Impact of Intensive High-Fat Ingestion in the Early Stage of Recovery from Exercise Training on Substrate Metabolism during Exercise in Humans

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Summary Not only increasing body carbohydrate (CHO) stores before exercise but also suppressing CHO oxidation during exercise is important for improving endurance performance. We tested the hypothesis that intensive high-fat ingestion in the early stage of recovery from exercise training (ET) for 2 d would suppress CHO oxidation during exercise by increasing whole body lipolysis and/or fat oxidation. In a randomized crossover design, on days 1 and 2, six male subjects performed cycle ET at 50% peak oxygen consumption ($\dot{V}O_2$ peak) for 60–90 min, and consumed a control diet (CON: 1,224 kcal, 55% carbohydrate, 30% fat) or the same diet supplemented with high fat (HF: 1,974 kcal, 34% carbohydrate, 56% fat) 1 h after ET, with the diet other than post-ET similar in both trials. On day 3, subjects performed cycle exercise at 65% $\dot{V}O_2$ peak until exhaustion. Exercise time to exhaustion was longer in the HF trial than in the CON trial (CON: 48.9±6.7 min, HF: 55.8±7.7 min, $p<0.05$). In the HF trial, total fat oxidation until exhaustion was higher, accompanied by higher post-exercise plasma glycerol concentration, than in the CON trial (CON: 213±54 vs. HF: 286±63 kcal, $p<0.05$), whereas total carbohydrate oxidation until exhaustion was not different between trials. These results suggest that intensive high-fat ingestion in the early stage of recovery from ET for a few days until the day before exercise was an effective means of eliciting a CHO-sparing effect during exercise by enhancing fat metabolism.

Key Words high fat, exercise, training, lipolysis

Muscle glycogen depletion and/or hypoglycemia during exercise are closely associated with fatigue, and so increasing body carbohydrate (CHO) stores before exercise and/or suppressing CHO oxidation during exercise are both essential for improving endurance performance (1, 2).

Recently, a simple and/or practical method of ingesting an adequate amount of CHO for a few days before competition has been recommended to effectively increase body CHO stores, in contrast to the classic 1-wk glycogen-loading regimen accompanied by exhausting exercise and/or ingestion of an extremely low-CHO diet for depleting body CHO stores (3). On the other hand, it has been demonstrated that ingestion of a high-fat, low-CHO diet throughout the recovery period after exercise training (ET) increased muscle triglyceride (TG) stores, thereby enhancing whole body lipolysis and/or fat oxidation during exercise, compared with an isoenergetic high-CHO, low-fat diet (4). In general, before a competition, endurance athletes gradually change ET at high intensity to ET at moderate intensity, at which a large amount of muscle TG is utilized as an important energy source (5, 6); therefore, ingestion of not only CHO but also fat until the day before exercise would be important to elicit a CHO-sparing effect during exercise by maintaining a higher muscle TG level.

TG uptake from the circulation to various tissues is highly related to lipoprotein lipase (LPL), which hydrolyzes TG in circulating chylomicrons and/or very low density lipoproteins, making free fatty acid (FFA) available for uptake by various tissues (7). It has been reported that ET increased muscle LPL activity (8, 9), and that insulin increased adipose tissue LPL activity (10), whereas it decreased muscle LPL activity (11), suggesting that elevating blood TG concentration with little effect of insulin after ET is effective for accelerating TG uptake into muscles. Since muscle glycogen stores were efficiently replenished if CHO was ingested as soon as possible after ET (12), CHO ingestion has been considered to be the first priority in the early stage of recovery from ET. On the other hand, experimentally, blood TG concentration reached a peak around 4 h after ingestion of a high-fat diet (13), and blood insulin concentration reached a peak within 1 h after ingestion of a CHO-containing diet and then returned to the baseline about 4 h later (12). Although the negative effect of insulin on muscle LPL activity cannot be completely excluded, ingestion of a CHO-containing diet supplemented with high fat in the early stage of recovery from ET would not only replenish body CHO stores but also effectively increase muscle TG stores by making use of the time lag between blood insulin and TG responses.

It was reported that ingestion of a high-fat, low-CHO diet for several days increased not only fat oxidation, but also heart rate (HR), rating of perceived exertion (RPE), and sympathetic activation during exercise, compared

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with ingestion of an isonenergetic high-CHO, low-fat diet (14). These results suggest that high-fat ingestion for several days might be stressful physically and/or mentally; in other words, the period of high-fat ingestion should be as short as possible. On the other hand, it was reported that high-fat ingestion within 1.5 d after ET significantly increased muscle TG stores, but did not affect HR during exercise (15); therefore, high-fat ingestion within a few days would sufficiently increase whole body lipolysis and/or fat oxidation during exercise with minimal physical and/or mental stress.

Based on these findings, in this study, we tested the hypothesis that intensive high-fat ingestion in the early stage of recovery from ET for 2 d would suppress CHO oxidation during exercise by increasing whole body lipolysis and/or fat oxidation. Although muscle TG stores could not be directly measured in this study, it was assumed that the increases in whole body lipolysis and/or fat oxidation during exercise by high-fat ingestion occurred in proportion to elevated muscle TG stores, as reported by previous studies (4, 16, 17).

**METHODS**

**Subjects.** Six healthy male volunteers gave written informed consent before participating in this study. Their age was 23 ± 1 (mean ± SE) y, height 170 ± 2 cm, weight 66.0 ± 3.4 kg, body mass index (BMI) 22.6 ± 0.8 kg/m², peak oxygen consumption (VO₂peak) 3.40 ± 0.21 L/min, ventilation threshold (VT) 2.24 ± 0.19 L/min, and maximal workload (W₇max) 263 ± 11 W. They were relatively active but did not participate in any regular ET program. All subjects were non-smokers. This study was approved by the Human Ethics Committee, Faculty of Human Sciences, Waseda University, and was performed in accordance with the Helsinki Declaration.

**Experimental design.** At least 4 d before the main experimental trials, a graded exercise test to determine VO₂peak, VT, and W₇max was performed. Thereafter, two experimental trials were performed in a randomized, crossover design, separated by at least a 1-wk washout period. As shown in Fig. 1, each experimental trial consisted of 2-d ET while ingesting each experimental diet, and 1-d submaximal exercise test to determine substrate metabolism and endurance performance.

**Graded exercise test.** VO₂peak, VT, and W₇max were measured with graded exercise using a cycle ergometer (Ergomedic 818E; Monark, Vansbro, Sweden) in an upright position at 25.3 ± 0.1°C ambient temperature (Ta) and 37.3 ± 1.6% relative humidity (RH). After 3-min baseline measurements at rest, subjects started pedaling at 60 revolutions per minute (rpm) without loading. Exercise intensity was increased by 60 W every 3 min until 180 W and, above this intensity, by 30 W every 2 min until 240 W and then by 15 W every 2 min until subjects were not able to maintain the rhythm due to exhaustion. Oxygen consumption (VO₂) and ventilation (VE) were measured every 15 s (Aeromonitor AE300S; Minato, Tokyo, Japan). HR was measured every minute using an electrocardiogram trace (Life Scope 6; Nihon Kohden, Tokyo, Japan). VO₂peak was determined after averaging three maximal values of VO₂ at the end of the graded exercise test. VT was determined by the VO₂ vs. VE relationship during the graded exercise test. This relationship was composed of two regression lines: a gentle line and a steep line. VT was determined by the crossing point of the two regression lines. W₇max was defined as the highest exercise intensity during the graded exercise test.

**ET and dietary intervention.** On the day before the start of the experimental trial, subjects had been told to refrain from any beverages containing caffeine or alcohol, and vigorous exercise, and to have their usual dinner by 21:00. On day 1, in two trials, subjects ate a light control diet (180 kcal: protein 0%, fat 0%, CHO 100%; Weider in jelly energy in; Morinaga, Tokyo, Japan) by 08:00 at home, came to the laboratory at 09:30, and then started the cycle ET at 50% VO₂peak (corresponding to 51.0 ± 0.5% W₇max) for 90 min at 60 rpm at 23.7 ± 0.7°C Ta and 40.3 ± 0.4% RH. Considering physical conditioning just before competition, we adopted moderate-intensity ET, which effectively elicits maximal fat oxidation (6, 18). After ET, at 12:00, subjects ate either a control diet (CON; 1,224 kcal: protein 16%, fat 30%, CHO 54%) or the same control diet supplemented with high fat (HF; 1,974 kcal: protein 10%, fat 56%, CHO 34%) in the laboratory. The control diet at noon consisted of a bowl of rice topped with beef, canned tuna, 100% pure orange juice, and calorie mate block (Otsuka Pharmaceutical Co., Ltd.). As for the high-fat diet, salt-free butter was added to the control diet. Thereafter, in the two trials, they went home and rested, and then ate a control diet (1,093 kcal: protein 10%, fat 25%, CHO 60%) at 20:30 at home. The control diet at night was the same as at noon, except for a bowl of rice with a chop-suey-like mixture on it. The amount of protein and CHO ingestion per day was therefore similar in two trials. They were instructed to drink 2 L of water per day throughout the experimental period.
On day 2, the experimental protocol was the same as on day 1, except that the ET for 60 min started at 10:00. Submaximal exercise test. On day 3, subjects came to the laboratory at 08:15, having fasted for 11 h, except for 250 mL water immediately after waking up. After resting for 60 min in a seated position, they rode the cycle ergometer in an upright position and all measurement devices were applied. After baseline measurements for 10 min, subjects exercised at 65% VO2 peak (corresponding to 66.9±0.6% WLmax) at 60 rpm in 24.9±0.1°C Ta and 40.1±1.3% RH until exhaustion. During exercise, subjects were not provided with verbal encouragement or a real-time display to exclude the effect of psychological factors on endurance performance. Exhaustion was defined as the point when the rhythm was below 55 rpm. HR was measured every minute. VO2, carbon dioxide production (VCO2), and V̇̇ were measured every 15 s. Blood samples were collected from the large antecubital vein using a 21 G butterfly needle (SV-21CL; Terumo, Tokyo, Japan) at rest and post-exercise (within 2 min after the end of exercise due to exhaustion). RER was determined by dividing VCO2 by VO2. CHO and fat oxidation (in g/min) were calculated from VCO2 and VO2. CHO and fat oxidation (in kcal) were calculated on the basis of carbohydrate (% CHO oxidation) and fat (% fat oxidation) oxidation rates. CHO oxidation and fat oxidation were calculated according to the following equations (19).

CHO oxidation = 4.210 V̇̇CO₂ – 2.962 V̇̇O₂

fat oxidation = 1.695 V̇̇O₂ – 1.701 V̇̇O₂

Statistics. The paired t-test was performed to examine the effects of high-fat ingestion on cardiopulmonary and metabolic responses, blood properties, and endurance performance (Tables 1 and 2; Fig. 2). The null hypothesis was rejected at \( p<0.05 \). Values are the means±SE for six subjects. HR, heart rate; VO₂, oxygen consumption; V̇̇, ventilation; RER, respiratory exchange ratio; CHO, carbohydrate; CON, control; HF, high fat; Ex 1–20, 1–20 min of exercise; Ex L5, the last 5 min of exercise before the end of exercise due to exhaustion. * \( p<0.05 \) vs. CON; $ p=0.05 \) vs. CON.

### Table 2. Blood properties at rest and at postexercise.

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Post Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood lactate (mmol/L)</td>
<td>CON 1.1±0.2</td>
<td>9.1±1.4</td>
</tr>
<tr>
<td></td>
<td>HF 1.0±0.1</td>
<td>8.5±1.9</td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>CON 5.6±0.1</td>
<td>6.0±0.5</td>
</tr>
<tr>
<td></td>
<td>HF 5.6±0.1</td>
<td>6.3±0.4</td>
</tr>
<tr>
<td>Plasma glycerol (mmol/L)</td>
<td>CON 0.05±0.004</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td></td>
<td>HF 0.04±0.003*</td>
<td>0.34±0.04*</td>
</tr>
<tr>
<td>Serum FF A (mEq/L)</td>
<td>CON 0.66±0.05</td>
<td>0.85±0.04</td>
</tr>
<tr>
<td></td>
<td>HF 0.62±0.06</td>
<td>1.10±0.22</td>
</tr>
</tbody>
</table>

Values are the means±SE for six subjects. FFA, free fatty acid; Post Ex, within 2 min after the end of exercise due to exhaustion. Other abbreviations are the same as in Table 1. * \( p<0.05 \) vs. CON.
During 1–20 min of exercise, fat oxidation was significantly lower by 9% during 1–20 min of exercise, compared with the CON trial. In the HF trial, CHO oxidation was significantly lower during the last 5 min of exercise, compared with the CON trial. In both trials, exercise time to exhaustion was not different between trials. In the HF trial, RER tended to be lower during 1–20 min of exercise (p=0.05), and was significantly lower during the last 5 min of exercise, compared with the CON trial. In the HF trial, CHO oxidation was significantly lower by 9% during 1–20 min of exercise, and by 14% during the last 5 min of exercise, compared with the CON trial. In the HF trial, fat oxidation was significantly higher by 25% during 1–20 min of exercise, and by 18% during the last 5 min of exercise, compared with the CON trial.

Endurance performance
Exercise time to exhaustion was 55.8±7.7 min in the HF trial, significantly longer than 48.9±6.7 min in the CON trial. In all subjects, exercise time to exhaustion was lengthened by high-fat ingestion.

Total CHO and fat oxidation
As shown in Fig. 2, total CHO oxidation (1–20 min and the last 5 min of exercise) was not different between trials. HR and VO2 during 1–20 min and the last 5 min of exercise were not different between trials. VO2 was significantly lower during 1–20 min of exercise in the HF trial than in the CON trial, but was similar during the last 5 min of exercise in both trials. In the HF trial, RER tended to be lower during 1–20 min of exercise (p=0.05), and was significantly lower during the last 5 min of exercise, compared with the CON trial. In the HF trial, CHO oxidation was significantly lower by 9% during 1–20 min of exercise, and by 14% during the last 5 min of exercise, compared with the CON trial. In the HF trial, fat oxidation was significantly higher by 25% during 1–20 min of exercise, and by 18% during the last 5 min of exercise, compared with the CON trial.

Blood substrate concentrations
As shown in Table 2, at rest and post-exercise, blood lactate and plasma glucose concentrations were not different between CON and HF trials. In the HF trial, plasma glycerol concentration was slightly but significantly lower at rest, whereas it was significantly higher post-exercise, compared with the CON trial. At rest and post-exercise, serum FFA concentrations were not different between trials.

Relationship between training status and a CHO-sparing effect
In six subjects, the decrease in CHO oxidation during 1–20 min of exercise by high-fat ingestion was not significantly correlated with VO2 peak. On the other hand, the decrease in CHO oxidation during the last 5 min of exercise by high-fat ingestion tended to be positively correlated with VO2 peak (r=0.81, p=0.05).

DISCUSSION
This is the first study to investigate the effect of intensive high-fat ingestion in the early stage of recovery from ET for a few days on substrate metabolism during exercise, in contrast to many studies which adopted intermittent high-fat ingestion throughout the recovery period after ET for several days or more. The major finding in this study was that intensive high-fat ingestion in the early stage of recovery from ET for only 2 d elicited a sufficient CHO-sparing effect during exercise.

Total fat oxidation was significantly higher in the HF trial than in the CON trial, whereas total CHO oxidation was similar in both trials (Fig. 2). Moreover, exercise time to exhaustion was significantly longer in the HF trial than in the CON trial. These results suggest that the depletion of body CHO stores was a major determinant of exercise time to exhaustion, and the improvement in endurance performance was at least partly associated with the suppression of CHO oxidation during exercise by the increase in fat oxidation due to high-fat ingestion.

In the HF trial, fat oxidation during 1–20 min and the last 5 min of exercise was significantly higher, compared with the CON trial (Table 1). Moreover, post-exercise plasma glycerol concentration, commonly reported as an indicator of whole body lipolysis (4, 20), was markedly higher in the HF trial than in the CON trial (Table 2). On the other hand, post-exercise serum FFA concentration, as an indicator of plasma fatty acid oxidation (21), was not different between trials. These results suggest that the increase in fat oxidation throughout exercise by high-fat ingestion was closely associated with the increases in whole body lipolysis and/or nonplasma fatty acid oxidation. Based on previous reports (4, 22), these responses by high-fat ingestion would be explained by the increases in muscle TG lipolysis and/or oxidation, which was closely associated with elevated muscle TG stores (16, 17).

CHO oxidation during 1–20 min and the last 5 min of exercise was significantly lower in the HF trial than in the CON (Table 1), suggesting that high-fat ingestion suppressed CHO oxidation throughout exercise; however, in this study, we could not distinguish muscle glycogen oxidation from plasma glucose oxidation. On the other hand, it is well known that increased VO2 during exercise is caused by elevated lactic acid in the vascular space due to enhanced glycolysis in contracting skeletal muscles because VO2 is stimulated by CO2 produced in these muscles. It has been also reported that the increase in fat oxidation during exercise by high-fat ingestion tended to be positively correlated with VO2 peak (r=0.81, p=0.05).

High-fat ingestion for several days was reported to
increase HR, RPE, or sympathetic activation, thereby compromising high-intensity exercise performance (14). In contrast, in this study, high-fat ingestion for 2 d improved exercise time to exhaustion, but did not increase HR during exercise, compared with no ingestion (Table 1); therefore, short-term high-fat ingestion would not have negative effects on sympathetic activation and/or a feeling of fatigue.

One study reported that high-fat ingestion for 1.66 d after ET elevated muscle TG stores above the pre-training level independently of the training status (24), while another study reported that the extent of elevation in muscle TG stores by high-fat ingestion for 1.5 d after ET was positively related to \( VO_2 \) peak (15). On the other hand, our data from six subjects showed that a CHO-sparing effect in the final stage of exercise by high-fat ingestion tended to be reduced with increased \( VO_2 \) peak. This result suggests that the elevation in muscle TG stores by high-fat ingestion might be attenuated in the subjects who had higher \( VO_2 \) peak because a CHO-sparing effect during exercise was closely associated with the increases in muscle TG lipolysis and/or oxidation due to elevated muscle TG stores (16, 17). Although the reason for discrepancy concerning the relationship between the repletion of muscle TG stores by high-fat ingestion and training status between studies is not clear, this discrepancy might be at least partly explained by the difference in experimental protocol. Since we adopted the experimental protocol that all subjects ingested the same amount of high fat irrespective of the difference in absolute intensity of ET, high-fat ingestion in this study might not be enough to replenish muscle TG stores in highly-trained subjects who possibly utilized more muscle TG because of ET at higher absolute intensity.

CONCLUSION

In conclusion, intensive high-fat ingestion in the early stage of recovery from ET for 2 d suppressed CHO oxidation during exercise by increasing whole body lipolysis and/or fat oxidation. These results may provide athletes with new concept that positive high-fat ingestion for a few days until the day before exercise is important from the viewpoint of efficiently utilizing the limited CHO stores in the body; however, our results was limited to relatively fit young men, so further research is needed to apply the dietary intervention developed in this study to endurance-trained men or women.

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