Effects of diet on muscle triglyceride and endurance performance

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Recent research using stable-isotope technologies has demonstrated that as much as 20–25% of exercise energy expenditure may be derived from muscle triglyceride during prolonged exercise at moderate intensities (29). Therefore, the purpose of this investigation was to examine the effects of different dietary compositions on muscle triglyceride concentration during an acute period after prolonged exercise. Thus, the purpose of this investigation was to examine the effects of a high-carbohydrate and a high-fat diet on muscle triglyceride storage 24 h after 120 min of cycling at 65% of \( \dot{V}O_2 \max \). A secondary purpose was to examine the effects of these different carbohydrate and fat intakes on muscle glycogen resynthesis and self-paced cycling performance.

METHODS

Subjects. Seven endurance-trained male cyclists participated in the investigation. The mean age, height, weight, and body fat percent of the subjects were 25 ± 3 (SD) yr, 181 ± 7 cm, 76 ± 9 kg, and 10 ± 4%, respectively. Their mean \( \dot{V}O_2 \max \) was 4.50 ± 0.35 l/min. Each subject was informed of the risks, stresses, and benefits of the study before signing a written consent form that had been approved by the Institutional Review Board.

Experimental testing. During this investigation, each subject completed two experimental trials that were separated by 10–12 days. The experimental testing protocol was completed over a 2-day period. For 2 days before trial 1, the subjects recorded their diet and exercise routine so that it could be repeated during trial 2 to reduce differences in pretesting intramuscular substrate concentrations between experimental trials. On day 1 of the experimental testing, each subject completed a 120-min cycling bout at 65% of \( \dot{V}O_2 \max \) in the morning (0600–0800) after an overnight fast. Before and immediately after the 120-min cycling bout, a biopsy of the vastus lateralis muscle was obtained by using a percutaneous biopsy needle (1) with modified suction (12).

After the postexercise biopsy, each subject in a randomized crossover design began a 12-h period of a high-carbohydrate diet [Hi-CHO; 14.97 ± 0.43 MJ, 83 energy% (E%) carbohydrate, 16 E% fat, 15 E% protein] or a high-fat diet (Hi-Fat; 15.02 ± 0.43 MJ, 16 E% carbohydrate, 68 E% fat, 16 E% protein) diet. All ingested food was provided for the subjects. The energy intake for each subject was the sum of the calculated energy expenditure during the 120-min cycling bout and an estimation of their daily resting energy expenditure (15) excluding expenditure for any other daily activities. The ingested calories were evenly spaced throughout the 12-h dietary period (i.e., breakfast, lunch, and dinner). The subjects ingested breakfast within 1 h of finishing the 120-min cycling bout.

After a 12-h overnight fast, each subject then completed a self-paced time trial on an isokinetic cycling ergometer (Cybex MET-100, Ronkonkoma, NY). The ergometer is designed to fix the cycling cadence but allows a variable resistance in response to the subject's effort. Immediately before the time trial, another biopsy of the vastus lateralis muscle was obtained. The time trial began after a 5-min warm-up at 150 W. All subjects completed 1,600 kJ of work during the time trial, which simulated ~2 h of cycling. Subjects were instructed to give an all-out effort that was similar to their previous efforts during the 120-min cycling bout.
time-trial efforts during road competitions. Furthermore, each subject completed a familiarization time trial 7–10 days before the first experimental trial to minimize any learning effect. The ergometer was interfaced with a computer that allowed the subjects to see the total work completed, with no feedback regarding total time elapsed. Total time to complete the self-paced cycling trial was recorded. In addition, respiratory gases and a heart rate via radiotelemetry (Polar Vantage XL, Polar Electro, Port Washington, NY) were obtained every 200 kJ. A blood sample was obtained from a forearm vein before and every 400 kJ throughout the time trial.

Analytic techniques. The biopsy samples were quickly dissected free of any visible fat and connective tissue and then were frozen and stored in liquid nitrogen (−190°C) until later analysis for glycogen and triglyceride concentrations. Before these analyses, the muscle samples were freeze-dried for 20 h and then were weighed on an electronic autobalance having a sensitivity of 0.001 mg. Total muscle glycogen was determined in triplicate after hydrolysis of the muscle in hydrochloric acid (26). The resultant glucose residues from the hydrolysis were measured fluorometrically with the use of the hexokinase-glucose-6-phosphatedehydrogenase reaction (27). Total muscle triglyceride was determined in quadruplicate with a dry weight sample size (mean ± SD) of 3.3 ± 0.5 mg. A modification of the chloroform-methanol method by Frayn and Maycock (13) was used to extract the triglyceride from the muscle samples. Extracts were dried, hydrolyzed in alcoholic KOH, and neutralized with HCl, and triglyceride concentration was then determined by measuring the liberated glycerol spectrophotometrically using a commercially available enzymatic method (kit no. 337, Sigma Chemical, St. Louis, MO).

The blood samples were centrifuged, and the serum was then stored at −20°C until later analysis. Serum glucose, free fatty acid (kit no. 990–75401, Wako Pure Chemical Industries, Osaka, Japan), and glycerol concentrations were measured on all serum samples by using commercially available enzymatic methods.

Respiratory gases were analyzed for oxygen (model S-3A, Applied Electrochemistry, Sunnyvale, CA) and carbon dioxide (model LB-2, Sensor Medics, Anaheim, CA) fractions by using electronic analyzers. Gas volume was determined via a dry-gas meter (Parkinson Cowan). From these data, oxygen consumption (Vo2) and respiratory exchange ratio (RER) were calculated.

Statistics. A two-way analysis of variance for repeated measures (treatment × time) was used to examine possible differences between the experimental trials for muscle glycogen and triglyceride concentrations and for all dependent variables obtained throughout the cycling time trial. When significant F-values were obtained, a Tukey’s post hoc analysis was administered to locate differences between means. In addition, possible differences between the experimental trials for total performance time were examined by using a paired t-test. Significance was accepted at the P < 0.05 level.

**RESULTS**

Muscle triglyceride and glycogen. Total muscle triglyceride concentration before the 120-min cycling bout was not significantly different between the Hi-CHO and Hi-Fat trials (Table 1). Furthermore, muscle triglyceride concentrations after the 120-min ride were not significantly different compared with the corresponding preexercise values for both the Hi-CHO and Hi-Fat trials. Muscle triglyceride concentration 24 h after the 120-min ride was significantly higher for the Hi-Fat compared with the Hi-CHO trial (Table 1).

Total muscle glycogen concentration before the 120-min cycling bout was not significantly different between the Hi-CHO and Hi-Fat trials (Table 1). Muscle glycogen concentration after the 120-min ride was significantly lower compared with the preexercise value for both the Hi-CHO and Hi-Fat trials. Furthermore, muscle glycogen concentration was significantly higher 24 h after the 120-min ride for the Hi-CHO compared with the Hi-Fat trial (Table 1).

Self-paced cycling time trial. Total time to complete the 1,600-kJ cycling time trial was significantly greater during the Hi-CHO trial (193.9 ± 7.1 min) compared with the Hi-Fat trial (117.1 ± 3.2 min). Figure 1 displays the elapsed time every 200 kJ throughout the cycling time trial for the Hi-Fat and Hi-CHO trials. Furthermore, V02 expressed as a percentage of V02max was not significantly different between the Hi-Fat and Hi-CHO trials during the first 1,000 kJ of the time trial.
In contrast, the percentage of $V_{O_{2max}}$ maintained was significantly lower during the Hi-Fat compared with the Hi-CHO trial at 1,200 (59 ± 3 vs. 75 ± 3%), 1,400 (59 ± 6 vs. 76 ± 4%), and 1,600 (53 ± 8 vs. 84 ± 5%) kJ, respectively. As with the $V_{O_{2}}$ data, heart rate values during the first 1,000 kJ of the time trial were not significantly different between the Hi-Fat and Hi-CHO trials (Fig. 2). Heart rate was significantly lower during the Hi-Fat compared with the Hi-CHO trial at 1,200 (143 ± 3 vs. 156 ± 5 beats/min), 1,400 (139 ± 6 vs. 162 ± 4 beats/min), and 1,600 (133 ± 9 vs. 167 ± 4 beats/min) kJ, respectively. RER was significantly lower at all analysis points during the time trial for the Hi-Fat vs. the Hi-CHO trials (Fig. 2). The average RER during the time trial for the Hi-CHO and Hi-Fat trials was 0.89 ± 0.01 and 0.82 ± 0.01, respectively.

Glucose concentration before the cycling time trial was not significantly different between the Hi-CHO (5.0 ± 0.05 mmol/l) and Hi-Fat (5.0 ± 0.08 mmol/l) trials (Fig. 3). In contrast, glucose concentration was significantly lower during the Hi-Fat compared with the Hi-CHO trial at 400 (4.4 ± 0.2 vs. 4.8 ± 0.2 mmol/l), 800 (4.0 ± 0.2 vs. 4.5 ± 0.2 mmol/l), 1,200 (3.6 ± 0.2 vs. 4.2 ± 0.2 mmol/l), and 1,600 (3.1 ± 0.3 vs. 4.1 ± 0.2 mmol/l) kJ, respectively.

Free fatty acid concentration before the cycling time trial was not significantly different between the Hi-CHO (0.38 ± 0.04 mmol/l) and Hi-Fat (0.54 ± 0.06 mmol/l) trials (Fig. 3). However, free fatty acid concentration was significantly higher during the Hi-Fat compared with the Hi-CHO trial at 1,200 (1.37 ± 0.14 vs. 1.03 ± 0.12 mmol/l) and 1,600 (1.76 ± 0.24 vs. 1.35 ± 0.21 mmol/l) kJ, respectively. Free fatty acid concentration before the cycling time trial was not significantly different between the Hi-CHO (0.38 ± 0.04 mmol/l) and Hi-Fat (0.54 ± 0.06 mmol/l) trials (Fig. 3). However, free fatty acid concentration was significantly higher during the Hi-Fat compared with the Hi-CHO trial at 400 (4.4 ± 0.2 vs. 4.8 ± 0.2 mmol/l), 800 (4.0 ± 0.2 vs. 4.5 ± 0.2 mmol/l), 1,200 (3.6 ± 0.2 vs. 4.2 ± 0.2 mmol/l), and 1,600 (3.1 ± 0.3 vs. 4.1 ± 0.2 mmol/l) kJ, respectively.

Glycerol concentration before the cycling time trial was not significantly different between the Hi-CHO (0.053 ± 0.015 mmol/l) and Hi-Fat (0.062 ± 0.012 mmol/l) trials (Fig. 3). In contrast, glycerol concentration was significantly lower during the Hi-Fat compared with the Hi-CHO trial at 400 (0.31 ± 0.03 vs. 0.14 ± 0.02 mmol/l), 800 (0.45 ± 0.04 vs. 0.22 ± 0.04 mmol/l), 1,200 (0.57 ± 0.07 vs. 0.33 ± 0.04 mmol/l), and 1,600 (0.60 ± 0.06 vs. 0.48 ± 0.05 mmol/l) kJ, respectively.
DISCUSSION

The results from the present investigation demonstrate that there was not a significant difference in muscle triglyceride concentration before and after a prolonged submaximal cycling bout. Nevertheless, the ingestion of a high-fat diet during the 24-h dietary-fasting period after the cycling bout increased muscle triglyceride concentration by 36%. In addition, the ingestion of 9.8 vs. 1.9 g carbohydrate/kg body wt during the 24-h dietary-fasting period after the cycling bout resulted in a greater glycogen storage and a subsequent improvement in self-paced cycling performance.

During the 120-min cycling bout at 65% of V\textsubscript{2\textsubscript{max}}, there was a reduction (P > 0.05) in muscle triglyceride concentration of 6 and 11% for the Hi-CHO and Hi-Fat trials, respectively (see Table 1). Other investigators have demonstrated similar percent changes in muscle triglyceride concentration during prolonged exercise (18, 20, 32). In contrast to the present and aforementioned studies, other investigators have demonstrated 20–50% reductions in muscle triglyceride concentration during prolonged exercise at intensities between ~55 and 75% of V\textsubscript{2\textsubscript{max}} (4, 5, 8, 9, 14, 16). Differences among these studies in exercise mode, intensity, and duration may explain the discrepant findings. Furthermore, preexercise intramuscular concentrations (11), heterogeneous storage of fat (10), and differences in utilization among muscle groups (28) may influence changes in muscle triglyceride during prolonged exercise.

Even though there was not a significant difference in muscle triglyceride before and after the 120-min cycling bout, the ingestion of a high-fat diet during the subsequent 24-h dietary-fasting period increased muscle triglyceride concentration by 36% (see Table 1). In contrast, a high-carbohydrate diet did not significantly change muscle triglyceride concentration. To our knowledge this is the first investigation to examine the effect of diet on muscle triglyceride concentration 24 h after prolonged exercise. Other investigators have examined the effect of dietary composition on muscle triglyceride concentration in humans during periods lasting 5 days to 4 wk (18, 21).

With use of a diet similar to the present investigation, Jansson and Kajiser (18) examined the effect of 5 days of a high-carbohydrate (75% of energy) and a high-fat (69% of energy) diet on muscle triglyceride concentration. Even though prediet biopsies were not obtained to examine percent changes, the resting muscle triglyceride concentration of the vastus lateralis after the 5-day dietary period was 80% higher with the high-fat (90.7 ± 20.1 mmol/kg dry wt) compared with the high-carbohydrate (50.4 ± 7.4 mmol/kg dry wt) diet. This mean difference was not statistically significant. After 4 wk of a high-fat diet (54% of energy), triglyceride concentration of the vastus lateralis muscle has been shown to increase 56% (30 ± 4 to 47 ± 8 mmol/kg dry wt) in a group of 10 healthy men (21). During a subsequent 4-wk period of a lower fat diet (29% of energy), Kiens et al. (21) reported that muscle triglyceride concentration decreased 13% to 41 ± 7 mmol/kg dry wt.

The ability to increase muscle triglyceride concentration may be linked to the activity of lipoprotein lipase. This enzyme catalyzes the hydrolysis of triglycerides in the capillary bed of adipose tissue and skeletal muscle (25). Several investigators have demonstrated an increased activity of skeletal muscle lipoprotein lipase after several days (17) or weeks (21, 31) of a higher fat diet. The activity of skeletal muscle lipoprotein lipase may be linked to circulating insulin levels. It has been demonstrated that skeletal muscle lipoprotein lipase activity is decreased when insulin concentrations are elevated (22). Thus a diet that does not raise insulin levels would be expected to increase the activity of skeletal muscle lipoprotein lipase, which might facilitate intramuscular triglyceride storage.

When compared with other studies utilizing longer dietary periods, it is interesting to note that only 1 day of a high-fat diet during the present investigation resulted in such a large increase in muscle triglyceride concentration (18, 21). In addition to diet, skeletal muscle lipoprotein lipase activity may be increased after prolonged exercise (25, 32). It has been demonstrated that skeletal muscle lipoprotein lipase activity is increased approximately two- to threefold immediately after a prolonged strenuous exercise bout (23, 24, 30). Furthermore, skeletal muscle lipoprotein lipase activity has been shown to be significantly elevated 4 h after 60 min of one-legged exercise (22). Thus the combination of the high-fat diet and the previous 120-min cycling bout may have had a synergistic effect with regard to increasing muscle triglyceride concentration during the present investigation.

The results from the present investigation demonstrate that an adequate amount of carbohydrate must be ingested after exhaustive exercise to maintain muscle glycogen concentration and prevent a reduction in subsequent endurance performance. The ingestion of 9.8 g carbohydrate/kg body wt during the Hi-CHO trial resulted in the resynthesis of 93% of the glycogen utilized throughout the 120-min cycling bout (see Table 1). In contrast, only 13% of the glycogen utilized throughout the 120-min cycling bout was resynthesized when 1.9 g carbohydrate/kg body wt were ingested during the Hi-Fat trial. These results are similar to other studies that have demonstrated complete resynthesis of muscle glycogen during a 24-h period after exhaustive exercise when between 8 and 10 g carbohydrate/kg body wt were ingested (2, 6). Similar to the present investigation, Costill et al. (6) reported no muscle glycogen resynthesis after exhaustive running when 2.4 g CHO·kg body wt\textsuperscript{-1}·24 h\textsuperscript{-1} were ingested.

With the incomplete resynthesis of muscle glycogen during the Hi-Fat trial, self-paced cycling performance was 19% slower compared with the Hi-CHO trial. To our knowledge, no one has examined the effect of different carbohydrate intakes during a 24-h period after prolonged exercise on self-paced cycling performance. These results are interesting because recent research has demonstrated that self-paced time trials are a more reliable measure of performance than are...
traditional time-to-fatigue tests (19). Inadequate muscle glycogen stores most likely resulted in the reduced performance during the Hi-Fat compared with the Hi-CHO trial. Even though we did not obtain post-time-trial muscle biopsies, the lower RER and serum glucose concentration and the higher serum free fatty acid and glycerol levels during the Hi-Fat vs. the Hi-CHO trials provide indirect evidence for reduced glycogen stores and thus an increased contribution to exercise energy expenditure from fat. Overall, these results demonstrate that the ingestion of ~10 g carbohydrate/kg body wt during a 24-h period after exhaustive exercise will resynthesize muscle glycogen stores and improve subsequent self-paced cycling performance.

In summary, muscle triglyceride concentration was not significantly different before and after 120 min of submaximal cycling. Nevertheless, the ingestion of a high-fat diet increased muscle triglyceride concentration by 36%, 24 h after the cycling bout. Furthermore, a high-carbohydrate diet did not increase muscle triglyceride concentration but did increase muscle glycogen storage and improve self-paced cycling performance compared with a high-fat diet.

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