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Enhancement of Fat Oxidation by Licorice Flavonoid Oil in Healthy Humans during Light Exercise

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Summary Licorice flavonoid oil (LFO) is a new functional food ingredient consisting of hydrophobic licorice polyphenols in medium-chain triglycerides. Recent studies reported that LFO prevented and ameliorated diet-induced obesity via the regulation of lipid metabolism-related gene expression in the livers of mice and rats, while it reduced body weight in overweight human subjects by reducing total body fat. However, the direct effects of LFO on energy metabolism have not been studied in human subjects. Therefore, we investigated the effects of ingestion of LFO on energy metabolism, including fat oxidation, by measuring body surface temperature under resting conditions and respiratory gas analysis under exercise conditions in healthy humans. We showed that ingestion of a single 600 mg dose of LFO elevated body trunk skin temperature when measured in a slightly cooled air-conditioned room, and increased oxygen consumption and decreased the respiratory exchange ratio as measured by respiratory gas analysis during 40% $\dot{V}o_2max$ exercise with a cycle ergometer. Furthermore, repeated ingestion of 300 mg of LFO for 8 d decreased respiratory exchange during the recovery period following 40 min of 30% $\dot{V}o_2max$ exercise on a treadmill. These results suggest that LFO enhances fat oxidation in humans during light exercise.

Key Words licorice, flavonoid, respiratory exchange ratio, fat oxidation, humans

Licorice is widely consumed as a food and natural medicine/herbal drug in both Eastern and Western countries (*1*). There are several species of licorice, including *Glycyrrhiza uralensis* Fischer, G. *glabra* Linne, and G. *inflata* Batalin, each of which contain specific flavonoids (*1*).

Licorice flavonoid oil (LFO) is a new functional food ingredient containing hydrophobic flavonoids derived from G. *glabra* (*2*). Our previous study suggested that LFO is effective in reducing visceral fat accumulation and suppressing elevated blood glucose levels in obese diabetic KK-Ay mice (*2*). Subsequently, a double-blind placebo-controlled clinical trial showed that LFO was effective in suppressing body weight gain by reducing body fat mass in overweight volunteers (*3*). These studies indicate that LFO may help to prevent lifestyle-related diseases such as obesity associated with metabolic syndrome. The mechanism of the antiobesity effect of LFO was previously investigated in a high-fat diet-induced obese C57BL/6J mouse model and the results suggest that the effect was mediated by modification of the expression levels of lipid metabolism-related genes in the liver (*4*). In particular, it has been shown that LFO increases the enzymatic activity of acyl-coA dehydrogenase, the rate-limiting enzyme in the fatty acid oxidative pathway, and simultaneously decreases the enzyme activity of acetyl-coA carboxylase and fatty acid synthase, the rate-limiting enzymes in the fatty acid synthetic pathway.

These studies suggest that LFO may alter energy metabolism, especially fat degradation. However, there is no information regarding the short-acting effects of LFO on energy metabolism at rest or during exercise in healthy subjects. In addition, the effects of LFO on body temperature have not been evaluated. Body temperature in humans is kept stable within a few tenths of a degree Celsius by the regulation of thermogenesis and heat diffusion (*5*). We hypothesized that an increase in fat oxidation would induce thermogenesis, which would in turn lead to elevation of tympanic membrane and body trunk surface temperatures; and these phenomena would be more responsive to changes in energy metabolism than changes in respiratory gas analysis.

The purpose of this study was to assess the effects of LFO ingestion on energy metabolism at rest and during exercise in healthy subjects. First, we investigated the effects of LFO on body surface temperature by measuring skin temperature in a slightly cool air-conditioned room. Next, we investigated the effects of LFO on energy metabolism by conducting respiratory gas analysis during exercise. To determine more effective and practical applications, we also investigated the effects of repeated ingestion of LFO on energy metabolism.

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MATERIALS AND METHODS

Subjects and informed consent. A total of 34 healthy Japanese females were included in the study and assigned to 4 experimental groups as follows: Experiment 1 (Ex. 1): $n=8$, age 18–22 y, height, 157.0±4.8 cm, body weight 49.6±3.7 kg, and BMI 20.1±1.5. Experiment 2 (Ex. 2): $n=10$, age 19–22 y, height 160.5±7.8 cm, body weight 57.3±6.8 kg, and BMI 22.2±2.0. Experiment 3 (Ex. 3): $n=9$, age 20–23 y, height 160.9±5.5 cm, body weight 52.9±5.6 kg, and BMI 20.9±1.2. Experiment 4 (Ex. 4): $n=7$, age 21–22 y, height 159.0±3.0 cm, body weight 50.0±3.8 kg and BMI 19.8±1.1 (values are mean±SD). None of the subjects was on any medication or was pregnant during the study. Subjects were instructed to avoid alcoholic and caffeinated beverages, irritant foods such as spices, and strenuous physical activity on the day before measurements. Prior to commencement of the experiments, the administrator provided an explanation to all subjects of the study purpose, test protocol, and bioactivity of LFO. The study was conducted with the approval of the ethics committee of the University of Shiga Prefecture in accordance with the guidelines of the Helsinki Declaration, and written consent was obtained from the subjects.

LFO test substance. To prepare the LFO test material, an ethanol extract of licorice (*G. glabra*) root was obtained. After filtration and concentration, the ethanolic layer was mixed with medium-chain triglycerides (MCT, C8 : C10=99 : 1). This solution, designated "LFO concentrate solution," is identical to the novel patented compound marketed under the proprietary brand name Kaneka Glavonoid™ and approved by the FDA for sale in the US as a new dietary ingredient (NDI). For this study the concentration of glabridin, the major component of LFO concentrate solution, was adjusted to 3% (w/w) prior to its use. Each test capsule contained 100 mg of LFO concentrate solution, 200 mg MCT and 33% beeswax in a soft gel, while each placebo capsule contained 300 mg MCT and 33% beeswax alone. Previous human safety studies confirmed the safety of 600 mg of LFO concentrate solution (*6*). The maximum recommended daily dose of Kaneka Glavonoid™ is 300 mg. In order to evaluate a potentially more effective dose we used 600 mg of LFO concentrate solution as a single dose while 300 mg was also used to test the standard repeat dose regimen.

Experimental conditions. All studies were conducted using a randomized double-blind, placebo-controlled, crossover method. A blinded third party generated the treatment sequence and allocated treatments randomly to each subject. Subjects in Ex. 1–3 ingested one of two samples on the first day and the other sample on the second day following a washout period of a minimum of 2 d. For Ex. 4, each subject ingested one of two samples during the first week and after a one-week wash out period the other sample was ingested. Subjects wore the same clothing (jerseys and socks for Ex. 1, shorts and T-shirt for Ex. 2–4) for every measurement to eliminate the influence of variation due to clothing. The body surface temperature and respiratory gas analysis were measured approximately 4 h after sample ingestion because previous pharmacokinetic studies of LFO showed this is the approximate amount of time required for the plasma concentration of glabridin to attain the maximum level after ingestion (*7*). In order to minimize the effect of diet in this study, the subjects were provided a simple breakfast (energy content approximately 440 kcal or 1,800 kJ. PFC=5 : 40 : 55, consisting of a wheat-bran cream sandwich cookie and vegetable juice) and a light lunch (energy content 330 kcal or approximately 1,400 kJ, PFC=8 : 24 : 68, consisting of tomato cream rice-gratin). Mealtimes were standardized for all subjects with lunch between 12:30 and 13:00. Each experiment commenced at 13:00 h to avoid the influence of the diurnal variation in body temperature or energy metabolism. All the subjects were in good health throughout the study period and there were no complaints of discomfort after ingestion of any sample. This study was conducted without strict restriction of physical exercise.

Measurements of body temperature (Ex. 1). Measurements of body surface temperature were performed as previously described with a slight modification (*8*, *9*). The measurements were carried out in a quiet air-conditioned, temperature-controlled room (23±0.5°C) with a humidity of approximately 50%, which induces a gradual decrease in the peripheral body surface temperature under resting conditions. The subjects ingested the LFO (600 mg) or placebo (MCT, 600 mg) capsules with 100 mL of water (37°C) at 13:00 h. After a 150 min rest period (free work in a seated position), subjects were moved to the air-conditioned room. Measurements of the body surface and tympanic temperature were performed using a thermometer (BTH-601 Bio Research Center Co., Nagoya, Japan) and an ear thermometer (M30, Terumo Corporation, Tokyo, Japan) respectively, between 15:30 and 17:30 h.

The time-course of changes in temperature at a constant room temperature were measured as follows: thermoprobes were fixed on the skin of the forehead, neck, abdomen, and back using surgical tape and temperature was measured from each site every min for 90 min following the commencement of the experiment. The time-course of changes in temperature of the tympanic membrane was monitored by the subjects themselves every 15 min for up to 90 min following the commencement of the measurements. The visual analog scale (VAS), used in behavioral science to measure a variety of subjective phenomena (*10*, *11*) was used to measure changes in bodily thermoception. The VAS is a horizontal line anchored by word descriptors about bodily thermoception on the left (cool) and right (warm) sides of the midpoint. The subjects indicated their perception of the current state of their whole body thermoception on the VAS. The sensation scores were determined by measuring the length from the midpoint of the line to the point marked by the subjects.

Respiratory gas analysis (Ex. 2–4). Expired gas exchange was measured by breath-by-breath analy-

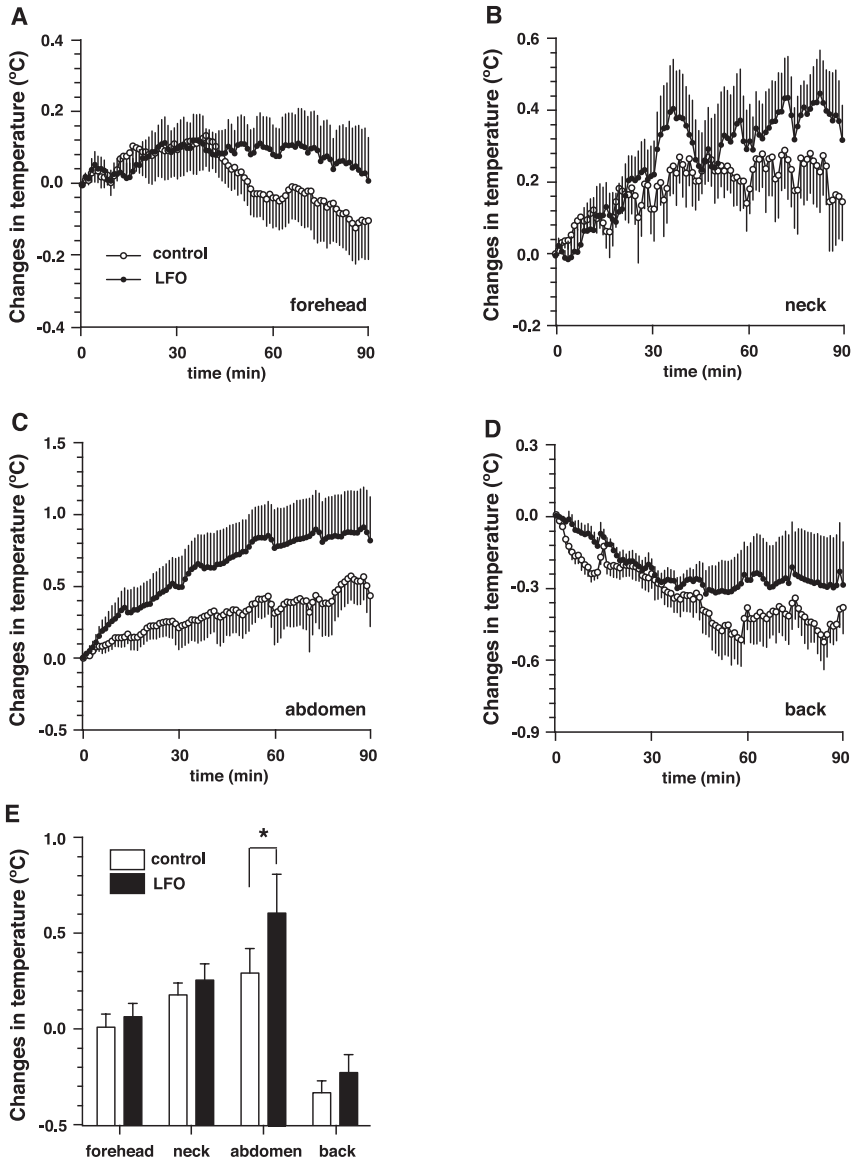

Fig. 1. (A–D) Changes in temperature at forehead (A), neck (B), abdomen (C) and back (D) in humans ingesting LFO (600 mg) or a placebo (control). Values are expressed as means±SE. $n=8$ (at forehead; $p<0.05$: two-way repeated-measures ANOVA). (E) Average changes in temperature at forehead, neck, abdomen and back for 90 min during cold exposure in humans ingesting LFO or a placebo (control). Values are expressed as means±SE. $n=8$; $^*p<0.05$ (paired t test).

sis using a metabolic cart (AE300s, MINATO, Tokyo, Japan). Oxygen uptake and the respiratory exchange ratio (RER) were calculated every 10 s.

On the basis of the volume of CO_2 production per unit of time (L/min) ($\dot{V}co_2$) and $\dot{V}o_2$, total glucose, and lipid oxidation were calculated using the stoichiometric equations of Frayn (*12*) as follows: total fat oxidation$=1.67\ \dot{V}o_2-1.67\ \dot{V}co_2$ and carbohydrate oxidation$=4.55\ \dot{V}co_2-3.21\ \dot{V}o_2$.

To determine the intensity level of exercise for each subject, preliminary tests were performed to determine the maximal oxygen uptake ($\dot{V}o_2max$) of each subject using a cycle ergometer (Ergometer 232CXL, Combi Co., Japan) for Ex. 2 and 3 or a treadmill (LIDO IWM treadmill, Greenmaster Japan Co., Japan) for Ex. 4. Preliminary tests for experiments using the cycle ergometer began with a 10 min rest period. This was followed by unloaded cycling for 2 min, after which the workload was increased by 25 W every 4 min starting at 50 W and going up to 100 W. The pedaling frequency was set at 60 rpm. The preliminary tests with the treadmill were started after a 10 min rest period and the treadmill speed was increased by 1 km/h every 4 min, starting at 2 km/h and going up to 6 km/h. We plotted the average $\dot{V}o_2$ versus the average heart rate (HR) under each workload or speed and a regression line was calculated. From the regression line, $\dot{V}o_2max$ was determined at the maximal HR point. Maximal HR was calculated using the Miller formula:

$$\text{Maximal HR}=217.4-(\text{age}\times 0.87)\ (13)$$

For these experiments, the exercise intensity level was tailored to subjects based on the workload or treadmill speed corresponding to the settled intensity of $\dot{V}o_2max$.

Protocol for single dose ingestion test (Ex. 2 and 3). We

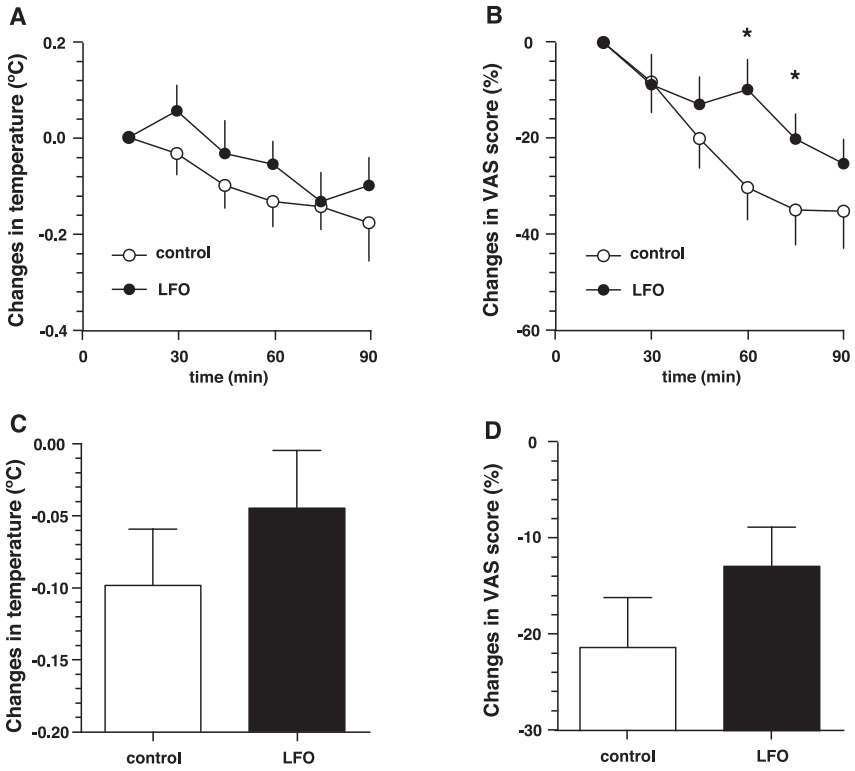

Fig. 2. (A and B) Changes in the tympanic membrane temperature (A) or VAS score (B) during cold exposure in humans ingesting LFO (600 mg) or a placebo (control). (C and D) Average changes in tympanic membrane temperature (C) or VAS score (D) for 90 min during cold exposure in humans ingesting LFO or a placebo. Values are expressed as means±SE. $n=8$; $^*p<0.05$ (two-way repeated-measures ANOVA, followed by paired t-test).

investigated the effects of a single dose of LFO on changes in energy metabolism using respiratory gas analysis. The subjects ingested 600 mg (Ex. 2) or 300 mg (Ex. 3) of samples (LFO or placebo) with 100 mL of water (37˚C) at 13:00. After a 150 min resting period (free of active movement in a seated position), subjects were moved to the air-conditioned room (approximately 24˚C) and the respiratory gas measurements were performed between 15:30 and 17:30.

Following a 30 min rest period after moving to the measurement room, the exercise sessions using a cycle ergometer at a pedaling frequency of 60 rpm commenced. To elucidate the optimal conditions for exercise intensity and/or time, exercises were performed at varying intensities. The exercise intensity began with 10% $\dot{V}o_2$max for 20 min and after a 5 min interval (rest period) continued with 20, 30 and 40% $\dot{V}o_2$max for a duration of 10 min for each intensity level. After the exercise, a 10-min recovery period followed (60 rpm with no work load). The average $\dot{V}o_2$, respiratory gas analysis (RER), fat oxidation, carbohydrate oxidation and $\dot{V}co_2$ for each intensity level of exercise work were calculated and the first 5 min was excluded from the calculation to account for instability of the respiratory gas during the switch in exercise intensities.

Protocol for 8-d repeated ingestion test (Ex. 4). We examined the effects of 8-d repeated ingestion of LFO on energy metabolism. The subjects ingested 300 mg of the samples (LFO or placebo) every day after lunch for 7 d until the measurement day. On the measurement day, the samples were ingested at 13:00. After a 150 min rest period (free of activity in a seated position), subjects were moved to the air-conditioned room (approximately 23˚C) and measurements were performed between 15:30 and 17:30.

Following a 10 min rest period after moving to a measurement room, the exercise began with 30% $\dot{V}o_2$max for 40 min followed by a 20 min recovery period. The average $\dot{V}o_2$, RER, fat oxidation, carbohydrate oxidation and $\dot{V}co_2$ during exercise or the recovery period were calculated with values for the first 5 min excluded.

Data analysis. All values are presented as the mean±SE. The effects of LFO on body surface temperature were analyzed by two-way repeated-measures analysis of variance (ANOVA, Prism 5.0, GraphPad Software, San Diego, CA). The effects of LFO on average body surface temperature for 90 min were analyzed by a paired t-test. The effects of LFO on tympanic membrane temperature and VAS score were analyzed by two-way repeated-measures ANOVA followed by a paired t-test. The effects of LFO on average tympanic membrane temperature and VAS for 90 min were analyzed by a paired t-test. For respiratory gas analysis, the effects of LFO on $\dot{V}o_2$, RER, fat oxidation, carbohydrate oxidation and $\dot{V}co_2$ were analyzed by two-way repeated-measures ANOVA. The effects of LFO on average $\dot{V}o_2$, RER, fat oxidation, carbohydrate oxidation and $\dot{V}co_2$ at each period (resting, each exercise intensity, and recovery) were analyzed by a paired t-test. Probability values of $p<0.05$ were regarded as significant.

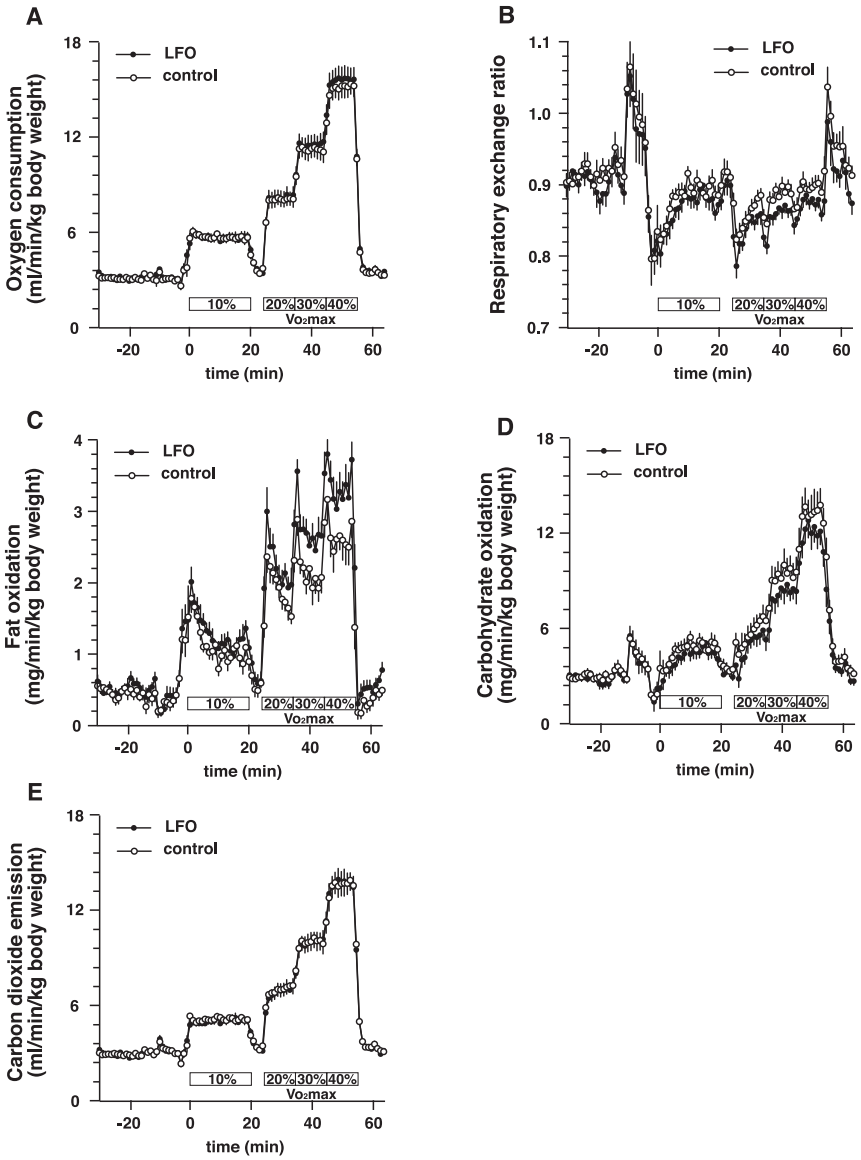

Fig. 3. Changes in the $\dot{V}o_2$ (A), RER (B), fat oxidation (C), carbohydrate oxidation (D) and $\dot{V}co_2$ (E) in humans ingesting LFO (600 mg) or a placebo (control). Values are expressed as means±SE (n=10).

RESULTS

Effects of a single dose of LFO on body surface temperature

To assess the effects of ingestion of a single dose of LFO on body surface temperature, subjects were exposed to slightly cold conditions for 90 min during which body surface temperatures gradually decreased under resting conditions. Body surface temperatures measured after the ingestion of LFO tended to be higher than those for the placebo (Fig. 1). Significant differences were observed in the forehead skin temperature (Fig. 1A). Investigation of the mean change in body surface temperature for 90 min during cold exposure revealed significant differences in abdominal skin temperature (Fig. 1E).

The tympanic membrane temperature of the LFO-treated group tended to be higher than that of the placebo-treated group; however, there was no significant difference between the two groups (Fig. 2A and C). The changes in VAS in the LFO-treated group were higher at 60 min and 75 min after the start of cold exposure compared to the placebo-treated group (Fig. 2B). Mean change in the VAS during cold exposure showed no significant difference between the two groups, although values of the LFO-treated group appeared to be higher than those of the placebo-treated group (p=0.0695).

Effects of ingestion of a single dose of LFO on energy metabolism during exercise

To elucidate the effects of LFO on energy metabolism, we analyzed the changes in respiratory gas during exercise using a cycle ergometer. Under resting conditions, the RER of the LFO-treated group tended to be lower than that of the placebo-treated group. However, there was no significant difference in RER between the two groups during the rest period (Fig. 4B). $\dot{V}o_2$, fat oxidation, carbohydrate oxidation and $\dot{V}co_2$ were not affected during the rest period under the present experimental conditions.

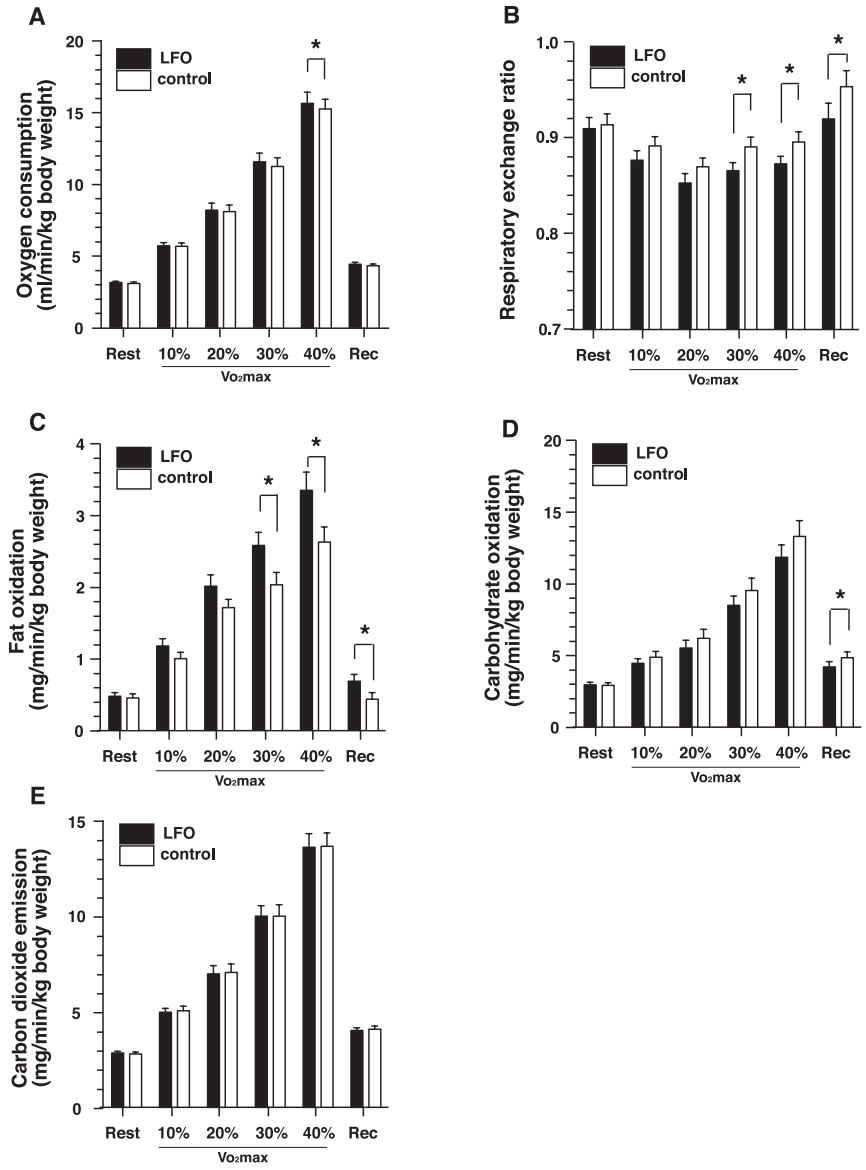

Fig. 4. Averages of the $\dot{V}o_2$ (A), RER (B), fat oxidation (C), carbohydrate oxidation (D) and $\dot{V}co_2$ (E) during rest, each intensity of exercise or recovery in humans ingesting LFO (600 mg) or a placebo (control). Values are expressed as means±SE (n=10). *p<0.05 (paired t test).

At a dose of 600 mg, LFO lowered RER during 30% and 40% $\dot{V}o_2$max exercise (Figs. 3B and 4B). Furthermore, LFO treatment increased $\dot{V}o_2$ during 40% $\dot{V}o_2$max exercise (Figs. 3A and 4A) and fat oxidation during 30% and 40% $\dot{V}o_2$max exercise compared to the control treatment. During the recovery period, the RER of the LFO-treated group was lower than that of the placebo-treated group (Fig. 4B), and fat oxidation was higher and carbohydrate oxidation was lower in the LFO-treated group than the placebo-treated group (Fig. 4C and D). $\dot{V}co_2$ was not affected under the present experimental conditions (Figs. 3E and 4E). There was no significant difference in HR between the two groups (data not shown).

At a dose of 300 mg, RER appeared slightly lower in the LFO-treated group compared to the control group; however, there was no significant difference between the two groups (Figs. 5B and 6B). Fat oxidation and carbohydrate oxidation appeared slightly higher and lower respectively in the LFO-treated group than in the placebo-treated group; however, there was no significant difference between the two groups (Figs. 5C, D and 6C, D). $\dot{V}o_2$ and $\dot{V}co_2$ were not affected under the present experimental conditions (Figs. 5A, E and 6A, E).

Effects of 8-d repeated ingestion of LFO on energy metabolism during exercise

To elucidate the efficacy of repeated ingestion of LFO on energy metabolism under different conditions, we varied experimental conditions such as ingestion period, exercise time, and nature of exercise. In previous studies, 1 wk of repeated administration of LFO increased the blood concentration of glabridin up to 1.7-fold compared to single-dose administration (*7*). Therefore, we investigated the effects of 8-d repeated ingestion of 300 mg of LFO, which did not show any effect from a single dose. In Ex. 2, the effects of LFO were observed clearly during 30–40% $\dot{V}o_2$max exercise. We suspected

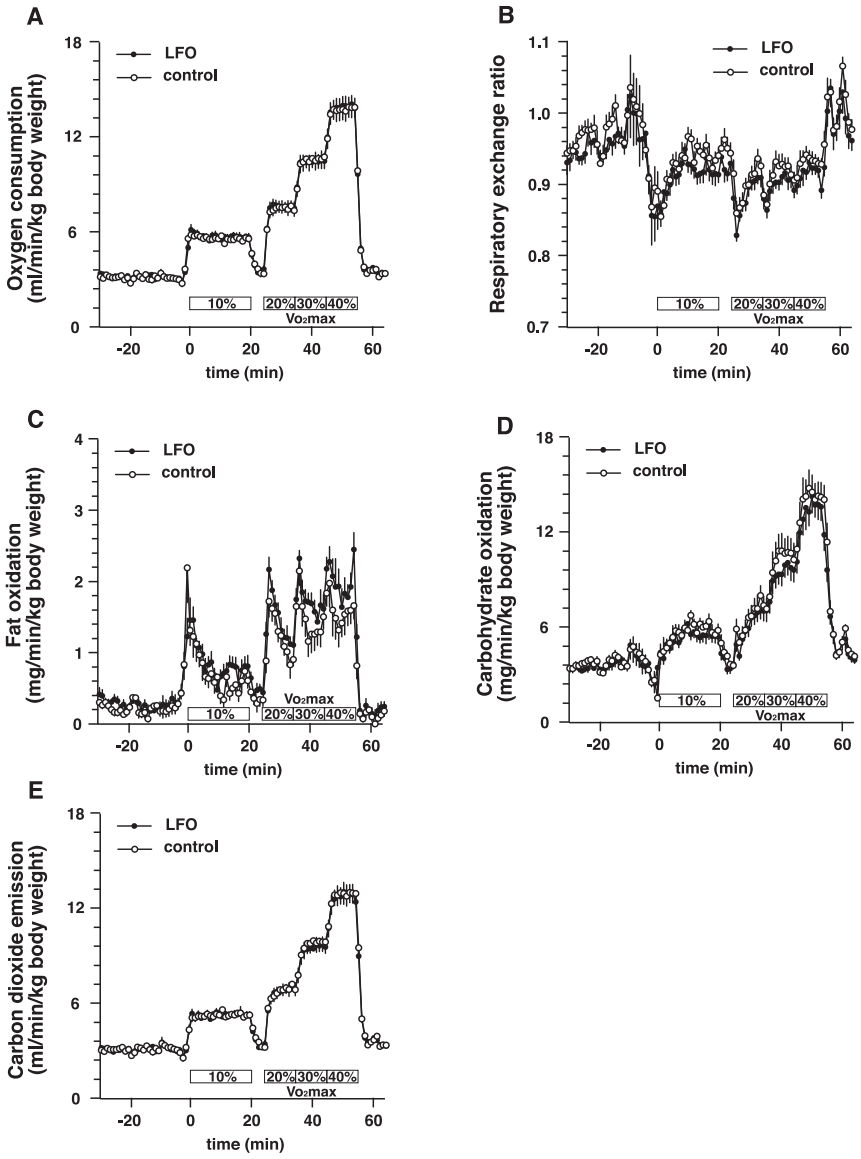

Fig. 5. Changes in the $\dot{V}o_2$ (A), RER (B), fat oxidation (C), carbohydrate oxidation (D) and $\dot{V}co_2$ (E) in humans ingesting LFO (300 mg) or a placebo (control). Values are expressed as means±SE (n=9).

that the effects of LFO on energy metabolism might be more effective in extended low-intensity aerobic exercise. Treadmill exercise causes less local muscle loading than cycle ergometer exercise, and is therefore suitable for prolonged aerobic exercise. Therefore, we performed the treadmill exercise test with 30% $\dot{V}o_2$max exercise for 40 min to evaluate the effects of 8-d repeated ingestion of LFO on energy metabolism during exercise.

Repeated ingestion of LFO (300 mg) over an 8-d period did not show any effects on $\dot{V}o_2$ or $\dot{V}co_2$ during exercise (Figs. 7A, E and 8A, E). RER was slightly lower in the LFO-treated group compared to the control group (Fig. 7B). The average RER during exercise showed no significant difference between the two groups; however, that of the LFO-treated group tended to be lower than that of the placebo-treated group (p=0.0937, Fig. 8B). Fat oxidation appeared slightly higher in the LFO-treated group compared to the control group; however, there was no significant difference between the two groups (Figs. 7C and 8C). On the other hand, carbohydrate oxidation during exercise was lower in LFO-treated group, compared to the control group (Figs. 7D and 8D).

During the recovery period, LFO treatment significantly decreased RER and carbohydrate oxidation and increased fat oxidation compared to control (Fig. 8C–E). $\dot{V}co_2$ during the recovery period was slightly lower in the LFO-treated group compared to the control group (Fig. 7E). The average of $\dot{V}co_2$ during the recovery period showed no significant difference between the two groups; however, the that of LFO-treated group tended to be lower than that of the control group (p=0.0934, Fig. 8E).

There was no significant difference in HR between the two groups in Ex. 4 (data not shown).

DISCUSSION

We showed that the ingestion of a single dose of 600 mg of LFO decreased RER and increased fat oxi-

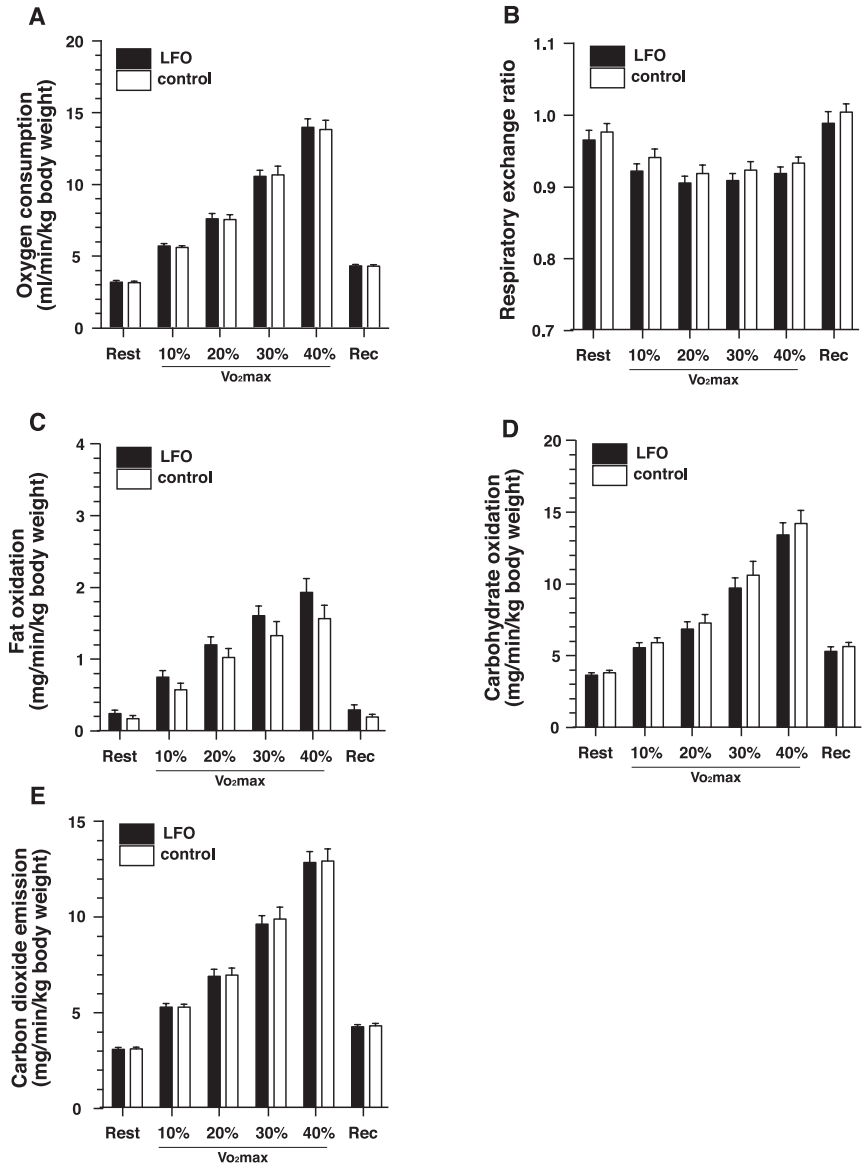

Fig. 6. Averages of the $\dot{V}o_2$ (A), RER (B), fat oxidation (C), carbohydrate oxidation (D) and $\dot{V}co_2$ (E) during rest, each intensity of exercise or recovery in humans ingesting LFO (300 mg) or a placebo (control). Values are expressed as means±SE (n=9). There is no significant difference between the groups.

dation during 30% and 40% $\dot{V}o_2$max exercise and also increased $\dot{V}o_2$ during 40% $\dot{V}o_2$max exercise. These results suggest that ingestion of LFO enhances energy consumption, especially fat oxidation. These results may be explained, at least in part, by the fact that in previous studies the administration of LFO suppressed fatty acid synthesis and activated peroxisomal beta-oxidation in the liver of mice and rats (*4*, *14*). It was postulated that the suppression of fatty acid synthesis and the enhancement of peroxisomal beta-oxidation resulted in the selective enhancement of fat oxidation. At 40% $\dot{V}o_2$max exercise, more energy substrates are needed than at 30% $\dot{V}o_2$max exercise. The enhancement of peroxisomal beta-oxidation might lead to an increase in the supply of fatty acids to mitochondria, where ATPs are generated from fatty acids, followed by the increase in $\dot{V}o_2$ compared to control. Although it remains unclear why $\dot{V}o_2$ was increased only at 40% $\dot{V}o_2$max exercise, our results suggest that the ingestion of LFO enhances energy consumption, especially fat oxidation.

Respiratory gas analysis showed no significant difference between LFO- and placebo-treated groups during the rest period; however, the LFO treatment increased the abdominal and forehead skin temperature as well as the VAS score (Figs.1 and 2). These results suggest the possibility that LFO might enhance energy metabolism during resting conditions, followed by thermogenesis. Respiratory gas analysis showed lower RER in the LFO-treated group during the rest period compared to the control (Fig. 4B), and this could be potentially attributed to a modest increase in fat oxidation during resting conditions. Although we could not confirm a difference in change in energy metabolism between the test and control groups in this study, the ingestion of LFO might be modestly effective during resting conditions and more effective during moderate exercise.

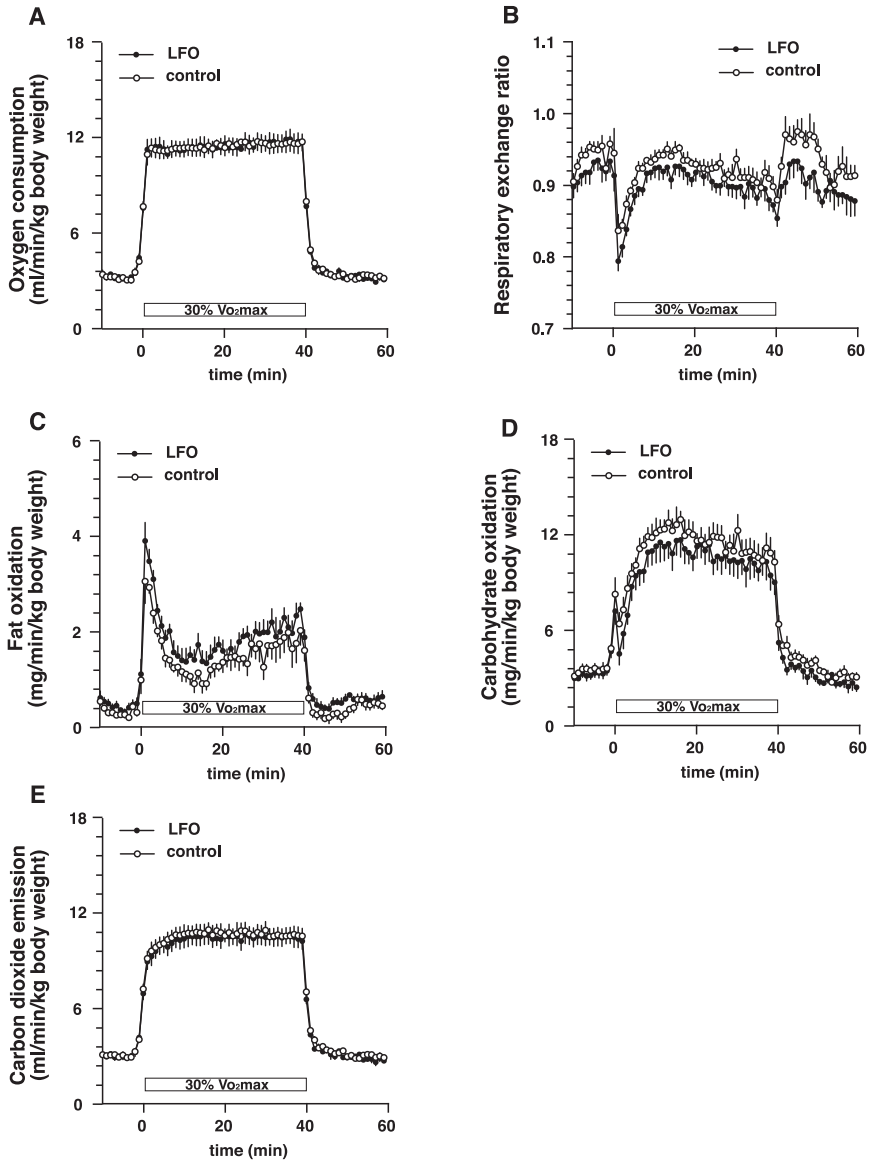

Fig. 7. Changes in the $\dot{V}O_2$ (A), RER (B), fat oxidation (C), carbohydrate oxidation (D) and $\dot{V}CO_2$ (E) in humans ingesting LFO (300 mg) or a placebo (control) for 8 d. Values are expressed as means±SE (n=7).

Ingestion of a single 300 mg dose of LFO showed no significant difference in RER, fat oxidation, or carbohydrate oxidation compared to the placebo-treated group. However, 8-d repeated ingestion of LFO appeared to have a tendency toward lower RER and a tendency toward higher fat oxidation as compared to the control group during exercise (Fig. 8B and C). Moreover, LFO treatment significantly decreased carbohydrate oxidation during exercise (Fig. 8D). During the recovery period, RER and carbohydrate oxidation were significantly decreased and fat oxidation was significantly increased (Fig. 8B–D). These results may suggest that repeated ingestion, which produces a higher blood concentration of glabridin than a single dose (*7*), would be more effective on energy metabolism. However, in addition to the duration of the ingestion period, differences in these parameters between single and repeated ingestion may be attributable to variations in the nature of exercise and exercise intensity as well as time, and not to differences in doses administered. Although the ingestion of LFO also appeared to be effective during prolonged exercise on a treadmill, further studies are required to elucidate the effectiveness of the 300 mg dose.

In the present study, we did not measure changes in the subject body weight or lipid profile makers in the 8-d repeated ingestion test. However, Aoki et al. reported that the blood concentration of glabridin was increased up to 1.7-fold after a 7-d repeated ingestion of LFO compared to single dose (*7*), and Tominaga et al. reported that a 4-wk repeated ingestion of the same dose of LFO decreased body weight and BMI in overweight subjects (*3*). The decrease in body weight is assumed to be caused by the increase in blood concentration of LFO. Therefore, an 8-d repeated ingestion of LFO might be more effective compared to single ingestion and lead to a moderate decrease in body weight or changes in lipid profiles.

Previous studies suggest that ingestion of LFO reduced body weight in overweight subjects by reduc-

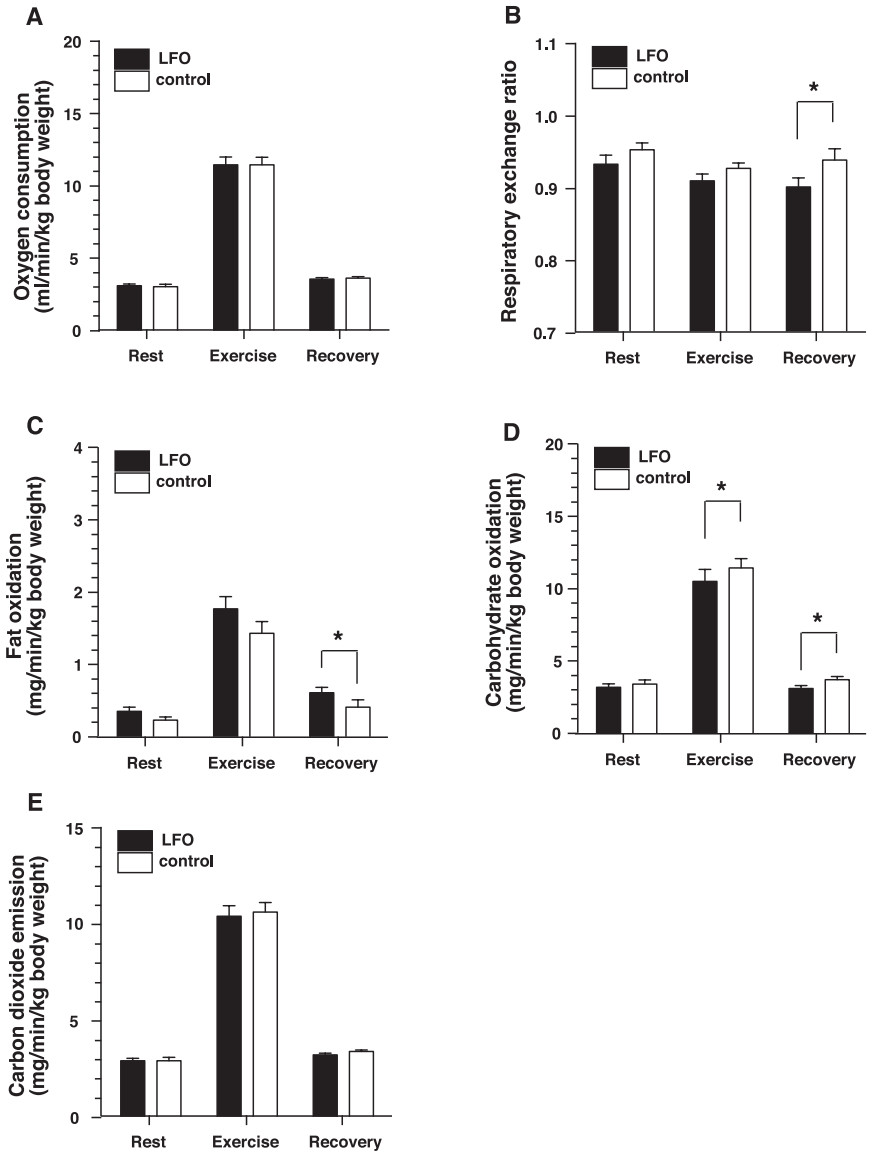

Fig. 8. Averages of the $\dot{V}o_2$ (A), RER (B), fat oxidation (C), carbohydrate oxidation (D) and $\dot{V}co_2$ (E) during rest, exercise or recovery in humans ingesting LFO (300 mg) or a placebo (control). Values are expressed as means±SE ($n=7$). *$p<0.05$ (paired t test).

ing total body fat (*3*). We postulated that LFO increases energy expenditure by enhancing beta-oxidation and inhibiting lipogenesis, resulting in the reduction of body fat and body weight. In the present study, we showed that the ingestion of LFO enhanced fat oxidation in humans. Therefore, the reduction in body fat accumulation caused by LFO might be attributable to its enhancement of fat oxidation.

We observed that the ingestion of LFO decreased RER during the recovery period in both the single-dose and repeated-ingestion tests. During the recovery period, RER was markedly increased in both the LFO- and placebo-treated groups. This increase in RER likely resulted from the increase in $\dot{V}co_2$ per $\dot{V}o_2$. The production of supplemental CO_2 would not be derived from the changes in metabolic state but from changes in the rate at which muscle and blood bicarbonate ($[HCO_3^-]$) decrease as a consequence of buffering of the H^+ associated with the lactate increase (*15*, *16*). In this case, the suppression of the CO_2 production could be considered to be associated with the suppression of the lactate production. Therefore, the ingestion of LFO might suppress lactate production. In this study, LFO treatment decreased carbohydrate oxidation during exercise (Fig. 8D). Presumably, the increase in fat oxidation caused the decrease in carbohydrate oxidation and this was followed by the suppression of lactate production, with a possible shift in the lactate threshold, creating an advantage in endurance exercise. More detailed studies are required in the future to elucidate these speculations regarding the effects on endurance exercise.

In conclusion, we demonstrated that the administration of a single 600 mg dose of LFO increased body trunk surface temperature during cold exposure, decreased RER during 30% and 40% $\dot{V}o_2$max exercise and during the recovery period, and also increased $\dot{V}o_2$ during

40% $\dot{V}o_2$max exercise. Repeated ingestion of 300 mg of LFO over an 8-d period appeared to have a tendency toward lower RER compared to the control group during exercise and a significant difference during the recovery period. These results suggest that LFO could enhance fat oxidation during moderate exercise.

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