Health, Labor and Welfare of Japan, 2017). Therefore, exercise training above a given exercise intensity has been recommended for older people (American College of Sports Medicine, 2006b).

However, to do this, they are expected to visit a gym or a hospital to use machines, such as a treadmill or bicycle ergometer, under the supervision by trainers, involving both time and cost (Campbell et al., 1997; Robertson et al., 2001). Additionally, it is often difficult for older people, by themselves, to visit a specific place equipped with the instruments due to not only their limited ability to use public transportation, but also the limited number of institutions (Booth et al., 2000).

To solve these problems, we have developed the e-Health Promotion System composed of IWT, a portable calorimeter (JD Mate), and an internet of things (IoT) system, which enables middle-aged and older people to perform IWT at their favorite time and place without going to any specific institutions (Masuki et al., 2020; Morikawa et al., 2011; Nose et al., 2009). By using the system, we have accumulated a database regarding the effects of IWT in 8700 participants, suggesting that fast walking during IWT more than 60 min per week for 5 months increased thigh muscle strength and VO_{2peak} by ~10% with improved symptoms of lifestyle-related disease by 20% in middle-aged and older people aged ~65 yrs. on average (Masuki et al., 2019; Nemoto et al., 2007; Yamazaki et al., 2009). In addition, we found in a pilot study using 8 older people aged over 80 yrs. (Ichihara et al., 2007), who were visiting a day-care service institution affiliated with Fujimikougen Hospital, that VO_{2peak} and thigh muscle strength increased by ~8% and ~4%, respectively, by performing the fast walking at an intensity above 70% relative to their VO_{2peak}, total ~ 11 min per day, but with irregularly intermittent short periods of slow walking and resting, 2 times a week, for 3 months. These results suggest that older people can attenuate their age-associated deterioration of physical fitness even if they perform the fast walking above a given intensity for a given period.

However, the adherence to the exercise program was reported to be inversely associated with age in older people aged ≥65 yrs. (Masuki et al., 2015). This might be because there are several physical and psychological barriers to hinder older people from performing the training. One of these barriers has been suggested as a feeling of "poor health" explained by easy fatigability due to reduced physical fitness usually accompanied by age-associated diseases (Hirvensalo et al., 1998; Moschny et al., 2011). Accordingly, in the present study, we hypothesized that if ALA + SFC supplementation attenuated the feeling of "poor health" by decreasing an increase in hydrogen ions dissociated from lactic acid during exercise, which otherwise induces muscle soreness through the polymodal receptors in the muscle and also induces panting for respiratory compensation to maintain pH in blood, it would make it easier for older people who do not have exercise habits to start training to increase physical fitness and improve their age-associated diseases. The study was conducted to examine the hypotheses.

4.1. The characteristics of subjects

Tables 1 and 2 show the physical characteristics of the subjects and the key measurements before and after the supplementation. Regarding their physical fitness levels, VO_{2peak} was 17.5 ml/kg/min, F_{EXT} and F_{FLX} were 1.77 and 0.74 Nm/kg, respectively, on average. In our previous study, Morikawa et al. (2011) divided 468 women aged 64 yrs. equally into 3 groups according to their VO_{2peak}, and reported that VO_{2peak} was 17.2, 21.5, and 25.9 ml/kg/min, F_{EXT} was 1.74, 1.96, and 2.20 Nm/kg, and F_{FLX} was 0.96, 1.06, and 1.13 Nm/kg, on average in the order of the low, middle and high VO_{2peak} groups, respectively. Thus, the subjects in the present study belong to the low VO_{2peak} group of older women aged 64 yrs. Since physical fitness reportedly decreases by 5–10% every decade, and also since the reduction rate is accelerated after 75 yrs. (Makizako et al., 2019; Sugiura et al., 1998), the physical characteristics of the subjects in the present study likely represented this demographic but with no overt symptoms disturbing voluntary IWT.

Regarding the cognitive function levels of the subjects, the MMSE marked no more than 27 points in 7 of 15 subjects, meeting the criteria for patients with mild cognitive impairment (Folstein et al., 1975; Kaufer et al., 2008). Since the prevalence of dementia including mild cognitive impairment was reported to be ~40% of the total for older women aged from 75 to 84 yrs. (Asada, 2012), the cognitive function in the subjects for the present study might also represent that of the population of this age who could perform the training with no help from others. Thus, the physical and cognitive characteristics of the subjects well represent those of the population of this age in Japan.

In addition, and importantly for the present study, it should be noted that the subjects were ~13 yrs. older and had ~31% lower VO_{2peak} than those in the previous ALA + SFC study (Masuki et al., 2016) and that they had no exercise habits while those in the previous study had performed IWT more than 12 months. These results suggest that the subjects in the present study might have elevated physical and psychological barriers for starting exercise compared with those in the previous study (Masuki et al., 2016).

4.2. Effects of ALA + SFC supplementation in the graded cycling test

As shown in Fig. 2, the increase in $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}_E$ and $[Lac^-]_p$ with increased workload were significantly attenuated by the ALA + SFC supplementation.

In the previous study stated above, Masuki et al. (2016) examined the effects of oral ingestion of ALA + SFC by the same protocol of intervention as in the present study, and suggested that $\dot{V}O_2$, $\dot{V}CO_2$ $\dot{V}_E$ and $[Lac^-]_p$ decreased during the graded cycling test, and that impulse and time at fast walking increased during IWT, similar to the results in the present study. Regarding the mechanisms, since our research group found that the gross and net exercise efficiencies during the graded cycling test, calculated based on the steady-state assumption that energy requirements were met $\dot{V}O_2$ (Brooks et al., 1996), both increased by ~12%, we suggested that the ALA + SFC supplementation enhanced heme biosynthesis (Ogura et al., 2011; Ota et al., 2013) to activate complex IV and to increase ATP production. In the present study, when we attempted to calculate approximately the gross and net exercise efficiencies from the relationship between the averaged values of $\dot{V}O_2$ and workload during the last 1 min of each exercise intensity at 10–40 W, the average gross and net efficiencies increased by ~3% and ~5%, respectively, results that were a little lower than those in the previous study (Masuki et al., 2016). The increases in the exercise efficiencies due to supplementation in the present study, smaller than those in our previous study (Masuki et al., 2016), were probably affected by the more limited condition for the determination and the lower range of exercise intensity during the graded cycling test. These results, however, suggest that the ALA + SFC supplementation activated mitochondrial ETC function and thereby increased ATP production to reduce $[Lac^-]_p$, which may lower the physical and psychological barriers for starting exercise above a given intensity required to increase physical fitness and improve lifestyle-related disease symptoms (Ministry of Health, Labor and Welfare of Japan, 2017; Ministry of Health, Labor and Welfare of Japan, 2019), which in turn will help older women start exercise training above a given intensity required to attain the effects (American College of Sports Medicine, 2006a; U.S. Department of Health and Human Services, 2008).

4.3. Effects of ALA + SFC supplementation in the exercise achievement

We found that the impulse (exercise volume) and time during fast walking of IWT were increased by supplementation (Fig. 3).

In the previous study stated above, Masuki et al. (2016) suggested that the ALA + SFC supplementation increased training days, impulse, and time at fast walking by 42%, 102%, and 69%, respectively, during the IWT for 6 days. Recently, Suzuki et al. (2018) examined the effects of the ALA + SFC supplementation in middle-aged depressive women aged 53 yrs. by a protocol similar to that of the present study and reported similarly improved respiratory responses during the graded cycling test and training achievements as in our previous (Masuki et al., 2016) and present studies, with improved depressive symptoms using the Montgomery-Asberg Depression Rating Scale (Snaith et al., 1986).

The precise mechanisms for the improved training achievements remained unclear. However, the ALA + SFC supplementation saved $\dot{V}O_2$ and $\dot{V}CO_2$ during the graded cycling test, attenuated the increases in $[Lac^-]_p$ (Fig. 2), and increased peak workload (Table 2). These results suggest that the subjective feeling of fast walking might thus be improved due to reduced panting and muscle pain, ultimately resulting in increases in impulse and time at fast walking by the supplementation (Fig. 3). This is consistent with a recent study showing that higher muscle mitochondrial efficiency was associated with a faster preferred walking speed in older adults (Coen et al., 2013). These mechanisms likely worked also in older women aged over 75 yrs. in the present study.

Alternatively, the increased training achievement by the ALA + SFC supplementation might be related to enhanced central mechanisms. ALA administration to rats reportedly induced increased levels of tryptophan and serotonin in the forebrain (Daya et al., 1990), which might enhance the melatonin concentration in the pineal gland and improve the circadian rhythm in older people (Reiter, 1986). Indeed, in a study using questionnaires, Rodriguez et al. (2012) suggested that, in middle-aged and older subjects, the ingestion of ALA + SFC (50 and 57 mg/day, respectively) for 6 weeks improve the quality of sleep, emotional state, and reaction to psychological stress. Therefore, in the present study, we could not exclude the possibility that the increased training achievements attributed to the ALA + SFC supplementation were caused by central mechanisms.

4.4. Experimental considerations

First, the possible effects of iron intake on the results were not excluded; however, we confirmed that no subjects showed any symptoms of iron deficiency in their medical records before participating in the study. Moreover, the dose of ALA and SFC used in the present study was the same as in the previous study which confirmed that this dose did not increase [Hb] in older women aged ~65 yrs. (Masuki et al., 2016). Therefore, it was unlikely that results in the ALA+SFC trial were caused by increase in [Hb].

Second, while the number of subjects is admittedly very small, especially given the large variation in older people with various diseases, we were able to confirm that physical characteristics of our subjects aged 75–85 yrs. well represent the population of this age in Japan, as noted earlier. Moreover, this study was conducted in a crossover design where each subject underwent both experimental and control trials; therefore, each participant acted as her own control. A key advantage of such a crossover design is that a smaller sample size is required compared with a parallel design in which each subject undergoes only one trial (Dwan et al., 2019). As a result, we found that ALA+SFC supplementation significantly increased fast-walking training achievement in older women aged over 75 yrs.

In conclusion, ALA + SFC supplementation improved respiratory and $[Lac^-]_p$ responses during graded exercise, resulting in increased fast-walking training achievement in older women aged over 75 yrs.

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CRediT authorship contribution statement

Yasuko Ichihara: Formal analysis, Investigation, Data curation, Writing – original draft. **Shizue Masuki:** Conceptualization, Methodology, Formal analysis, Writing – review & editing. **Koji Uchida:** Validation. **Kiwamu Takahashi:** Conceptualization, Resources. **Motowo Nakajima:** Conceptualization, Resources. **Hiroshi Nose:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition.

Declaration of competing interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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