

Postexercise Muscle Triacylglycerol and Glycogen Metabolism in Obese Insulin-Resistant Zucker Rats

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Abstract

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Objective: To determine the impact of insulin resistance and obesity on muscle triacylglycerol (IMTG) and glycogen metabolism during and after prolonged exercise.

Research Methods and Procedures: Female lean (*fa/?*; $N = 40$, ZL) and obese insulin-resistant (*fa/fa*; $N = 40$, ZO) Zucker rats performed an acute bout of swimming exercise (8 times for 30 minutes) followed by 6 hours of carbohydrate supplementation (CHO) or fasting (FAST). IMTG and glycogen were measured in the extensor digitorum longus (EDL) and red vastus lateralis (RVL) muscles.

Results: Despite resting IMTG content being 4-fold higher in ZO compared with ZL rats, IMTG levels were unchanged in either EDL or RVL muscles immediately after exercise. Resting glycogen concentration in EDL and RVL muscles was similar between genotypes, with exercise resulting in glycogen use in both muscles from ZL rats ($\sim 85\%$, $p < 0.05$). However, in ZO rats, there was a much smaller decrease in postexercise glycogen content in both EDL and RVL muscles ($\sim 30\%$). During postexercise recovery, there was a decrease in EDL muscle levels of IMTG in ZL rats supplemented with CHO after 30 and 360 minutes ($p < 0.05$). In contrast, IMTG content was increased above resting levels in RVL muscles of ZO rats fasted for 360 min-

utes. Six hours of CHO refeeding restored glycogen content to resting levels in both muscles in ZL rats. However, after 6 hours of FAST in ZO animals, RVL muscle glycogen content was still lower than resting levels ($p < 0.05$). At this time, IMTG levels were elevated above basal ($p < 0.05$).

Discussion: In both healthy and insulin-resistant skeletal muscle, there was negligible net IMTG degradation after a single bout of prolonged exercise. However, during postexercise recovery, there was differential metabolism of IMTG between phenotypes.

Key words: skeletal muscle lipids, insulin resistance

Introduction

Skeletal muscle contains significant quantities of lipid stored as triacylglycerol, with the intramuscular triacylglycerol (IMTG)¹ pool representing a potentially large energy reserve for muscle contraction. Investigations in humans using stable isotope techniques have shown that IMTG can contribute 20% to 30% of total energy expenditure during prolonged submaximal exercise (1,2). However, findings from studies that have directly measured IMTG use from biopsy samples are equivocal, with some reporting decreases during prolonged (1 to 7 hours) exercise (3–6) and others finding little change (7–11).

Although attention has been focused on determining whether IMTG is used during exercise, little is known about the effect of exercise on IMTG metabolism in insulin-resistant states. This is somewhat surprising given that IMTG is increased with obesity and type 2 diabetes and is strongly associated with insulin resistance (12,13). Insulin-

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¹ Nonstandard abbreviations: IMTG, intramuscular triacylglycerol; FFA, free fatty acid; ZO, obese Zucker rat; CHO, carbohydrate; FAST, fasting; ZL, lean Zucker rat; REST, sedentary control; POST, fasted and killed immediately after exercise; FAST-30, fasted and killed 30 minutes after exercise; FAST-360, fasted and killed 6 hours after exercise; CHO-30, CHO supplemented and killed 30 minutes after exercise; CHO-360, CHO supplemented and killed 6 hours after exercise; EDL, extensor digitorum longus; RVL, red vastus lateralis; FA, fatty acid; BM, body mass.

resistant skeletal muscle is characterized by low rates of fat oxidation at rest and during exercise (14–16), and, therefore, it may be expected that insulin-resistant muscle would have less reliance on IMTG as a substrate during exercise. However, greater use of IMTG has been reported in skeletal muscle from a rodent model of type 2 diabetes compared with that of control animals during a treadmill run to exhaustion (17). Furthermore, there is evidence suggesting that higher initial IMTG content is associated with an increased rate of hydrolysis of IMTG during exercise (17,18).

Although the focus of many studies has been to determine whether IMTG is used during exercise, there is an absence of information pertaining to the contribution of muscle lipids to metabolism after prolonged exercise. Recently, several studies have determined the time-course changes in IMTG content during recovery from exercise (19,20). Kiens and Richter (19) reported that IMTG remained unchanged after a bout of prolonged, exhaustive exercise in well-trained individuals. However, there was a significant reduction in IMTG content during the initial 18 hours of recovery. In contrast, Kimber et al. (20), using a similar experimental protocol to that employed by Kiens and Richter (19), reported that IMTG content remained unchanged and suggested that plasma free fatty acids (FFA) and very-low-density lipoproteins are important fuel sources during recovery (20).

Despite these findings, no information is available regarding lipid metabolism in insulin-resistant skeletal muscle during recovery from exercise. Therefore, the aim of this study was to examine IMTG metabolism in muscle from obese Zucker (ZO) rats after a prolonged bout of exercise. In addition, we wished to examine the impact of carbohydrate (CHO) refeeding vs. fasting (FAST) on IMTG metabolism in insulin-resistant muscle. In the postexercise recovery period, it has been reported that the ZO rat relies on lipids for energy (21), despite exhibiting altered fatty acid disposal toward storage in the basal state (22). We, therefore, hypothesized that IMTG would be used during the postexercise recovery period to a greater extent in insulin-resistant skeletal muscle from ZO rats than in muscle from their lean counterparts.

Research Methods and Procedures

Animal Care and Overview of Experimental Design

Analyses were carried out on tissues of rats previously studied for other purposes (23). Female lean (ZL; *fa/?*; $N = 40$) and ZO (*fa/fa*; $N = 40$) rats (age, 10 to 11 weeks; weight, 180 and 300 grams, respectively) were obtained from Monash University Animal Services (Victoria, Australia) and housed two per cage in environmentally controlled conditions (temperature, 22 °C; relative humidity, 51%), with a 12-hour light-dark cycle (light, 7:00 AM to 7:00 PM). All animals were fed the same diet (standard

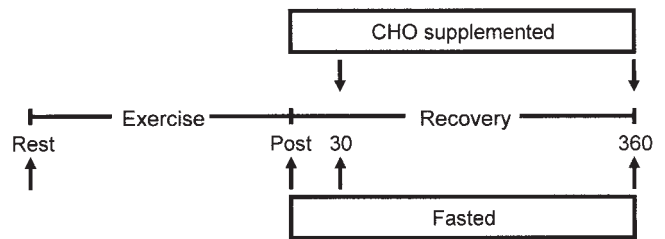


Figure 1: Schematic representation of the study protocol. ZL and ZO rats were killed at rest after exercise (eight 30-minute bouts of swimming) and after 30 and 360 minutes of recovery. During the postexercise recovery period, rats were either supplemented with CHO or remained fasted. Arrows indicate time of death.

rodent chow; 67.5% carbohydrate, 11.7% fat, 20.8% protein; Barastock Ltd., Victoria, Australia) and had ad libitum access to water. Before experimentation, animals were familiarized to laboratory conditions by swimming for 10 min/d for 3 days. The Animal Experimentation Ethics Committee of RMIT University approved all experimental procedures. Animals were assigned to one of six subgroups on the basis of whether they were to remain as sedentary controls (REST) or were exercised; whether they were FAST or received CHO postexercise; and the time of post-exercise death.

Experimental Protocol

At 5:00 PM on the day before an experiment, ZL animals were restricted to 10 grams of chow and ZO animals to 12 grams of chow; this amount was 60% of the animals' average daily food intake from the previous 7 days. ZL and ZO rats were randomly assigned to one of six experimental groups (Figure 1): sedentary control who performed no exercise (REST; $n = 7$); fasted and killed immediately after exercise (POST; $n = 7$); fasted and killed 30 minutes after exercise (FAST-30; $n = 6$); fasted and killed 6 hours after exercise (FAST-360; $n = 7$); CHO supplemented and killed 30 minutes after exercise (CHO-30; $n = 7$); and CHO supplemented and killed 6 hours after exercise (CHO-360; $n = 6$).

Exercise and Refeeding Protocol

The exercise model chosen for this study was a swimming protocol previously used in our laboratory (23). In brief, three rats swam together in a steel barrel filled to a depth of ~60 cm. Water temperature was maintained at 35 ± 1 °C. Rats swam for eight 30-minute bouts, separated by 5-minute rest periods during which time they were dried and placed in their cages. In the case of the ZO rats, a weight equal to ~2.5% of body mass (BM) was attached to the base of the tail after the first 30-minute exercise bout (24) to compensate for their increased buoyancy. For the remaining seven exercise bouts, ZO rats swam with the

weight attached. Weights were selected so that, during the swimming protocol, the body angles relative to the surface of the water were similar for both the ZO and ZL rats (25). After the eighth exercise bout, rats were dried, placed back in their cages, and killed at the time-points previously described. At the onset of the recovery period, rats assigned to the CHO-supplemented groups received an intraperitoneal glucose injection (0.5 mg/g BM) and were subsequently allowed free access to chow and water ad libitum. Rats in the fasted groups were allowed ad libitum access to water. Postexercise food consumption was quantified.

Animal Death

Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 mg/kg BM), and the extensor digitorum longus (EDL; 38% type IId/x and 38% type IIB fibers) (26) and red vastus lateralis (RVL; 33% type IIA fibers and 32% type IId/x) (26) muscles were rapidly excised and clamp frozen with tongs cooled in liquid N₂. At this time, a blood sample (~2 mL) was obtained from the femoral artery. Muscle samples were freeze dried, dissected free of nonmuscular components, and powdered.

Blood Biochemistry

Whole blood (~2 mL) was transferred to an EDTA-administered tube and spun in a centrifuge at 12,000 rpm for 3 minutes. The plasma was immediately analyzed in duplicate for plasma glucose and plasma lactate concentration using an automated analyzer (2300 Stat Plus Glucose and L-Lactate Analyzer; Yellow Springs Instruments, Yellow Springs, OH). The remaining plasma was stored at -80 °C and was subsequently analyzed for plasma FFA concentration, using an enzymatic colorimetric method (NEFA C test kit; Wako, Richmond, VA), and plasma insulin concentration by radioimmunoassay, using a commercially available kit (Phadeseph, Insulin RIA; Pharmacia & Upjohn Diagnostics, Uppsala, Sweden).

Muscle Analyses

Before biochemical analyses, muscle samples were freeze dried and dissected free of visible fat, connective tissue, and blood and were powdered and mixed. IMTG was determined from ~5 mg (dry mass) sampled from the 10 to 15 mg of mixed powder. Glycerol from the degraded triglycerides was assayed as previously described (9). Muscle glycogen concentration was determined as glucose residues after hydrolysis of the muscle sample in 1 M HCl at 100 °C for 2 hours (27).

Statistical Analyses

Analysis of differences between the two treatments (FAST or CHO) within a genotype was performed using a paired Student's *t* test. An unpaired Student's *t* test was used to assess differences between ZL and ZO animals. All other

differences were determined using a one-way ANOVA with Tukey's post hoc analysis. Significance was accepted when $p < 0.05$. All data are presented as mean \pm SE.

Results

Postexercise Food Intake

Food consumption was similar between ZL and ZO rats after both 30 (0.7 \pm 0.2 vs. 0.5 \pm 0.2 grams) and 360 minutes (5.6 \pm 0.7 vs. 5.2 \pm 0.8 grams) of refeeding. The relative (milligram per gram BM) postexercise food intake was not different between ZL CHO-30 and ZO CHO-30 rats (3.9 \pm 1.1 vs. 1.7 \pm 0.7 mg/gram BM). However, after 360 minutes of CHO refeeding, the relative food intake was greater in ZL than in ZO rats (30.2 \pm 4.4 vs. 18.3 \pm 2.2 mg/gram BM, $p < 0.05$).

Muscle Triacylglycerol Content

IMTG content was increased ~4-fold in the EDL and RVL muscles of ZO rats compared with ZL rats (Figures 2A and 3A). However, despite these differences, exercise did not affect IMTG content in either the EDL or RVL muscles in either genotype. During recovery from exercise, there was a significant decrease in IMTG in the EDL from ZL animals supplemented with CHO after both 30 and 360 minutes ($p < 0.05$; Figures 2A and 4A). IMTG content was also reduced in the RVL muscle from ZL rats fasted for 30 minutes after exercise ($p < 0.05$; Figure 4B). In contrast, RVL muscle IMTG was increased in ZO rats after 360 minutes of fasting ($p < 0.05$; Figure 4B).

Muscle Glycogen Concentration

Resting EDL muscle glycogen content was similar between genotypes (Figure 2B). Exercise reduced glycogen concentration by 84% ($p < 0.05$) in ZL but only 30% ($p < 0.05$) in ZO rats. In ZL rats, CHO or FAST had little effect on muscle glycogen resynthesis in the 30-minute period after exercise (Figure 2B). However, 360 minutes of CHO supplementation restored EDL muscle glycogen content to resting levels in ZL rats but did not result in glycogen supercompensation. Glycogen content of the EDL muscle during the recovery period was not different from REST in ZO rats regardless of postexercise dietary intervention. However, CHO refeeding for 360 minutes in ZO rats resulted in an increase in glycogen content compared with postexercise levels (41%; $p < 0.05$). Glycogen concentration of the EDL muscle was greater in ZO compared with ZL rats at all time-points during recovery, except for ZO CHO-360 (Figure 2B).

Glycogen content of the RVL muscle was similar between ZL and ZO rats at rest (Figure 3B). Exercise depleted glycogen content of the RVL muscle by 87% ($p < 0.05$) in ZL rats. However, no significant reduction in RVL muscle glycogen content was observed immediately after exercise

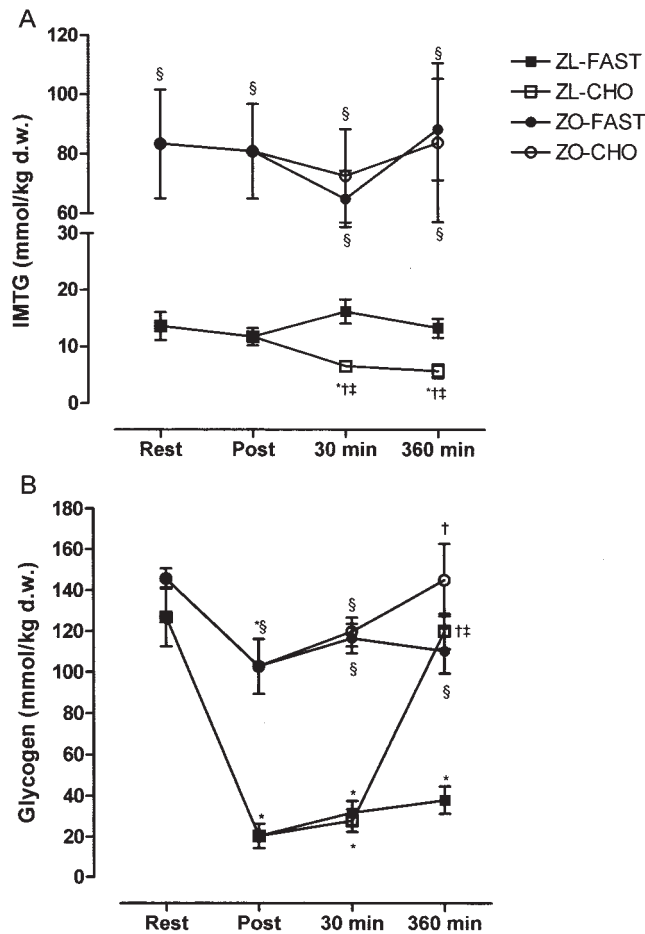


Figure 2: EDL muscle triacylglycerol (A) and glycogen (B) content in ZL and ZO rats. Values are mean \pm SE. * p < 0.05 vs. REST for each genotype. † p < 0.05 vs. POST for each genotype. ‡ p < 0.05 vs. FAST at same time-point for each genotype. § p < 0.05 vs. LEAN at same time-point.

in ZO rats (Figure 3B). In ZL rats, glycogen concentration remained at postexercise levels after 30 minutes of recovery, irrespective of diet. After 360 minutes of CHO refeeding, RVL muscle glycogen content was restored to resting levels in ZL rats. After 360 minutes and in the absence of CHO, muscle glycogen content for ZO rats was reduced to 55% of resting values. Glycogen concentration was greater in ZO rats compared with ZL rats immediately after exercise and after 30 minutes of recovery, regardless of dietary intervention (Figure 3B).

Concentrations of Plasma Metabolites

The concentrations of plasma glucose, lactate, insulin, and FFAs at rest and during recovery for the two treatment interventions are shown in Table 1. Animals from both genotypes exhibited a modest hyperglycemia (i.e., 8 to 9 mM). Exercise resulted in a significant reduction in plasma

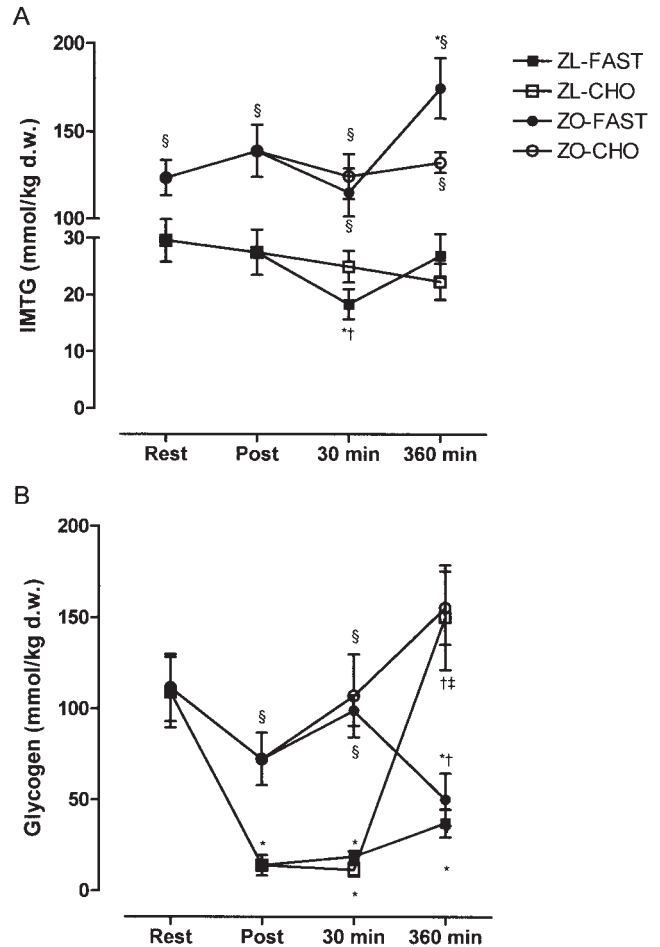


Figure 3: RVL muscle triacylglycerol (A) and glycogen (B) content in ZL and ZO rats. Values are mean \pm SE. * p < 0.05 vs. REST for each genotype. † p < 0.05 vs. POST for each genotype. ‡ p < 0.05 vs. FAST at same time-point for each genotype. § p < 0.05 vs. LEAN at same time-point.

glucose concentration in ZL rats (Table 1). After 30 minutes of CHO refeeding, plasma glucose concentration was greater in ZL rats compared with FAST and was still higher than POST values after 360 minutes of CHO supplementation. Postexercise plasma glucose concentrations in ZO rats were similar to REST (Table 1). During recovery, apart from CHO-360, plasma glucose concentration was greater in ZO than in ZL rats (Table 1). After 360 minutes of CHO refeeding, plasma glucose concentration was similar for both genotypes.

Resting plasma lactate concentration was increased in ZO compared with ZL rats. Exercise had little effect on plasma lactate concentrations in either genotype (Table 1). However, plasma lactate was significantly higher in obese animals at all time-points, regardless of the treatment intervention. Only after 30 minutes of CHO refeeding was plasma lactate lower than REST for ZO rats.

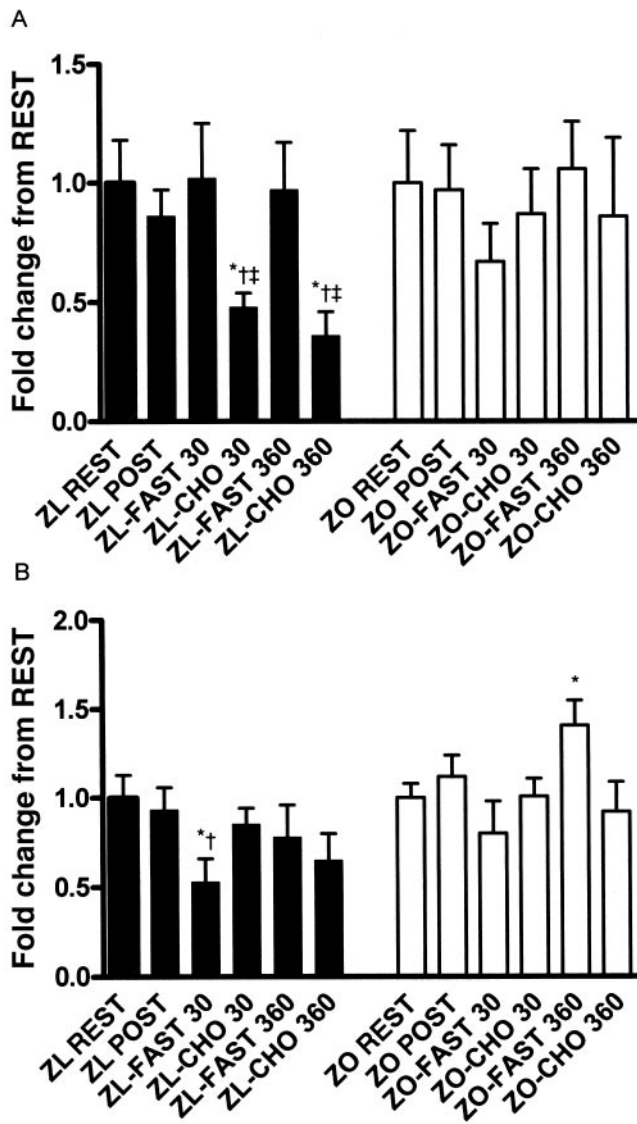


Figure 4: Change in EDL (A) and RVL (B) muscle triacylglycerol content in ZL and ZO rats compared with resting values. Values are mean \pm SE. * $p < 0.05$ vs. REST for each genotype. † $p < 0.05$ vs. POST for each genotype. ‡ $p < 0.05$ vs. FAST at same time-point for each genotype.

Resting plasma insulin concentrations were elevated in ZO compared with ZL rats and remained higher at all time-points, regardless of treatment intervention (Table 1). Exercise resulted in a significant reduction in plasma insulin concentrations in the ZL rats, such that at all time-points, concentrations were lower than REST. After 360 minutes of CHO refeeding, plasma insulin concentration was greater than POST in ZL rats.

Plasma FFA concentrations were elevated in ZO compared with ZL rats at rest. Exercise increased plasma FFA concentration in ZL rats (Table 1). Exercise did not affect plasma FFA levels in ZO rats. Plasma FFA levels in ZO rats

remained between 1.2 and 1.4 mM during the postexercise period, regardless of dietary intervention. In contrast, FFA concentration was increased after 30 minutes of FAST and CHO supplementation and also after 360 minutes of FAST after exercise in ZL rats (Table 1). Only after 360 minutes of CHO refeeding were plasma FFA levels back to resting values in ZL rats.

Discussion

The first novel finding of this study was that, despite markedly elevated resting levels of IMTG in obese insulin-resistant Zucker rats, we failed to detect any significant net degradation of this metabolite after 4 hours of exercise. Indeed, for both muscles under study (EDL and RVL muscles) and for both genotypes (lean and obese), there was little use of IMTG in response to a single bout of prolonged swimming exercise.

Our results contrast to those recently reported by Strackowski et al. (17), who found that, in a rodent model of type 2 diabetes, there was a significant reduction in triacylglycerol levels in the white and red gastrocnemius muscles after a single bout of exhaustive treadmill running. Furthermore, in that study (17), there was a positive relationship between the initial muscle triacylglycerol levels, as determined by biochemical extraction, and the subsequent rate of use during exercise. It is possible that slight adipose tissue contamination of the muscle sample may explain some of the difference in results. It has been argued that the biochemical extraction technique to determine IMTG content may result in the inadvertent contamination of the sample with fat and connective tissue (28). However, it should be noted that the technique used in this study includes a microscopic visualization of samples to carefully remove adipose tissue and other contaminants from the muscle specimens before extraction of lipid for IMTG quantification. Differences in results between studies could also be due to the model of insulin resistance used (obese Zucker rat vs. streptozotocin-injected high-fat-fed rat), the mode of exercise employed (swimming vs. treadmill running), and the associated muscle recruitment patterns, muscle tension generation, and/or differences in the relative exercise intensity.

In the obese insulin-resistant animals, there was only a modest (~30%) depletion in the EDL muscle and trivial glycogen use in the RVL muscle after exercise. This raises the possibility that the *relative* exercise intensity during the prolonged swimming protocol may have been different between the lean and obese animals. However, in an attempt to match the relative intensity between the two genotypes, we attached a tail weight equal to ~2.5% of BM to the obese animals after the first bout of exercise. Such an intervention has previously been reported to compensate for the increased buoyancy of the obese animals and was selected so that, during swimming, the body angles relative to the

Table 1. Concentration of plasma metabolites

	Glucose (mM)		Lactate (mM)		Insulin ($\mu\text{U}/\text{mL}$)		FFA (mM)	
	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese
REST	8.8 \pm 0.1	9.3 \pm 0.3	1.7 \pm 0.2	4.1 \pm 0.2 \ddagger	13.1 \pm 0.4	48.8 \pm 6.2 \ddagger	0.4 \pm 0.1	1.6 \pm 0.3 \ddagger
POST	5.3 \pm 0.7*	10.3 \pm 0.7 \ddagger	2.4 \pm 0.7	5.0 \pm 0.8 \ddagger	4.6 \pm 1.0*	38.2 \pm 5.1 \ddagger	1.0 \pm 0.1*	1.4 \pm 0.1 \ddagger
FAST 30	5.2 \pm 0.4*	10.3 \pm 0.8 \ddagger	1.5 \pm 0.4	3.4 \pm 0.3 \ddagger	2.6 \pm 0.6*	46.9 \pm 9.9 \ddagger	0.9 \pm 0.1*	1.3 \pm 0.2 \ddagger
CHO 30	7.6 \pm 0.7 \S	11.9 \pm 0.9* \ddagger	1.3 \pm 0.2	2.9 \pm 0.4* \ddagger	5.5 \pm 1.3*	55.2 \pm 10.8 \ddagger	1.0 \pm 0.1*	1.2 \pm 0.1 \ddagger
FAST 360	6.3 \pm 0.1*	9.0 \pm 0.6 \ddagger	1.0 \pm 0.1	4.0 \pm 0.1 \ddagger	3.3 \pm 0.5*	61.1 \pm 13.0 \ddagger	0.9 \pm 0.1*	1.4 \pm 0.1 \ddagger
CHO 360	7.7 \pm 0.3 \ddagger \S	8.0 \pm 0.4	2.4 \pm 0.5	4.2 \pm 0.2 \ddagger	9.9 \pm 1.1* \ddagger \S	55.0 \pm 4.7 \ddagger	0.5 \pm 0.1 \ddagger \S	1.4 \pm 0.1 \ddagger

Plasma glucose, lactate, insulin, and FFA concentrations were measured in lean and obese Zucker rats as described in Research Methods and Procedures. Values are mean \pm SEM.

* $p < 0.05$ vs. REST for each genotype.

\ddagger $p < 0.05$ vs. POST for each genotype.

\ddagger $p < 0.05$ vs. lean group under identical conditions.

\S $p < 0.05$ vs. FAST at same time-point for each genotype.

surface of the water were similar for both obese and lean rats (25). In a previous study (23), using an identical swimming protocol, we observed comparable levels of glycogen depletion in the soleus muscle of both lean and obese animals, strongly suggesting that recruitment patterns in that muscle were similar between genotypes. Based on those findings (23), we did not hypothesize that there would be significant differences in glycogen depletion patterns in either of the hindlimb muscles chosen for study in this experiment. Accordingly, we acknowledge that a potential limitation of this study is the lack of any direct measure of relative exercise intensity between lean and obese animals (i.e., direct measures of O_2 consumption during the swimming protocol). For example, differences in plasma catecholamine concentrations during swim exercise may explain some of the discrepancies in the rates of glycogenolysis and IMTG hydrolysis between genotypes. From these data, we can only suggest that recruitment patterns (i.e., differential activation of muscle fiber types between genotypes), at least for the RVL muscle, are different in ZL and ZO rats during exhaustive swimming exercise.

It has recently been reported that insulin plays a pivotal role in the regulation of fatty acid (FA) oxidation and IMTG use during intense muscle contraction (29). Specifically, it has been suggested that the decline in insulin concentration early in exercise may be a necessary event to fully increase FA oxidation and permit increased lipid use by muscle at this time (29). In this study, exercise did not result in a decline in plasma insulin concentration in the obese animals. Thus, it is possible that the prevailing high levels of circulating insulin (40 to 60 $\mu\text{U}/\text{mL}$) in these animals may have had a direct effect on lipid partitioning toward esterifi-

cation rather than oxidation. If this was the case, we would have failed to detect any net use of IMTG during the exercise bout. However, in lean animals, the swimming protocol resulted in a 3-fold drop in plasma insulin concentration, yet IMTG content was similar before and after exercise. Taken collectively, these data provide evidence that, during a single bout of prolonged swimming, there is negligible net depletion of IMTG, even in insulin-resistant skeletal muscle with an elevated concentration of IMTG.

It is well accepted that after a bout of prolonged, sub-maximal exercise, rates of whole body fat oxidation are increased (19,20,30,31). Accordingly, recent interest has focused on determining the source of this elevated postexercise lipid oxidation and, specifically, whether IMTG is used in this period (19,20). In this study, we observed differential regulation of IMTG metabolism in muscles from lean rats during recovery from prolonged exercise: there was a significant decrease in IMTG in the EDL muscle from lean animals supplemented with CHO after both 30 and 360 minutes (Figure 2A). These data support the findings of Kiens and Richter (19), who reported use of IMTG in the postexercise recovery period in well-trained humans despite a large intake of CHO. Such a result is, perhaps, somewhat surprising in that it might be expected that the activity of hormone-sensitive lipase in skeletal muscle would be inhibited by the CHO-induced elevation in plasma insulin concentration (32), thus reducing IMTG hydrolysis. However, in this study, plasma insulin levels in lean rats fed CHO during recovery remained lower than resting (pre-exercise) concentrations (Table 1), despite the greater relative voluntary food intake in the lean compared with the obese animals after 360 minutes of CHO refeeding.

In contrast to the finding that IMTG was used in the postexercise recovery period after CHO refeeding in the EDL muscle, IMTG content was reduced in the RVL muscle from lean animals fasted for the first 30 minutes after exercise (Figure 3A). EDL muscle IMTG decreased with CHO; however, in the RVL muscle, it decreased while fasting. Such a finding is somewhat difficult to explain, especially considering that both muscles under investigation did not differ in their respective amounts of type I fibers, which seem to be preferentially involved in IMTG metabolism with exercise (33).

Whereas we were able to detect a significant disappearance of IMTG during recovery after prolonged exercise in skeletal muscle from lean animals, IMTG was actually increased in the RVL muscle of obese animals after 360 minutes of fasting (Figure 3A). At this time, muscle glycogen levels were still significantly lower than preexercise (resting) concentrations. Skeletal muscle insulin resistance in ZO rats has previously been reported to result in increased rates of FA uptake (34), which have been associated with the relocalization of the FA transport protein, FAT/CD36, from an intracellular membrane pool to the plasma membrane (34). In addition to the increased rates of skeletal muscle FA uptake, the rate of triacylglycerol synthesis in red muscle from ZO rats is increased compared with lean rats (22). Thus, skeletal muscle FA metabolism in ZO rats is altered, favoring increased rates of FA uptake and FA disposal toward storage. Such a phenomenon may, in part, explain the increase in IMTG content observed in the RVL muscle of obese rats after 360 minutes of fasting. However, such premises are largely speculative because FA turnover was not determined in this study, and there was no change in plasma FFA concentration in ZO animals at any time-point or after the dietary intervention.

In conclusion, this study examined lipid and carbohydrate metabolism in skeletal muscle from both normal healthy and obese insulin-resistant rats after a single bout of prolonged exercise, with and without CHO refeeding. Our results show that, in both healthy and insulin-resistant skeletal muscle, there is negligible net IMTG degradation after a single bout of prolonged (4 hours) swimming exercise. However, during recovery from exercise, we observed a differential metabolism of IMTG between phenotypes. In muscle from lean, healthy animals, IMTG was decreased in the EDL muscle after CHO supplementation, whereas in the RVL muscle, there was a net disappearance of IMTG during fasting. In contrast, IMTG was not used in muscle from obese insulin-resistant animals either during exercise or throughout the subsequent recovery period.

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References

1. Klein S, Coyle EF, Wolfe RR. Fat metabolism during low-intensity exercise in endurance-trained and untrained men. *Am J Physiol Endocrinol Metab.* 1994;267:E934–40.
2. Romijn JA, Coyle EF, Sidossis LS, et al. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am J Physiol Endocrinol Metab.* 1993;265:E380–91.
3. Essen B, Hagenfeldt L, Kaijser L. Utilization of blood-borne and intramuscular substrates during continuous and intermittent exercise in man. *J Physiol.* 1977;265:489–506.
4. Fröberg SO, Mossfeldt F. Effect of prolonged strenuous exercise on the concentration of triglycerides, phospholipids and glycogen in muscle of man. *Acta Physiol Scand.* 1971; 82:167–71.
5. Hurley BF, Nemeth PM, Martin WH III, Hagberg JM, Dalsky GP, Holloszy JO. Muscle triglyceride utilization during exercise: effect of training. *J Appl Physiol.* 1986;60: 562–7.
6. Watt MJ, Heigenhauser GJF, Spriet LL. Intramuscular triacylglycerol, glycogen and acetyl group metabolism during 4 h of moderate exercise in man. *J Physiol.* 2002;541:969–78.
7. Bergman BC, Butterfield GE, Wolfel EE, Casazza GA, Lopaschuk GD, Brooks GA. Evaluation of exercise and training on muscle lipid metabolism. *Am J Physiol Endocrinol Metab.* 1999;276:E106–17.
8. Kiens B, Essen-Gustavsson B, Christensen NJ, Saltin B. Skeletal muscle substrate utilization during submaximal exercise in man: effect of endurance training. *J Physiol.* 1993;469: 459–78.
9. Kiens B, Richter EA. Types of carbohydrate in an ordinary diet affect insulin action and muscle substrates in humans. *Am J Clin Nutr.* 1996;63:47–53.
10. Starling RD, Trappe TA, Parcell AC, Kerr CG, Fink WJ, Costill DL. Effects of diet on muscle triglyceride and endurance performance. *J Appl Physiol.* 1997;82:1185–9.
11. Wendling PS, Peters SJ, Heigenhauser GJF, Spriet LL. Variability of triacylglycerol content in human skeletal muscle biopsy samples. *J Appl Physiol.* 1996;81:1150–5.
12. Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes.* 1991;40:280–9.
13. Pan DA, Lillioja S, Kriketos AD, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes.* 1997;46:983–8.
14. Blaak EE, van Aggel-Leijssen DPC, Wagenmakers AJM, Saris WHM, van Baak MA. Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. *Diabetes.* 2000;49:2102–7.
15. Colberg SR, Simoneau J-A, Thaete FL, Kelley DE. Skeletal muscle utilization of free fatty acids in women with visceral obesity. *J Clin Invest.* 1995;95:1846–53.
16. Torgan CE, Brozinick JT Jr, Willems MET, Ivy JL. Substrate utilization during acute exercise in obese Zucker rats. *J Appl Physiol.* 1990;69:1987–91.
17. Straczkowski M, Kowalska I, Górski J, Kinalska I. The effect of a single bout of exhaustive exercise on muscle

- carbohydrate and lipid metabolism in a rat model of type 2 diabetes mellitus. *Acta Diabteol.* 2000;37:47–53.
18. **Standl E, Lotz N, Dixel T, Janka H-U, Kolb HJ.** Muscle triglycerides in diabetic subjects: effects of insulin deficiency and exercise. *Diabetologia.* 1980;18:463–9.
 19. **Kiens B, Richter EA.** Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *Am J Physiol Endocrinol Metab.* 1998;275:E332–7.
 20. **Kimber NE, Heigenhauser GJF, Spriet LL, Dyck DJ.** Skeletal muscle fat and carbohydrate metabolism during recovery from glycogen-depleting exercise in humans. *J Physiol.* 2003; 548:919–27.
 21. **Ardévol A, Adán C, Remesar X, Fernández-López JA, Alemany M.** Carbohydrate handling in exercising muscle of obese Zucker rats. *Int J Obes Relat Metab Disord.* 1997;21: 239–49.
 22. **Turcotte LP, Swenberger JR, Tucker MZ, Yee AJ.** Increased fatty acid uptake and altered fatty acid metabolism in insulin-resistant muscle of obese Zucker rats. *Diabetes.* 2001; 50:1389–96.
 23. **Bruce CR, Lee JS, Hawley JA.** Postexercise muscle glycogen resynthesis in obese insulin-resistant Zucker rats. *J Appl Physiol.* 2001;91:1512–9.
 24. **Cartee GD, Young DA, Sleeper MD, Zierath JR, Wallberg-Henriksson H, Holloszy JO.** Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol Endocrinol Metab.* 1989;256:E494–9.
 25. **Walberg JL, Molé PA, Stern JS.** Effect of swim training on development of obesity in the genetically obese rat. *Am J Physiol Regul Integr Comp Physiol.* 1982;242:R204–11.
 26. **Delp MD, Duan C.** Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. *J Appl Physiol.* 1996;80:261–70.
 27. **Lowry OH, Passonneau JV.** *A Flexible System of Enzymatic Analysis.* New York: Academic Press, 1972, pp. 189–192.
 28. **Goodpaster BH, He J, Watkins S, Kelley DE.** Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab* 2001;86:5755–61.
 29. **Dyck DJ, Steinberg G, Bonen A.** Insulin increases FA uptake and esterification but reduces lipid utilization in isolated contracting muscle. *Am J Physiol Endocrinol Metab.* 2001;281: E600–7.
 30. **Bielinski R, Schutz Y, Jequier E.** Energy metabolism during the postexercise recovery in man. *Am J Clin Nutr.* 1985;42: 69–82.
 31. **Krzentowski G, Pirnay F, Luyckx AS, et al.** Metabolic adaptations in post-exercise recovery. *Clin Physiol Funct Imaging.* 1982;2:277–88.
 32. **Watt MJ, Krstrup P, Secher NH, Saltin B, Pedersen BK, Febbraio MA.** Glucose ingestion blunts hormone-sensitive lipase activity in contracting human skeletal muscle. *Am J Physiol Endocrinol Metab* 2004;286:E144–50.
 33. **Van Loon LJ, Koopman R, Stegen JH, Wagenmakers AJ, Keizer HA, Saris WH.** Intramyocellular lipids form an important substrate source during moderate intensity exercise in endurance trained males in a fasted state. *J Physiol.* 2003;553: 611–25.
 34. **Luiken JJFP, Arumugam Y, Dyck DJ, et al.** Increased rates of fatty acid uptake and plasmalemmal fatty acid transporters in obese Zucker rats. *J Biol Chem.* 2001;276:40567–73.