Failure to Repeatedly Supercompensate Muscle Glycogen Stores in Highly Trained Men

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ABSTRACT

MCINERNEY, P., S. J. LESSARD, L. M. BURKE, V. G. COFFEY, S. L. LO GIUDICE, R. J. SOUTHGATE, and J. A. HAWLEY. Failure to Repeatedly Supercompensate Muscle Glycogen Stores in Highly Trained Men. *Med. Sci. Sports Exerc.*, Vol. 37, No. 3, pp. 404–411, 2005. **Purpose:** It is not known whether it is possible to repeatedly supercompensate muscle glycogen stores after exhaustive exercise bouts undertaken within several days. **Methods:** We evaluated the effect of repeated exercise-diet manipulation on muscle glycogen and triacylglycerol (IMTG) metabolism and exercise capacity in six well-trained subjects who completed an intermittent, exhaustive cycling protocol (EX) on three occasions separated by 48 h (i.e., days 1, 3, and 5) in a 5-d period. Twenty-four hours before day 1, subjects consumed a moderate (6 $g \cdot kg^{-1}$)-carbohydrate (CHO) diet, followed by 5 d of a high (12 $g \cdot kg^{-1} \cdot d^{-1}$)-CHO diet. Muscle biopsies were taken at rest, immediately post-EX on days 1, 3, and 5, and after 3 h of recovery on days 1 and 3. **Results:** Compared with day 1, resting muscle [glycogen] was elevated on day 3 but not day 5 (435 ± 57 vs 713 ± 60 vs 409 ± 40 $mmol \cdot kg^{-1}$, $P < 0.001$). [IMTG] was reduced by 28% ($P < 0.05$) after EX on day 1, but post-EX levels on days 3 and 5 were similar to rest. EX was enhanced on days 3 and 5 compared with day 1 (31.9 ± 2.5 and 35.4 ± 3.8 vs 24.1 ± 1.4 $kJ \cdot kg^{-1}$, $P < 0.05$). Glycogen synthase activity at rest and immediately post-EX was similar between trials. Additionally, the rates of muscle glycogen accumulation were similar during the 3-h recovery period on days 1 and 3. **Conclusion:** We show that well-trained men cannot repeatedly supercompensate muscle [glycogen] after glycogen-depleting exercise and 2 d of a high-CHO diet, suggesting that the mechanisms responsible for glycogen accumulation are attenuated as a consequence of successive days of glycogen-depleting exercise. **Key Words:** CARBOHYDRATE, GLYCOGEN SYNTHESIS, MUSCLE TRIACYLGLYCEROL, GLYCOGEN SYNTHASE

Almost 40 years ago, Swedish investigators described an extreme regimen involving submaximal exercise to exhaustion, a period of restricted carbohydrate (CHO) intake, and subsequent consumption of large quantities of dietary CHO to increase the stores of muscle and liver glycogen (2). This exercise-diet regimen dramatically elevated or "supercompensated" muscle glycogen levels and prolonged submaximal exercise time to exhaustion, compared with ingestion of a low-CHO diet (1). The positive relationship between the preexercise levels of muscle (and liver) glycogen and the improvement in exercise capacity led to subsequent experiments designed to determine the optimal methods for maximally increasing glycogen stores before glycogen-depleting exercise (14,18,29).

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Submitted for publication July 2004.
Accepted for publication November 2004.

0195-9131/05/3703-0404
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/01.MSS.0000155699.51360.2F

Depending on the extent of glycogen depletion, and provided that adequate CHO is consumed and the individual reduces his or her exercise training, the complete restoration of muscle glycogen can occur within 24 h after prolonged, continuous submaximal exercise (1). Whereas exercise-diet interventions to achieve supercompensated muscle glycogen stores and their effect on a variety of single exercise tasks have been well described (14), these procedures do not address the problem of athletic competitions that require repeated maximal efforts: athletes in many events often have to undertake prolonged, submaximal, and/or intermittent high-intensity exercise bouts on successive days for several weeks (19). In this case, the restoration of glycogen stores after exhaustive exercise is probably the single most important factor determining the time needed to recover before intense activity can be continued or resumed (5). Presently, it is not known whether trained individuals can supercompensate their muscle glycogen stores after bouts of glycogen-depleting exercise that are undertaken in rapid (~48 h) succession.

Whereas much attention has focused on determinants of postexercise glycogen accumulation (18), less is known about muscle triacylglycerol (IMTG) metabolism during recovery. Kiens and Richter (20) reported that during the postexercise recovery period, muscle glycogen synthesis has such high metabolic priority that IMTG is broken down at

an increased rate to supply lipid fuel for oxidative metabolism. However, others have reported that IMTG has a negligible role in metabolism during recovery from exhaustive exercise (21). Recently, van Loon et al. (31) showed that IMTG repletion after prolonged, submaximal exercise was impaired when subjects consumed a typical CHO-rich diet in the postexercise period. Furthermore, they speculated that such a phenomenon "could prove to result in suboptimal performance capacity during periods in which prolonged endurance exercise trials are performed repeatedly." Accordingly, in the present study, we determined the effects of repeated bouts of exhaustive exercise followed by a high-CHO diet on muscle glycogen and IMTG metabolism in well-trained subjects. We hypothesized that if competitive athletes were able to repeatedly supercompensate their muscle glycogen and IMTG stores after exhaustive exercise and a high-CHO diet, subsequent exercise capacity would be maintained. As running has been associated with an inhibition of glycogen accumulation due to muscle microtrauma (16), we chose to study muscle glycogen balance in well-trained cyclists rather than runners, as Costill et al. (6) have previously reported impaired muscle glycogen resynthesis after eccentric exercise.

METHODS

Subjects. Nine endurance-trained cyclists/triathletes who were cycling >15 h·wk^{-1} volunteered to participate in this study. Of the original nine subjects who entered the study, two sustained injuries/illness after one or more experimental trials, and another subject withdrew after the first trial. Accordingly, a total of six subjects completed the entire testing protocol. The subjects' age, body mass (BM), peak oxygen consumption ($\dot{V}O_2$ peak), and peak power output (PPO) were 28.3 ± 1.8 yr, 72.5 ± 3.1 kg, 63.4 ± 1.6 mL·kg·min^{-1}, and 361 ± 11 W (values are mean ± SE). The experimental procedures and possible risks of the study were explained to each subject who gave their written informed consent before participation. The study was approved by the RMIT University Human Research Ethics Committee.

Study overview. Subjects completed four prolonged, exhaustive cycling trials (EX) after appropriate diet and exercise control. The first EX was used to familiarize subjects with the laboratory testing protocols, and no invasive procedures were performed. One week after the familiarization trial, subjects commenced a 5-d intervention period that involved three EX trials, with each exercise bout separated by 48 h. Before and immediately upon completion of each of these three exercise bouts, a muscle biopsy was taken. Subjects were then fed a high-CHO meal and monitored during the subsequent 3 h of recovery. Blood samples were taken at regular intervals during this time, and at the completion of the 3-h recovery period, a further biopsy was taken (but not on day 5, due to subject considerations). On the days between EX, subjects maintained light training (described subsequently).

Preliminary testing. Subjects performed a maximal incremental cycling test on a Lode ergometer (Groningen, The Netherlands) for the determination of PPO and $\dot{V}O_{2peak}$. The maximal incremental test protocol commenced at a workload equivalent to 3.3 W·kg^{-1} BM, and the work rate was increased by 50 W after the first 150 s, and then by 25 W every 150 s thereafter until exhaustion (13). Throughout the maximal test subjects breathed through a mouthpiece attached to a metabolic cart (MedGraphics CardiO$_2$, St Paul, MN). Expired gas was passed through a flowmeter, an O_2 analyzer, and a CO_2 analyzer that were calibrated before testing using a 3-L Hans-Rudolph syringe and gases of known concentration (4.00% CO_2 and 16.00% O_2). The flowmeter and gas analyzers were connected to a computer that calculated minute ventilation, oxygen uptake ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), and the respiratory exchange ratio (RER) from conventional equations. The test was terminated at the point of volitional fatigue, defined as the inability to maintain a cadence >70 rpm. $\dot{V}O_{2peak}$ was reported as the highest $\dot{V}O_2$ for any 60 s. PPO was calculated by adding the work completed on the final workload to the last successfully completed workload (13). Individual PPO values were used to determine the appropriate exercise intensities for the subsequently described experimental trials.

Dietary and exercise control. On the day before the familiarization trial (FAM) and the first experimental trial (day 1), each subject was supplied with a moderate-CHO diet with a total energy content of 0.25 MJ·kg^{-1} BM that provided 6 g CHO·kg^{-1} BM·d^{-1}. After the first experimental trial, and throughout the remaining 5 d of the intervention period, subjects consumed an energy-matched high-CHO diet providing 12 g CHO·kg^{-1} BM·d^{-1}. Immediately after EX on days 1 and 3 (described subsequently), subjects were fed 4 g CHO·kg^{-1} BM of CHO-rich foods and fluids of moderate to high glycemic index consumed within 30 min. The balance of each day's dietary intake (i.e., 8 g CHO·kg^{-1} BM) was consumed throughout the rest of the day. All diets were constructed by a dietitian, and dietary control included the individualization of food plans for each subject relative to BM and food preferences. All food and drinks were supplied to subjects and were prepackaged to minimize the amount of food preparation required. Subjects were supplied with a food checklist to record their daily intake of the foods supplied, any portions of meals left unconsumed, and additional intake of fluids. On the day before an experimental trial, subjects performed 60–120 min of continuous low- to moderate-intensity cycling ($\sim$70% $\dot{V}O_{2peak}$). The duration of this training session was dependent upon each subject's current training volume and varied between individuals. However, the duration of these training sessions was rigorously controlled and remained constant for each subject throughout the experimental period.

Experimental trials. Subjects completed three EX bouts in the laboratory at the same time of day, and after a 10- to 12-h overnight fast. Upon arrival at the laboratory, subjects voided and after resting quietly for 5 min, a 20-gauge Teflon Catheter (Terumo, Tokyo, Japan) was inserted into a forearm vein and a resting blood sample ($\sim$5 mL) taken. The catheter was then flushed with $\sim$1 mL sterile saline (Astra Zeneca, North Ryde, Australia) to keep the

catheter patent and sterile, a procedure that was completed after each subsequent blood draw. Local anesthesia (2–3 mL of 1% Xylocaine (lignocaine)) was administered to the skin, subcutaneous tissue, and fascia of the vastus lateralis, scalpel incisions made, and a resting biopsy taken using a 6-mm Bergstrom needle modified with suction. Approximately 100–125 mg of muscle was removed, immediately frozen in liquid N_2 and stored at −80°C until analysis. At this time, two separate sites on the same leg (~5 cm distal) were prepared for subsequent biopsies to be taken immediately upon termination of exercise, and after 3 h of recovery.

Subjects then mounted the cycle ergometer and began a 5-min warm-up at 50% PPO before commencing EX as described previously (23). Briefly, subjects completed 2 min of high-intensity cycling at 90% PPO (~95% $\dot{V}O_{2peak}$) followed by 2 min at 50% PPO (~60% $\dot{V}O_{2peak}$). This work:recovery protocol was maintained until subjects were unable to complete 2 min of cycling at 90% PPO, determined as an inability to maintain a cadence of 60 rpm for 15 s. At this time the work rate was lowered to 80% PPO (~85% of $\dot{V}O_{2peak}$) with the same recovery employed. When subjects were unable to complete 2 min of high-intensity cycling, the work rate was lowered to 70% of PPO (~75% of $\dot{V}O_{2peak}$) and finally 60% PPO (~65% of $\dot{V}O_{2peak}$) with the same work:recovery ratio. Exercise was terminated when subjects could not complete 2 min of cycling at 60% of PPO. Such an exercise protocol was chosen to maximally deplete both Type I and II muscle fibers of their glycogen stores. During EX, subjects received no feedback with respect to either the number of repetitions they had performed at each power output, nor the elapsed time. During these rides, water was consumed *ad libitum* and an electric fan (wind speed 7 $m \cdot s^{-1}$) positioned to increase air circulation and evaporative cooling. At the point of exhaustion, with the subject remaining seated on the cycle ergometer, a second muscle biopsy was taken (<15 s) and rapidly frozen. A 5-mL blood sample was also taken at this time point. Subjects then dismounted the ergometer and rested in a supine position while consuming a standardized meal consisting of 4 g $CHO \cdot kg^{-1}$ BM. This meal was consumed within ~30 min of the termination of exercise. Blood samples (~5 mL) were taken at regular intervals throughout the 3-h recovery period. After 3 h of rest, a third muscle biopsy and final blood sample were taken. This exact experimental procedure was repeated on days 3 and 5, with the exception that a 3-h postexercise muscle biopsy was not taken on day 5. Laboratory conditions remained constant for all testing (21–22°C, 40–50% RH).

Analytical procedures. A small piece of frozen muscle (40–50 mg) was removed, and an attempt was made to dissect this free of all visible blood, connective tissue, and fat. This sample was then freeze-dried and powdered. One aliquot (~3 mg) of freeze-dried muscle was extracted and, with 250 μL of 2 M hydrochloric acid, incubated at 100°C for 2 h and then neutralized with 750 μL of 0.667 M sodium hydroxide for subsequent determination of glycogen concentration via enzymatic analyses with fluorometric detection (24). Glycogen concentration was expressed as millimoles of glycogen per kilogram d.w. The intraassay coefficient of variation (CV) for this assay in our laboratory is 6%.

A second aliquot (5–7 mg) of freeze-dried muscle was extracted for determination of intramuscular triacylglycerol (IMTG) concentration. Briefly, the IMTG was extracted and the chloroform phase evaporated (10). After reconstitution, phospholipids were removed upon the addition of silicic acid. The IMTG was saponified and the free glycerol was assayed fluorometrically (26). IMTG concentration was expressed as millimoles per kilogram d.w. The intraassay CV for this assay in our laboratory is 9%.

A second piece of frozen muscle (10 mg) was extracted and homogenized (1:50 dilution) in 50% glycerol, 20 mM phosphate buffer (pH 7.4), 5 mM 2-mercarptoethanol, 0.5 mM EDTA, and 0.02% bovine serum albumin for determination of glycogen synthase activity, as previously described (15). Glycogen synthase activity was measured in the absence (active form) and in the presence of 10 mM (total activity) of glucose-6-phosphate. Glycogen synthase activity is reported as fractional activity of the enzyme (active/total).

One milliliter of whole blood was immediately analyzed for glucose and lactate concentration using an automated glucose/lactate analyzer (YSI 2300 STAT PLUS, Yellow Springs, OH). Five milliliters of whole blood was placed into a tube containing fluoride EDTA, and mixed and spun in a centrifuge at 4000 rpm for 8 min at 0°C. The plasma was stored at −80°C for later analysis of plasma insulin concentration by radioimmunoassay (Coat-A-Count, Insulin RIA, Diagnostics Products Corporation, Los Angeles, CA) and plasma FFA concentration by an enzymatic colorimetric method (Wako, NEFA C code 279-75409, Tokyo, Japan). Plasma glycerol concentration was determined by placing 250 μL of plasma in a tube containing 250 μL of ice-cold 3 M perchloric acid, which was then mixed vigorously on a vortex mixer and spun; 400 μL of this supernatant were added to a tube containing 100 μL of 6 M potassium hydroxide (KOH), mixed, and spun. The resultant supernatant was analyzed for plasma glycerol concentration using an enzymatic spectrophotometric analysis (26).

Cycling performance. Cycling performance was determined from the total amount of work performed during each trial and expressed relative to each subjects BM (i.e., $kJ \cdot kg^{-1}$).

Statistical analysis. Blood metabolites and hormones, and glycogen and IMTG concentration were analyzed by a one-way ANOVA with repeated measures. Where significance was detected, *post hoc* analysis was performed using the Student–Newman–Keuls test. Analyses of the concentrations of glucose, insulin, and FFA between trials were analyzed by a Student's paired *t*-test, and also compared by examination of the area under the concentration versus time curve. Analyses of the rates of glycogen and IMTG resynthesis and blood metabolite concentrations between trials at individual time points were analyzed by a Student's paired *t*-test.

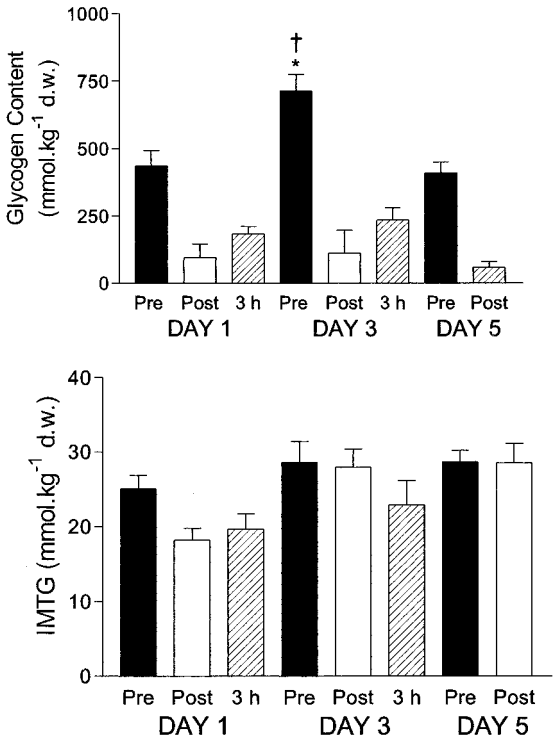

FIGURE 1—Muscle glycogen (A) and IMTG concentration (B) before (pre), immediately after prolonged, intense, exhaustive cycling exercise (post), and after 3 h of recovery (3 h). * Preexercise glycogen concentration significantly different from day 1, $P < 0.001$; † glycogen utilization significantly different from day 1, $P < 0.001$.

RESULTS

All subjects maintained dietary and exercise compliance for the duration of the study period.

Muscle glycogen and triacylglycerol concentrations. Figure 1A displays resting and postexercise muscle glycogen concentration, whereas Figure 1B shows muscle triacylglycerol concentration before and after EX. On day 1 after 1 d of a moderate (6 g·kg^{-1})-CHO diet, resting muscle glycogen concentration was 435 ± 57 mmol·kg^{-1} d.w. EX resulted in a marked depletion of muscle glycogen to 96 ± 50 mmol·kg^{-1} d.w. ($P < 0.001$). After 2 d of a high (12 g·kg^{-1})-CHO diet, resting muscle glycogen content on day 3 was supercompensated to 713 ± 60 mmol·kg^{-1} d.w. ($P < 0.01$). EX resulted in a reduction of muscle glycogen to 111 ± 84 mmol·kg^{-1} d.w. ($P < 0.001$) such that the net utilization was greater on day 3 compared with day 1 (601 ± 83 vs 339 ± 83 mmol·kg^{-1} d.w., $P < 0.05$). Despite a further 2 d of a high (12 g·kg^{-1})-CHO diet, resting muscle glycogen concentration on day 5 was similar to that on day 1 (after a moderate-CHO diet), and lower than on day 3 (409 ± 40 vs 713 ± 60 mmol·kg^{-1} d.w., $P < 0.001$). Accordingly, the rate of glycogen resynthesis was greater in the time between trials undertaken on days 1 and 3 than between days 3 and 5 (14.56 ± 1.11 vs 9.61 ± 0.6 mmol·kg^{-1} d.w. h^{-1}, $P < 0.05$). Subsequently, glycogen utilization during exercise was also less on day 5 than day 3 (350 ± 115 vs 601 ± 83 mmol·kg^{-1} d.w., $P < 0.001$). The rate of exercise-induced glycogen depletion on day 5 was significantly less than day 3 (138.8 ± 20.2 vs 213.6 ± 20.2, mmol·kg^{-1} d.w. h^{-1}, $P < 0.05$) but not different from day 1 (204.7 ± 26.1, mmol·kg^{-1} d.w. h^{-1}). As would be expected from the similar extent of glycogen depletion and an identical intake of CHO, there were no differences in the rates of muscle glycogen resynthesis during the 3-h recovery period on days 1 or 3 (36.2 ± 6.9 and 41.3 ± 5.4 mmol·kg^{-1} d.w. h^{-1}, respectively).

On day 1, resting IMTG concentration was 25 ± 2 mmol·kg^{-1} d.w., and was reduced by 28% ($P < 0.05$) after EX (Fig. 1B). After 2 d of a high-CHO diet, resting IMTG concentration was 29 ± 3 mmol·kg^{-1} d.w. on day 3, and 28 ± 2 mmol·kg^{-1} d.w. on day 5. These resting values were not different from day 1. Postexercise IMTG content on days 3 and 5 was not different from resting levels. There were no changes in IMTG concentration during the 3-h recovery period for days 1 or 3 (data not shown).

Glycogen synthase activity. The fractional activity of glycogen synthase was similar at rest (0.57 ± 0.06, 0.53 ± 0.08, and 0.54 ± 0.06) and immediately after exercise (0.76 ± 0.06, 0.69 ± 0.06, and 0.76 ± 0.01) on days 1, 3, and 5, respectively. After 3 h of recovery, glycogen synthase activity was significantly increased when compared with rest on both days 1 and 3 (0.57 ± 0.06 vs 0.83 ± 0.08, $P < 0.05$; and 0.53 ± 0.08 vs 0.90 ± 0.04, $P < 0.001$, respectively).

Blood metabolite and hormone concentrations. Figure 2 displays blood glucose (A), plasma insulin (B), and plasma FFA (C) concentrations at rest, immediately after exercise, and at regular intervals throughout 3 h of recovery for the three experimental trials. Resting blood glucose concentration on day 1 was ~5 mmol·L^{-1}, and subjects remained euglycemic throughout exercise. With the ingestion of the postexercise CHO meal (4 g CHO·kg^{-1} BM), blood glucose concentration increased so that between 30 and 120 min it was higher than either resting or immediately postexercise values ($P < 0.05$). As would be expected with an identical postexercise CHO ingestion protocol, there were no differences in blood glucose concentrations (area under the curve) between days 1 and 3 (recovery bloods not taken on day 5).

Resting plasma insulin concentrations for the three experimental trials were not different (Fig. 2B). Furthermore, resting plasma insulin was not different from the values measured immediately postexercise. However, ingestion of the postexercise CHO meal resulted in a steady rise in plasma insulin concentration throughout the first 60 min of recovery, whereafter levels remained elevated (at ~30 μU·mL^{-1}) for the following 2 h of the recovery period. There were no differences in plasma insulin concentrations (area under the curve) between days 1 and 3.

Resting plasma FFA concentrations were ~0.30 mmol·L^{-1} and were not different between experimental trials (Fig. 2C). Prolonged exercise on day 1 resulted in a fourfold increase in plasma FFA levels ($P < 0.001$) compared with rest. Plasma FFA concentrations after exercise on day 3 (1.9 ± 0.2 mmol·L^{-1}) and day 5 (1.9 ± 0.1 mmol·L^{-1}) were higher than at the end of exercise on day 1 ($P < 0.05$). Plasma FFA concentrations remained elevated for 30 min after exercise on day 1 ($P < 0.001$) and 15 min

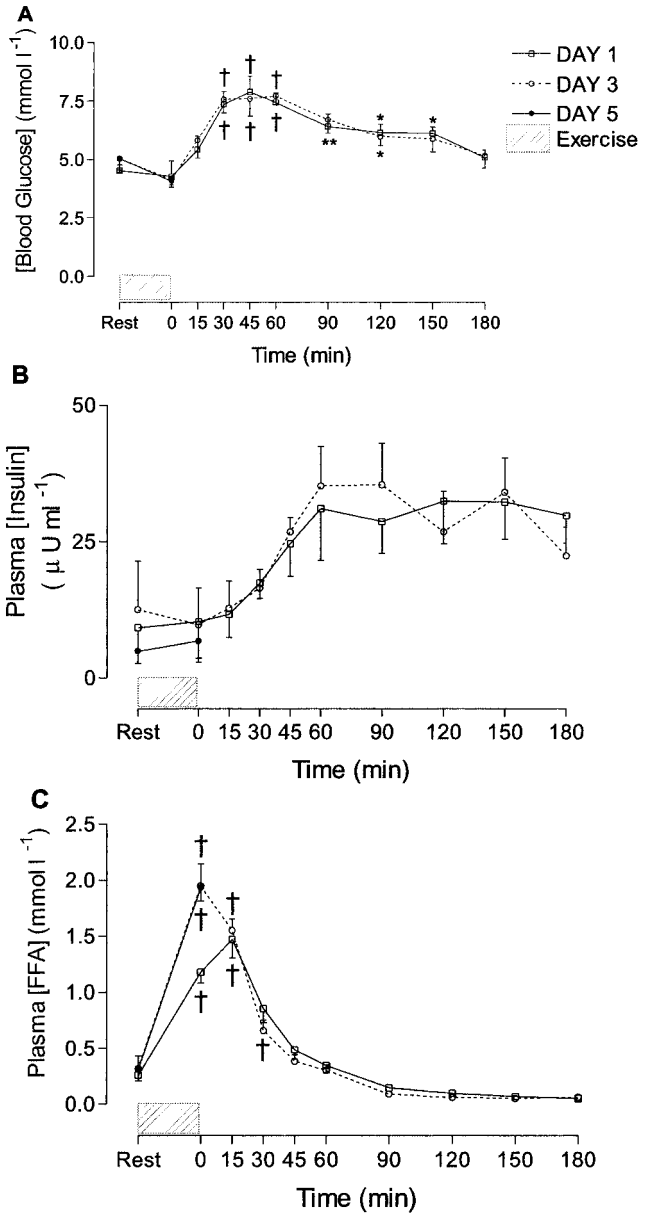

FIGURE 2—Blood glucose (A), plasma insulin (B), and plasma FFA concentrations (C) during 3 h of postexercise recovery. Postexercise values significantly different from rest; * $P < 0.05$, ** $P < 0.01$, † $P < 0.001$.

on day 3 ($P < 0.001$), and over the next 150 min returned to resting values (Fig. 2C).

Resting plasma glycerol concentration on day 1 was 0.27 ± 0.08 mmol·L^{-1} and was increased to 0.58 ± 0.06 mmol·L^{-1} at the end of exercise ($P < 0.001$). By the end of the 3-h postexercise recovery period, plasma glycerol concentration had returned to resting levels. There was no difference in either resting or immediate postexercise plasma glycerol concentrations between day 1, 3, or day 5, or at the end of the 3-h recovery period for days 1 and 3 (data not shown).

Exercise performance. Figure 3 displays exercise performance for each of the four trials. There were no differences in exercise performance between the familiarization trial and day 1 (23.6 ± 2.4 vs 24.1 ± 1.4 kJ·kg^{-1}, respectively). After 2 d of a high-CHO diet, exercise performance was enhanced on days 3 and 5 compared with day 1 (31.9 ± 2.5, 35.4 ± 3.8 vs 24.1 ± 1.4 kJ·kg^{-1} respectively, $P < 0.05$). Compared with day 1 (22.4 ± 4.3), the number of high-intensity work bouts at 90% of PPO (~95% of $\dot{V}O_{2peak}$) was higher on both day 3 (29.3 ± 1.5, $P < 0.01$) and day 5 (26.3 ± 3.3, $P < 0.05$). There was no difference in exercise time to exhaustion between the familiarization trial and day 1 (88.9 ± 16.6 vs 111.0 ± 6.4 min). However, time to exhaustion on day 1 was less than both days 3 and 5 (111.0 ± 6.4 vs 163.5 ± 10.3 and 174.7 ± 16.8 min, $P < 0.05$)

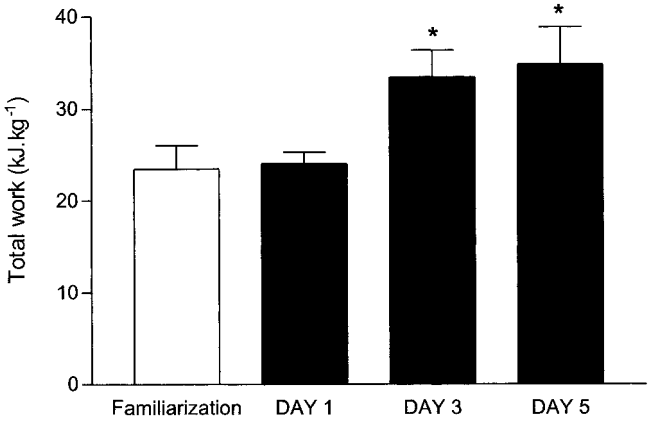

FIGURE 3—Exercise performance during the experimental rides expressed as total work (kJ·kg^{-1}). * Significantly different from day 1 and familiarization, $P < 0.05$.

DISCUSSION

The importance of endogenous CHO stores for the performance of prolonged (>90 min), submaximal exercise has been recognized since the classic studies conducted by Scandinavian researchers over a quarter of a century ago (1). At this time, it was also demonstrated that CHO feeding after a single bout of glycogen-depleting exercise resulted in a substantial increase in muscle glycogen concentration, a phenomenon known as glycogen supercompensation. Because of these pioneering investigations, a number of different dietary-exercise regimens have been evaluated in an attempt to attain supercompensated levels of muscle (and liver) glycogen stores and enhance subsequent exercise performance (14,18). A common feature of all glycogen-loading studies to date is that exercise capacity has been evaluated in a single exercise task. Accordingly, it is presently unknown whether trained individuals can supercompensate their muscle glycogen stores following bouts of glycogen-depleting exercise that are undertaken within rapid (48 h) succession. As endurance training in humans markedly enhances the capacity of muscle to accumulate glycogen (11), particularly during the first 6 h after glycogen-depleting exercise, we hypothesized that highly trained subjects would be able to supercompensate their glycogen stores after repeated exercise-diet intervention. Hence, the first novel finding of the present investigation was that under the conditions of the

current experiment, competitive athletes who performed several bouts of prolonged, exhaustive cycling to deplete muscle glycogen stores and then consumed a high-CHO diet, could not supercompensate their muscle glycogen stores a second time (2 d after initial supercompensation).

Previous studies of the effects of prolonged, intense (but not exhaustive) exercise undertaken on successive days have shown a marked reduction in both resting muscle glycogen content and its subsequent utilization (5,22). Indeed, Kirwan et al. (22) reported that resting muscle glycogen content declined by ~35% over 9 d of intense daily running. Of note was that this progressive glycogen depletion occurred despite attempts to match the subjects CHO intake to their daily energy expenditure. This observation suggests that either inadequate dietary CHO was provided to subjects or that the mechanisms responsible for muscle glycogen resynthesis are attenuated as a consequence of successive days of glycogen-depleting running.

Although it cannot be completely ruled out, we feel confident that our high-CHO diet would have provided ample substrate for glycogen resynthesis: subjects consumed 12 g CHO·kg^{-1} BM for four successive days: such an amount is 20–35% more than typical glycogen-loading protocols (14). Accordingly, the failure of muscle glycogen stores to reach supercompensated values on day 5 compared with day 3 of the experimental protocol strongly suggests an impairment in one or more of the mechanisms responsible for glycogen storage, possibly as a direct consequence of the cumulative effect of repeated, exhaustive exercise. Furthermore, because the rates of muscle glycogen resynthesis in the first 3 h after exhaustive exercise were similar to those previously reported by others (11,17,30), this effect was confined to the "slow" or "insulin-dependent" phase of glycogen resynthesis (27). A potential limitation of the current study is the lack of information regarding liver glycogen accumulation. However, although no direct measures of liver glycogen were made, it seems safe to assume that glycogen stores were adequate: resting blood glucose concentrations on the morning of a performance ride were typically ~5 mmol·L^{-1} and subjects remained euglycemic throughout prolonged exercise.

It has been proposed that the activation of glycogen synthase is responsible for the glycogen supercompensation that occurs after glycogen-depleting exercise (4). However, the results from the present study, as well as those from other investigations (9,28) do not support the concept that glycogen synthase activity is rate limiting for glycogen resynthesis, unless glycogen synthase activity is below the fasting range (8). In the current study, glycogen synthase activity was similar at rest and immediately postexercise. The values observed in the present study are in close agreement with those previously reported (3). Although glycogen synthase is likely to play an important role in the rapid initial increase in muscle glycogen, it plays only a permissive role in the glycogen supercompensation phenomenon. This is demonstrated by the finding that glycogen concentration continues to increase and reach supercompensated levels in the face of a decrease in the glycogen synthase activity (25).

It is difficult to speculate on the precise mechanism that might explain the failure of muscle glycogen stores to reach supercompensated values on day 5 compared with day 3 of the experimental protocol. We deliberately chose to have our well-trained subjects train on the days between the exhaustive "performance" rides to exhaustion (60–120 min of continuous low- to moderate-intensity cycling), as this is common practice for competitive athletes (19). As noted, the duration of these training sessions varied between subjects and was dependent upon their current training volume. However, the duration of these training sessions was rigorously controlled and was constant for each subject throughout the experimental period. Whereas training at an intensity requiring substantial CHO oxidation would obviously be counterproductive to glycogen synthesis, this did not appear to be the case, as glycogen stores reached supercompensated levels on day 3. Accordingly, these "recovery" training sessions cannot be the reason for the lack of muscle glycogen supercompensation on day 5.

As would be expected, exercise capacity was improved on day 3 compared with day 1 in the face of muscle glycogen supercompensation (Fig. 1A). However, on day 5 after 2 d of a high-CHO diet, muscle glycogen stores were similar to day 1 after a moderate-CHO intake. Given the lack of supercompensation on day 5 compared with day 3, and the concomitant reduction in glycogen utilization, it is difficult to explain the similar exercise capacity during these two rides. We did not determine RER values during the intermittent, exhaustive rides because subjects never attained the steady-state conditions necessary to make such measures valid. However, blood glucose concentrations were similar each day, suggesting that changes in glucose uptake were minimal. Accordingly, a likely explanation for the maintenance of exercise capacity (on day 5) would be a substantial increase in the contribution from lipid oxidation to total energy requirements. Another possible reason for the preservation of exercise capacity on day 5 is that there was a training effect during the intervention period. However, this seems unlikely; our subjects were all competitive athletes who had a prolonged history of endurance training. Furthermore, there were no differences in the performance of a familiarization trial with exercise performance on day 1. If any training-induced adaptations occurred, such changes would be small and would not have any direct effect on the measures of muscle glycogen metabolism determined in this study. Finally, it is possible that glycogen supercompensation may not be required in the trained athlete during successive days of competition: such a premise is borne out by the similar exercise performances on days 3 and 5 despite the lack of glycogen accumulation on day 5.

In contrast to the numerous studies on postexercise muscle glycogen accumulation (18), far less data are available on postexercise IMTG repletion (7,20,31). In a recent investigation, van Loon et al. (31) reported that in well-trained subjects, IMTG content declined significantly during 3 h of submaximal exercise and its accumulation was impaired when subjects consumed a low-fat, high-CHO diet compared with a normal-fat diet. In contrast, Kiens and Richter (20) reported that IMTG

levels remained unchanged upon completing a bout of prolonged, exhaustive, glycogen-depleting exercise in well-trained individuals. However, in that study there was a rapid and marked (~20%) decrease in IMTG content postexercise: such an observation was particularly surprising in the face of a large intake of CHO and the concomitant elevation in plasma insulin concentration, conditions that would be expected to inhibit IMTG hydrolysis. In the current study, we observed a significant (~30%) decrease in IMTG concentration during exhaustive exercise undertaken on day 1, but were unable to detect any significant decrease in IMTG levels after exhaustive exercise undertaken on days 3 and 5. It should be noted that the exhaustive exercise bout on day 1 was undertaken after subjects consumed a mixed diet containing a moderate amount of CHO (6 $g \cdot kg^{-1}$ BM), whereas they commenced exercise on days 3 and 5 after consuming a high-CHO (12 $g \cdot kg^{-1}$ BM), low-fat diet. Unlike muscle glycogen resynthesis, there were no changes in IMTG concentration during the 3-h recovery period for day 1 or 3 (recovery biopsies were not obtained on day 5). These findings are in close agreement with those of Kimber et al. (21), who reported that IMTG content does not decrease during recovery from exhaustive exercise in well-trained individuals in the presence of elevated glucose and insulin concentrations. Our data regarding the use, or lack thereof, of IMTG during and after exercise adds to the controversy regarding the utilization of this substrate.

Although it might be tempting to speculate that the lack of measurable utilization of IMTG during exercise was due to a lower preexercise value (i.e., impaired repletion) after 2 d of a high-CHO diet (31), this was not the case. Resting IMTG levels were similar on all 3 d (~30 $mmol \cdot kg^{-1}$ d.w.), whereas postexercise levels on days 3 and 5 were not different to preexercise concentrations. The lack of detectable changes in IMTG levels during or after exercise has often been attributed to the methodological limitations associated with the muscle biopsy technique, which does not discriminate between IMTG and fat contained between adjacent muscle fibers. However, using well-trained subjects with similar physiological and training characteristics to those participating in the present investigation, Watt et al. (32) reported a CV of 12% for IMTG measured on paired biopsies from the same individual. Accordingly, we acknowledge that a 12% or greater reduction in IMTG is required for changes to be considered meaningful. Another potential explanation for any lack of change in IMTG levels is that the IMTG pool can undergo esterification during low-intensity (~45% peak $\dot{V}O_2$) exercise when FFA levels are elevated (12). As plasma FFA levels at the completion of EX on days 3 and 5 increased to ~2.0 $mmol \cdot L^{-1}$ in the present study, we cannot exclude the possibility of IMTG/FFA cycling during our intermittent (low- and high-) intensity exercise protocol. Such a scenario would potentially mask the hydrolysis of IMTG.

In conclusion, the results of the current study demonstrate a failure of well-trained subjects to repeatedly supercompensate muscle glycogen stores after repeated bouts of exhaustive exercise and 2 d of a high-CHO diet during which normal training was undertaken. This effect was largely confined to the "slow" phase of glycogen resynthesis, as rates of glycogen resynthesis during the initial (3 h) stages of recovery from exhaustive exercise were similar to those reported previously. Accordingly, our data do not support the concept that glycogen synthase activity was rate limiting for glycogen resynthesis. Instead, the mechanisms responsible for glycogen resynthesis appear to be attenuated as a consequence of successive days of glycogen-depleting exercise. We found no evidence of impaired IMTG repletion after prolonged, intense, exhaustive cycling followed by a high-CHO diet. Finally, the similar exercise performance on days 3 and 5 despite the lack of glycogen accumulation on day 5 suggests that glycogen supercompensation may not be required in the trained athlete during successive days of competition.

We thank the subjects for their excellent cooperation and physical efforts in a difficult study.

This study was supported by a research grant from GlaxoSmithKline Nutrition (U.K) to John A. Hawley.

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