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# Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans

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**Kiens, Bente, and Erik A. Richter.** Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *Am. J. Physiol. Endocrinol. Metab.* 38: E332–E337, 1998.—The utilization of muscle triacylglycerols was studied during and after prolonged bicycle ergometer exercise to exhaustion in eight healthy young men. Two days before exercise and in the postexercise recovery period, subjects were fed a carbohydrate-rich diet (65–70% of energy from carbohydrates). Exercise decreased muscle glycogen concentrations from  $533 \pm 18$  to  $108 \pm 10$  mmol/kg dry wt, whereas muscle triacylglycerol concentrations were unaffected ( $49 \pm 5$  before vs.  $49 \pm 8$  mmol/kg dry wt after exercise). During the first 18 h after exercise, muscle glycogen concentrations were restored to  $409 \pm 20$  mmol/kg dry wt. In contrast, muscle triacylglycerol concentrations decreased ( $P < 0.05$ ) to a nadir of  $38 \pm 5$  mmol/kg dry wt, and muscle lipoprotein lipase activity increased by 72% compared with values before exercise. Pulmonary respiratory exchange ratio values of 0.80–0.82 indicated a relatively high fractional lipid combustion despite the high carbohydrate intake. From 18 to 42 h of recovery, muscle glycogen synthesis was slow and muscle triacylglycerol concentrations and lipoprotein lipase activity were restored to the preexercise values. It is concluded that muscle triacylglycerol concentrations are not diminished during exhaustive glycogen-depleting exercise. However, in the postexercise recovery period, muscle glycogen resynthesis has high metabolic priority, resulting in postexercise lipid combustion despite a high carbohydrate intake. It is suggested that muscle triacylglycerols, and probably very low density lipoprotein triacylglycerols, are important in providing fuel for muscle metabolism in the postexercise recovery period.

muscle triglycerides; glycogen; muscle lipoprotein lipase activity

TRIACYLGLYCEROLS (TG) stored within skeletal muscle cells represent a potentially large energy source. However, from the available studies, it is controversial whether muscle triacylglycerols are utilized during exercise in men. From recent whole body experiments in humans using stable isotope techniques, it has been estimated that intramuscular TG ( $TG_m$ ) contribute as much as 20–25% of energy expenditure during prolonged submaximal exercise (16, 24). Some studies in which direct measurements of  $TG_m$  concentrations in muscle tissue have been performed have reported a decrease in  $TG_m$  of 15–50% during exercise lasting from 1–7 h (4, 5, 11, 23), whereas other such studies have reported no utilization during prolonged submaximal exercise (12, 27, 30, 31). Thus the focus has primarily been on whether  $TG_m$  are utilized during prolonged exercise. Less is known about whether  $TG_m$  contribute to the energy metabolism during the postexercise recovery period after prolonged submaximal

exercise, when muscle glycogen stores are depleted. Studies have shown, however, that utilization of fat for energy was elevated after 60–90 min of different types of exercise (21, 28). Furthermore, the study by Tuominen et al. (29) revealed a twofold elevated lipid oxidation rate, compared with the basal state, the morning after a competitive marathon race.

One of the sources of lipid fuel during recovery is thought to be circulating fatty acids (29). But other sources, such as very low density lipoprotein (VLDL) TG and  $TG_m$ , might be of significance too. The aim of the present investigation, therefore, was to study the role of  $TG_m$  for energy metabolism during a postexercise recovery period during which muscle glycogen stores are resynthesized. In addition, although the contribution of VLDL TG was not directly evaluated, the activity of the VLDL TG-degrading enzyme lipoprotein lipase was measured in skeletal muscle before and after exercise and in the postexercise recovery period.

## MATERIALS AND METHODS

Eight well-trained male athletes (1 rower, 3 runners, 2 cyclists, and 2 swimmers) participated in the study. Five of these subjects participated in competition. Subjects were 20–30 yr of age, body weight averaged 68 kg (63–75 kg), and height averaged 182 cm (175–188 cm). Maximal oxygen uptake ( $\dot{V}O_{2max}$ ; measured rowing or on a Krogh bicycle ergometer or treadmill) averaged 4.5 l/min (range 3.9–5.4 l/min).

To establish daily energy intake and composition of the subject's habitual diet, 4-day diet records were carried out by all subjects (3 weekdays and 1 weekend day). All food intake and beverages were weighed and recorded, and energy intake and composition of the diets were calculated with a computer database (Dankost II, the Danish Catering Center, Copenhagen, Denmark). In addition, individual energy intakes were determined from the World Health Organization's equation for calculation of energy needs (32). All subjects were fully informed of the nature of the study and the possible risks associated with it before they volunteered to participate, and written consent was given. The study was approved by the Copenhagen Ethics Committee and conforms with the code of ethics of the World Medical Association (Declaration of Helsinki). Subjects were covered by state medical insurance and also by the insurance that covers hospitalized patients in case of complications.

**Protocol.** During the 2 days before the experiments, the subjects abstained from all sport activities and consumed a carbohydrate-rich diet [65–70% of energy (E%) from carbohydrates (CHO), 20 E% from fat, and 10–15 E% from protein] to ensure filled glycogen stores. On the experimental day ( $D_0$ ), the subjects reported to the laboratory either by bus or car in the morning after an overnight fast. After 30 min of rest in the supine position, resting oxygen uptake ( $\dot{V}O_2$ ) and respiratory exchange ratio (RER) were measured. A needle biopsy was then taken from the vastus lateralis muscle under local

anesthesia with lidocaine. Then a light breakfast (800 kJ) was consumed, consisting mainly of CHO with a high glycaemic index (GI). After 2 h of rest, exercise was initiated on a Krogh bicycle ergometer. Exercise was performed at 75% of  $\dot{V}O_{2\max}$  for 20 min followed by alternating 2-min bouts of 90 and 50% of  $\dot{V}O_{2\max}$ , as previously described (17), for ~90 min until exhaustion to ensure depleted muscle glycogen stores. At termination of exercise, another muscle biopsy was taken in the same leg as the morning biopsy through a new incision spaced 4–5 cm from the first. Blood was drawn from a catheter inserted in the antecubital vein. For the following 42 h subjects were asked to abstain from all sport activities. In this period the subjects continued to follow the well-controlled diet. During the rest of  $D_0$ , forearm venous blood samples, muscle biopsies from the vastus lateralis muscle, and resting oxygen uptake were obtained frequently. The following day ( $D_1$ ), samples were obtained before breakfast in the fasting state (morning  $D_1$ , hour 18 of recovery) and before dinner (evening  $D_1$ , hour 30 of recovery). On  $D_1$  subjects were allowed to leave the laboratory between samplings, and they slept at home. On  $D_2$  samples were obtained before breakfast only (morning  $D_2$ , hour 42 of recovery). Muscle biopsies were taken, with alternation of these between right and left thighs, through different incisions spaced 4–5 cm apart.

**Diet.** All food ingested by the subjects during the recovery period was prepared and weighed in a metabolic kitchen. It was prepared on an individual basis, to one gram of accuracy. The composition of the diet in the postexercise recovery period was aimed at providing 65–70 E% from CHO, 10–15 E% from protein, and 20 E% from fat. The first meal was begun 1 h after termination of exercise. The amount of CHO, as well as energy contained in this meal, was calculated to provide that utilized during the exercise bout. A light meal was consumed 3 h later, and dinner after another 3 h. On the following day ( $D_1$ ) four meals were ingested, distributed over the day. Common food items were used in the diet, consisting of CHO-rich food items varying in GI and averaging a GI of 62 with glucose as reference. The subjects consumed 8–10 g CHO·kg body wt<sup>-1</sup>·day<sup>-1</sup>. Total energy intake during the experimental days was based on the energy intake calculated from the individual self registrations.

**Blood analyses.** Blood glucose was analyzed by enzymatic fluorometric methods (19) after whole blood had been deproteinized in ice-chilled perchloric acid and neutralized by KOH. Plasma free fatty acids (FFA) were measured fluorometrically as described by Kiens et al. (12). Insulin in plasma was determined using a radioimmunoassay kit, kindly donated by Novo-Nordisk (Copenhagen, Denmark), and catecholamines in plasma were determined by a radioenzymatic procedure (2).

**Muscle analyses.** The biopsy samples were frozen in liquid nitrogen within 10–15 s and were stored at  $-80^\circ\text{C}$  until further analysis. Before biochemical analysis, muscle biopsy samples were freeze-dried and dissected free of connective tissue, visible fat, and blood with a stereomicroscope and were then powdered and mixed.  $\text{TG}_m$  concentration was determined from  $\approx 2$  mg (dry wt) muscle sampled from the  $\approx 15$  mg (dry wt) mixed powder. Glycerol from the degraded TG was assayed fluorometrically as described by Kiens and Richter (15). Muscle glycogen concentration was determined as glucose residues after hydrolysis of the muscle sample in 1 M HCl at  $100^\circ\text{C}$  for 2 h (19). Lipoprotein lipase activity in muscle ( $\text{LPLA}_m$ ) was determined as described (13).

**$\dot{V}O_2$  and heart rate.** Pulmonary  $\dot{V}O_2$  at rest and during exercise was determined by collection of expired air in Douglas bags. The volume of air was measured in a Collins bell-spirometer (according to the Tissot principle), and the

fractions of oxygen and carbon dioxide were determined with paramagnetic (Servomex) and infrared (Beckmann LB-2) systems, respectively. Two gas samples with known compositions were used to calibrate both systems regularly. Heart rate was recorded with a PE 3000 Sports Tester (Polar Electro, Finland).

**Statistical evaluation.** Results are given as means  $\pm$  SE, if not otherwise stated. For each variable measured, a one-way ANOVA with repeated measures for the time factor was performed to test for changes during recovery. Differences between time points were detected with an all pairwise multiple comparison procedure (Student-Newman-Keuls method). In all cases, an  $\alpha$  of 0.05 was used as level of significance.

## RESULTS

$\text{TG}_m$  concentrations averaged  $49 \pm 5$  mmol/kg dry wt at rest ( $D_0$ , Fig. 1) and remained unchanged at termination of the exercise bout. After 3 h of recovery,  $\text{TG}_m$  concentrations had decreased significantly and reached a nadir 18 h after the end of exercise (morning  $D_1$ ), at which point  $\text{TG}_m$  concentrations were 20% lower than at rest (Fig. 1).  $\text{TG}_m$  remained lower than initial concentrations for 30 h after termination of exercise (evening  $D_1$ , Fig. 1). Muscle glycogen concentrations amounted to  $533 \pm 18$  mmol/kg dry wt at rest and decreased to  $108 \pm 10$  mmol/kg dry wt at termination of exercise (Fig. 2). After 6 h of recovery, muscle glycogen concentrations had increased to  $268 \pm 15$  mmol/kg dry wt ( $P < 0.05$ ). After 30 h of recovery (evening  $D_1$ ), muscle glycogen concentrations averaged  $500 \pm 25$  mmol/kg dry wt, which was similar to initial values (Fig. 2). After exercise,  $\text{LPLA}_m$  was slightly but significantly higher than the value before exercise (Table 1).  $\text{LPLA}_m$  increased to a maximum value 18 h after termination of exercise and returned to basal levels by 42 h of recovery (Table 1).

Initially blood glucose concentrations averaged  $4.42 \pm 0.10$  mmol/l (Table 2). After 2 h of recovery, blood glucose concentrations were significantly higher than baseline values and remained elevated for the following 2 h. Plasma insulin concentrations were higher ( $P <$

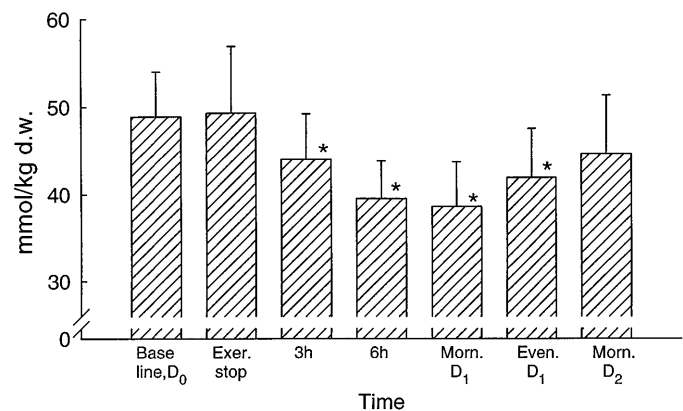


Fig. 1. Concentrations of triacylglycerol in vastus lateralis muscle ( $\text{TG}_m$ ) at rest ( $D_0$ ), immediately after exercise (Exer. stop), and during postexercise recovery. Morning (Morn.)  $D_1$  = hour 18 of recovery, evening (Even.)  $D_1$  = hour 30 of recovery, and Morn.  $D_2$  = hour 42 of recovery. Values are means  $\pm$  SE of 8 observations. dw, Dry wt. \*  $P < 0.05$  vs. resting values.

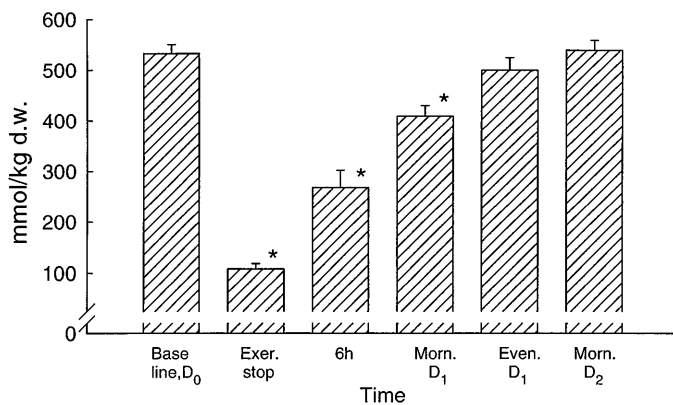


Fig. 2. Concentrations of muscle glycogen in vastus lateralis muscle at rest (D<sub>0</sub>), immediately after exercise, and during postexercise recovery. Times are like those in Fig. 1. Values are means  $\pm$  SE of 8 observations. \* $P < 0.05$  vs. resting values.

0.05) than baseline values after 2 h of recovery and remained higher during the following 4 h (Table 2). Plasma FFA concentrations were initially  $288 \pm 38$   $\mu\text{mol/l}$ . At the end of exercise and during the first 2 h of recovery, plasma FFA concentrations were significantly higher than baseline values (Table 2). During the recovery period until the end of the experiment, the concentrations of epinephrine and norepinephrine averaged 0.045 (range 0.04–0.05) ng/ml and 0.27 (0.24–0.29) ng/ml, respectively, and were not different from initial resting concentrations. RER values averaged  $0.85 \pm 0.02$  at baseline (Table 2). During the rest of D<sub>0</sub> and in the morning of D<sub>1</sub>, RER averaged 0.81 (Table 2). Resting  $\dot{V}\text{O}_2$  measured in the morning 4 h before exercise start averaged 0.26 l/min (Table 2). Four and six hours after exercise stop, resting  $\dot{V}\text{O}_2$  was significantly higher than baseline values. During the remainder of the experimental period, resting  $\dot{V}\text{O}_2$  was similar to baseline values (Table 2).

On the basis of 4 days of self-registration records, the habitual diet of the subjects averaged 14.5 (13–18) MJ. Total daily energy intake averaged  $16.0 \pm 2.4$  and  $14.4 \pm 0.6$  MJ on D<sub>0</sub> (exercise day) and on D<sub>1</sub> (resting day), respectively. Calculated dietary CHO intake averaged 641 (560–761) g and 552 (538–567) g on D<sub>0</sub> and D<sub>1</sub>, respectively. This amounts to an average of 8.9 (8.0–10.0) g/kg on D<sub>0</sub> and 7.6 (7.1–8.9) g/kg on D<sub>1</sub>, which results in an average of  $8.3 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$  during the postexercise recovery period.

Table 1. Activity of LPLA in vastus lateralis muscle before and after exercise and during postexercise recovery

LPLA, mU/g wet wt	Day 0		Day 1			Day 2
	Exercise		Recovery			
	Before	After	6 h	18 h Morn.	30 h Eve.	42 h Morn.
	46 $\pm$ 6	52 $\pm$ 8*	63 $\pm$ 12*	79 $\pm$ 10*	50 $\pm$ 3*	41 $\pm$ 7

Values are means  $\pm$  SE of 8 observations. LPLA, lipoprotein lipase activity. Morn, morning; eve, evening. \* $P < 0.05$  vs. before exercise.

On the basis of chemical analysis, the diet in the postexercise recovery period consisted of 70–73 E% of CHO, 15 E% of protein, and 12–15 E% of fat. The first meal (1 h after termination of exercise) contained  $47 \pm 7\%$  of the total energy intake of D<sub>0</sub> and  $49 \pm 6\%$  of total CHO intake for D<sub>0</sub>.

## DISCUSSION

The main finding in the present study is that skeletal muscle TG concentrations decrease in the postexercise recovery period despite a large intake of CHO ( $8.3 \text{ g CHO} \cdot \text{kg body wt}^{-1} \cdot \text{day}^{-1}$ , amounting to  $\sim 570 \text{ g/day}$ ). In contrast, no TG<sub>m</sub> breakdown could be detected during exercise. The rapid and marked decrease in TG<sub>m</sub> concentrations during the postexercise recovery period was surprising because, in accordance with the literature, intake of diets rich in CHO for shorter or longer periods is associated with a high fractional CHO oxidation at rest and during exercise (1, 8). Thus it might be expected that during the present postexercise recovery period there would be no need for significant fat oxidation. Nevertheless, the RER values of an average of 0.81 in the postexercise recovery period indicate a substantial fractional fat oxidation during the first 18 h of recovery. It appears that muscle glycogen resynthesis has such high metabolic priority during recovery that utilization of lipids is necessary to cover energy expenditure in muscle and that TG<sub>m</sub> accounts for a substantial part of it.

The mechanisms involved in activating the TG<sub>m</sub> breakdown in postexercise recovery are elusive, because the regulation of the responsible lipase is not known. It has been proposed that a hormone-sensitive TG lipase (HSL) enzyme similar to the adipose tissue HSL could regulate TG<sub>m</sub> hydrolysis (26). After the production of an antibody raised against the purified rat adipose tissue HSL, immunological evidence has been presented to support this hypothesis. In rat skeletal muscle extracts, immunoblotting with this antibody revealed the presence of an antigenic protein with a molecular mass similar to that of the adipose tissue HSL (9). The use of a cDNA clone to perform Northern blotting showed that HSL mRNA in heart and skeletal muscle was also similar in size to that found in adipose tissue (10). The activity of HSL in adipose tissue is increased by  $\beta$ -adrenergic stimulation and decreased by insulin (7). If the lipase in resting muscle is regulated as it is in adipose tissue, then activation would be expected to occur if sympathetic nervous activity to the muscle is increased in the postexercise recovery period, if circulating concentrations of catecholamines are high and/or plasma insulin concentrations are low. In fact, because of the CHO feeding in the present postexercise recovery period, plasma insulin concentrations were markedly increased above fasting levels after meals (Table 2), and plasma concentrations of catecholamines were not significantly elevated compared with fasting resting levels (data in text). Thus, even though a local increase in skeletal muscle sympathetic activity may not be re-

Table 2. Blood glucose, plasma insulin, FFA, RER, and  $\dot{V}O_2$  before and after exercise and during postexercise recovery

	Day 0						Day 1		Day 2	
	Exercise		Recovery				18 h	30 h	42 h	
	Before	After	1 h	2 h	3 h	4 h	6 h	Morn.	Eve.	Morn.
Glucose, mmol/l	4.42±0.10	4.56±0.28	3.94±0.24	6.03±0.15*	5.68±0.31*	4.89±0.11*	4.48±0.14	4.36±0.16	4.38±0.16	4.31±0.12
Insulin, $\mu$ U/ml	9.3±4.5	5.4±1.5*	4.5±2.0*	15.9±7.9*	36.1±7.8*	30.9±12.9*	14.8±3.6*	7.8±2.1	9.5±2.8	8.2±2.4
FFA, $\mu$ mol/l	288±38	1,326±192*	2,038±198*	546±95*	285±28	348±25	317±47	426±59*	340±61*	409±99*
RER	0.85±0.02	ND	ND	ND	ND	0.82±0.01	0.81±0.02	0.80±0.03*	0.87±0.02	0.85±0.03
Resting $\dot{V}O_2$ , l/min	0.26±0.01	ND	ND	ND	ND	0.32±0.02*	0.30±0.01*	0.24±0.01	0.28±0.02	0.26±0.01

Values are means  $\pm$  SE of 8 observations. FFA, free fatty acids. Respiratory exchange ratios (RER) and resting oxygen uptake ( $\dot{V}O_2$ ) were obtained before exercise and during selected recovery times. ND, not determined. \*  $P < 0.05$  vs. before exercise.

flected in measurable increases in forearm venous plasma norepinephrine concentrations, from which our values stem, it is unlikely that sympathetic nervous activity was markedly increased to the muscles in postexercise recovery. Thus increased sympathetic activity may not play a major role for the observed breakdown of TG<sub>m</sub> observed in the present postexercise recovery period.

Regarding insulin, it might seem surprising that the TG<sub>m</sub> content in the early recovery period decreased in the face of increased plasma insulin levels, because, if anything, one might expect the lipase to be inhibited by insulin as is the case in adipose tissue. This assumption is supported by recent findings using microdialysis, which show that the interstitial glycerol concentration in muscle is decreased during infusion of insulin (6). Thus, in the postexercise recovery period, the apparent inhibitory effect of insulin on muscle lipolysis is in effect overruled by some unknown stimulatory signal probably related to muscle glycogen depletion. There are other examples of activation or inhibition of enzymes by substrate. For instance, the activity of glycogen synthase in muscle is influenced by the glycogen concentration. Thus low glycogen concentrations are associated with a high glycogen synthase activity (22). The molecular mechanism linking low glycogen concentrations to glycogen synthase activation is not known, but it cannot be excluded that this mechanism might also cause activation of the TG lipase in skeletal muscle. Another intriguing example of substrate-enzyme interaction is found after exercise: orally ingested CHO escapes the liver and is used for muscle glycogen replenishment until the glycogen stores in muscle are refilled (20). Only then does the liver retain absorbed CHO to replenish its own glycogen stores (20). This example of how replenishment of muscle glycogen stores after exercise has priority over replenishment of other substrate stores is still biochemically unexplained but serves to support the notion that glycogen stores in muscle may also influence activity of the TG lipase in skeletal muscle.

It might be argued that the transient decrease in TG<sub>m</sub> content in the postexercise recovery period was due to the CHO-rich diet per se rather than the

preceding exercise-induced muscle glycogen depletion. However, essentially the same CHO-rich diet was fed 2 days before the exercise bout as during the recovery period. It is very unlikely that a transient decrease in TG<sub>m</sub> content would suddenly occur after 2 days on the diet if no exercise had been performed. Furthermore, if the decrease in TG<sub>m</sub> were due to the CHO-rich diet by itself, then it would not be expected to be a transient effect, because the CHO-rich diet was consumed throughout the recovery period. Therefore, it is unlikely that the CHO-rich diet by itself led to the decrease in the TG<sub>m</sub> content in the postexercise recovery period.

In the present study we demonstrated an increase in LPLA<sub>m</sub> immediately after exercise (Table 1). This is in accordance with previous findings by Lithell et al. (18) after exhaustive prolonged exercise. The 4-h delayed increase in LPLA<sub>m</sub> after exercise previously reported by Kiens et al. (14) might be explained by a shorter exercise bout than in the present study and the study of Lithell et al. (18). In the present study, LPLA<sub>m</sub> was also increased in the postexercise recovery period, as observed previously (14), and the maximum activity was found at the same time that the TG<sub>m</sub> content was decreased the most. Because LPL is responsible for VLDL TG hydrolysis, our findings suggest that, in addition to TG<sub>m</sub> providing lipid fuel in the postexercise recovery period, the breakdown of VLDL TG was probably also increased in muscle, providing supplementary long-chain fatty acids as fuel. Seip et al. (25) recently described increased muscle LPL mRNA and protein 4 and 8 h, respectively, after 72 min of exercise at 63%  $\dot{V}O_{2max}$ . These findings indicate that it is not only activity of the muscle LPL that is increased after exercise, but LPL gene transcription is also increased.

Several studies have previously addressed the question of whether TG<sub>m</sub> is utilized as a fuel during exercise. The answer has been equivocal, because some studies have demonstrated an exercise-induced decrease in TG<sub>m</sub> concentrations (3–5, 11, 23), whereas others have not (12, 27, 31). Part of the uncertainty regarding utilization of muscle TG stores during exercise probably stems from the difficulty in measuring TG<sub>m</sub> concentrations. It has recently been described that the aver-

age coefficient of variation for TG<sub>m</sub> concentrations sampled three times from the same muscle in eight subjects was 24% (31). During exercise for 90 min at 65%  $\dot{V}O_{2\max}$ , it was reported that the average difference in TG<sub>m</sub> concentrations from rest to after exercise was <24% (31). The authors concluded that differences in TG<sub>m</sub> concentrations of <24% cannot be reliably measured with their biopsy technique. In our hands, the TG method allowed us to detect a difference of 10% between resting and 3-h postexercise concentrations (Fig. 1), possibly because we used a fraction ( $\approx 2$  mg dry wt) of a large powdered and mixed biopsy ( $\approx 15$  mg dry wt). Still, even though we can pick up relatively small differences after exercise, our data show no tendency toward a decrease in TG<sub>m</sub> concentrations after exhausting glycogen-depleting exercise. These data thus support evidence that, during such exercise, net utilization of intramuscular triglycerides even in well-trained subjects is negligible, in agreement with our earlier findings during 2 h of one-legged knee extensions in both trained and untrained muscle (12).

It is concluded that, in the recovery period after prolonged glycogen-depleting exercise, oxidation of lipids covers >50% of oxidative metabolism despite a large intake of CHO. It appears that resynthesis of muscle glycogen in the postexercise recovery period has such high metabolic priority that TG<sub>m</sub>, and possibly VLDL TG, are broken down at an increased rate to supply lipid fuel for oxidative muscle metabolism.

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