Effects of training duration on substrate turnover and oxidation during exercise

S. M. PHILLIPS, H. J. GREEN, M. A. TARNOPOLSKY,
G. J. F. HEIGENHAUSER, R. E. HILL, AND S. M. GRANT
Department of Kinesiology, University of Waterloo, Waterloo, Ontario N2L 3G1; and Departments of Kinesiology, Cardiorespiratory Medicine, and Pathology, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

Phillips, S. M., H. J. Green, M. A. Tarnopolsky, G. J. F. Heigenhauser, R. E. Hill, and S. M. Grant. Effects of training duration on substrate turnover and oxidation during exercise. *J. Appl. Physiol.* 81(5): 2182–2191, 1996.—Adaptations in fat and carbohydrate metabolism after a prolonged endurance training program were examined using stable isotope tracers of glucose ([6,6-$^2H_2$]glucose), glycerol ([$^2H_5$]glycerol), and palmitate ([$^2H_2$]palmitate). Active, but untrained, males exercised on a cycle for 2 h/day [60% pretraining peak O_2 consumption ($\dot{V}O_{2peak}$) = 44.3 ± 2.4 $ml \cdot kg^{-1} \cdot min^{-1}$] for a total of 31 days. Three cycle tests (90 min at 60% pretraining $\dot{V}O_{2peak}$) were administered before training (PRE) and after 5 (5D) and 31 (31D) days of training. Exercise increased the rate of glucose production (R_a) and utilization (R_d) as well as the rate of lipolysis (glycerol R_a) and free fatty acid turnover (FFA R_a/R_d). At 5D, training induced a 10% ($P < 0.05$) increase in total fat oxidation because of an increase in intramuscular triglyceride oxidation (+63%, $P < 0.05$) and a decreased glycogen oxidation (−16%, $P < 0.05$). At 31D, total fat oxidation during exercise increased a further 58% ($P < 0.01$). The pattern of fat utilization during exercise at 31D showed a reduced reliance on plasma FFA oxidation (FFA R_d) and a greater dependence on oxidation of intramuscular triglyceride, which increased more than twofold ($P < 0.001$). In addition, glucose R_a and R_d were reduced at all time points during exercise at 31D compared with PRE and 5D. We conclude that long-term training induces a progressive increase in fat utilization mediated by a greater oxidation of fats from intramuscular sources and a reduction in glucose oxidation. Initial changes are present as early as 5D and occur before increases in muscle maximal mitochondrial enzyme activity [S. M. Phillips, H. J. Green, M. A. Tarnopolsky, G. J. F. Heigenhauser, and S. M. Grant. *Am. J. Physiol.* 270 (*Endocrinol. Metab.* 33): E265–E272, 1996].

isotope; glucose; glycerol; palmitate oxidation

DURING EXERCISE the two primary substrates available for use by the working skeletal muscle are carbohydrates (CHO) and fats (1, 3, 15, 31). These substrates can be supplied from the muscle (glycogen and triglycerides) or from the liver in the case of glucose (6, 23) and from the adipose tissue in the case of fatty acids (19, 22, 31). Prolonged exercise of moderate intensity has been shown to result in a time-dependent increase in fat oxidation and a decrease in CHO oxidation (1, 22, 31). After training the dependency on fat oxidation becomes even more pronounced (3, 5, 6, 18, 31).

In the untrained state the increase in the reliance on fat oxidation with exercise appears to be (at least partly) dependent on increases in the availability of plasma free fatty acids (FFA) (22, 31). Training has been shown to result in a reduced concentration of circulating plasma FFA (19, 20, 22, 28). On the basis of this finding, it has been speculated that the increase in intramuscular triglyceride (IMTG) oxidation that apparently occurs with training is due to a reduction in circulating FFA that are available to the muscle for oxidation during exercise (19, 20, 22).

Prolonged exercise in the untrained state also results in a progressive reduction in total CHO (16) and blood glucose oxidization (3). The reduction in oxidation of these sources would appear to depend on reduced availability consequent to reductions in muscle glycogen reserves and blood glucose concentration (11). With training, muscle glycogen (14) and blood glucose utilization (3, 23) during sustained exercise are reduced. The shift in substrate selection after training is undoubtedly under multiple regulatory control (6, 20, 22, 23, 28). As an example, adaptations within the working muscle and, in particular, increases in the potential for oxidative phosphorylation and β-oxidation are believed to be mechanistically involved (18). Other factors also appear important, because we have recently shown that alterations in substrate selection can occur before increases in metabolic potential. Training for as little as 3–4 days, as an example, results in pronounced reductions in glycogen degradation in the absence of changes in the respiratory exchange ratio (RER) (13). Conceivably, these findings could be explained by increases in blood glucose oxidation or a reduced glycolytic flux, resulting in less lactate formation. As the training period is extended to 10–12 days, reductions in the RER occur, indicating an increase in total fat oxidation (15, 16, 28).

The purpose of this study, therefore, was to examine the time course of changes in whole body substrate turnover and oxidation that occur during prolonged exercise, after a short (5 days, 5D) and longer period (31 days, 31D) of endurance training. The 5D time point was chosen as a time when RER was unaffected (13) and when muscle mitochondrial potential, expressed as maximal mitochondrial enzyme activity, was not significantly increased from pretrained (PRE) levels (12). We have hypothesized that exercise after short-term training would result in increases in IMTG and glucose oxidation. As training is prolonged and increases in mitochondrial potential occur, increases in IMTG oxidation become more pronounced while glucose oxidation is decreased (18, 19).

METHODS

Subjects

Seven healthy, but untrained, males [23 ± 1 (SE) yr of age] volunteered for the study. All participants were from the student population at the University of Waterloo. After being informed of the procedures involved and the possible risks associated with each procedure, each participant signed a consent form, as approved by the Office of Human Research. During the study the participants were asked to maintain a normal weight-maintaining diet. Participants were also instructed to refrain from consumption of alcohol for the duration of the study. In addition, consumption of foods and liquids containing caffeine was not permitted on the days when exercise tests were administered.

Experimental Design

The testing and training protocol was similar in nature to that employed in previous studies (2, 12, 14–16). Two exercise tests were utilized. A progressive cycle test was administered 1–2 wk before beginning training and at 8 and 30 days of training. This test was used to establish pretraining workloads (using regression analyses) and to assess changes in peak O_2 consumption ($\dot{V}O_{2peak}$, peak aerobic power), ventilation, and heart rate. $\dot{V}O_{2peak}$ obtained during a maximal progressive exercise test to fatigue, before training, was 3.52 ± 0.2 l/min (44.3 ± 2.4 $ml \cdot kg^{-1} \cdot min^{-1}$) and was not significantly different after 8 days of training. $\dot{V}O_{2peak}$ did, however, increase significantly ($P < 0.05$) at 31D by 10% compared with PRE. The second test, which involved 90 min of cycling at 59 ± 2.1% of pretraining $\dot{V}O_{2peak}$, was administered at PRE, 5D, and 31D. Each challenge ride was performed under as near-identical conditions as possible and at the same absolute power output.

At least 6 h before each challenge ride, the subjects selected a snack (from a list of recommended foods provided for them: bread, cereal, juice), which was recorded, and the identical snack was consumed at the same time before each subsequent challenge ride. The snack provided 1,760 ± 200 kJ and was, on average, 62% CHO, 12% protein, and 26% fat.

Training consisted of cycling for 5 consecutive days, for 2 h/day at ~60% of pretraining $\dot{V}O_{2peak}$, on a Lode cycle ergometer, resting for 1 day, and then repeating the cycle training (5:1:5 training cycle). Training was performed at the same power output throughout the study. Training was initiated 2 days after the first prolonged exercise test and terminated 1 day before each subsequent test at 5D and 31D. On a given day, training was interrupted only if the subjects could not complete the prescribed 2 h of cycling, and in such cases the subjects were allowed rest pauses until they could complete the full 2 h of cycling. All subjects could complete the training session without pauses by *day 4* of the training protocol. Subjects were allowed ad libitum access to water during the training sessions.

Respiratory Measurements

$\dot{V}O_{2peak}$ was determined as described previously (12, 15, 16). During prolonged exercise, respiratory gas measurements (O_2 consumption and CO_2 production) were made using a gas collection system that has been described previously (8). Respiratory gases were collected and analyzed every 15 min during all 90-min challenge rides and at rest. Four-minute collection periods were used, with the values averaged every 30 s over the final 2 min of sampling.

Blood

Arterialized blood samples were obtained from a 20-gauge catheter inserted into a heated dorsal hand vein. Blood samples were taken at rest, before infusion, after 110 min of resting primed constant infusion 15 min before (−15 min) and just before exercise (0 min), and at 5 min (4 min) and every 15 min (15, 30, 45, 60, 75, and 90 min) during the challenge ride. These samples were used for the determination of blood lactate, glucose, glycerol, plasma catecholamine, and serum FFA concentration. For the metabolites (lactate, glucose, and glycerol), blood was placed into lithium heparinized tubes, and a 100-μl sample was removed and pipetted into ice-cold perchloric acid. The sample and perchloric acid were centrifuged [10,000 revolutions/min (rpm) for 10 min], and the resulting supernatant was neutralized using ice-cold $KHCO_3$ (15). For analysis of FFA concentration, 1.5 ml of blood were allowed to clot, the sample was centrifuged (8,000 rpm for 15 min), and the resulting serum was extracted and stored until analysis. All samples were stored at −80°C before analysis. All assays for the determination of blood lactate, glucose, glycerol, and serum FFA concentration were fluorometric assays and were performed as described previously (15). Plasma catecholamine concentration was determined using reverse-phase high-performance liquid chromatography procedures that have been described previously (15).

Isotopes

All tracers, [6,6-$^2H_2$]glucose, [$^2H_5$]glycerol, and [$^2H_2$]palmitate as a potassium salt (all 99% enriched), were purchased from CDN Isotopes (Pointe Claire, PQ, Canada). Glucose and glycerol were mixed into 0.9% sterile saline and filtered through a 0.2-μm filter before infusion. Isotopes were infused into a proximal forearm vein via an 18-gauge catheter, which was not occluded by arm bending, with use of a constant-rate infusion pump (Harvard Apparatus, Natick, MA). Priming doses of glucose (17 μmol/kg) and glycerol (1.5 μmol/kg) were given before initiation of the constant infusion (0.22 ± 0.02, 0.09 ± 0.01, and 0.04 ± 0.001 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for [6,6-$^2H_2$]glucose, [$^2H_5$]glycerol, and [$^2H_2$]palmitate, no prime, respectively). Specific infusion rates for each tracer were calculated by multiplying the exact infusate concentration, determined by gas chromatography-mass spectrometry (GC-MS), by the measured infusion rate. Before infusion, palmitate was bound to albumin (Miles Pharmaceuticals, Etobicoke, ON, Canada) by following procedures described previously (38). Subjects were infused for ≥110 min at rest, before exercise was initiated. When exercise was initiated, the infusion rate was tripled (compared with rest) for glucose and glycerol and doubled for palmitate (37).

Tracer Enrichment

Glucose and glycerol enrichments were determined (with some modifications) by making the trimethylsilyl (TMS) derivatives of each compound as described previously (37). Briefly, 100 μl of plasma were deproteinized with barium hydroxide (0.3 N) and zinc sulfate (0.3 N). The resulting supernatant was then deionized by passing it over a mixed-bed anion-cation exchange chromatographic column (AG-1-X8 and AG 50W-X8, Sigma Chemical, St. Louis, MO). The eluted extract from this column was then lyophilized to dryness. To ensure complete resolution of glucose from other sugars present in the plasma sample, the methyloxime form of glucose was made before derivatization to form the TMS derivative. To the lyophilized extract, 50 μl of methoxyamine hydrochloride (MOX reagent; Pierce Chemical, Rockford, IL)

were added, and the samples were heated at 80°C for 2 h. Before the samples were cooled, 50 µl of *N,O*-bis(trimethylsilyl)trifluroacetamide with 1% trimethylchlorosilane (Pierce Chemical) were added, and the samples were incubated at 80°C for a further 15 min. By making the TMS derivatives of glucose and glycerol, the enrichment of both derivatives could then be measured from a single injection into the GC-MS. This was easily accomplished, because the elution times from the capillary column for the two compounds were 3.25 min for TMS-glycerol and 7.75 min for TMS-glucose.

Palmitate enrichments and concentrations were determined by making the TMS derivative of palmitate. Serum (100 µl) was deproteinized with addition of absolute ethanol and allowed to stand for 10 min. The samples were then centrifuged (10 min at 10,000 rpm), and the supernatant was removed and dried under dry N_2. The remaining residue was then resuspended in 2 ml of $CHCl_3$-methanol (2:1), and samples were shaken vigorously for 2 h. After the addition of 2 ml of 0.05 mM NaCl, the samples were again centrifuged (15 min at 8,000 rpm) to separate the aqueous and organic phases. The organic phase was carefully decanted into fresh test tubes, and ~250 µg of silic acid were added. Samples were spun (10 min at 10,000 rpm), and the supernatant was removed and dried under N_2. The dry residue was extracted twice more in $CHCl_3$-methanol (2:1). To the remaining residue, 50 µl of *N,O*-bis(trimethylsilyl)trifluroacetamide with 1% trimethylchlorosilane (Pierce Chemicals) and 50 µl of reagent-grade pyridine were added. Samples were then heated at 70°C for 15 min. TMS-palmitate (elution time = 3.6 min) was completely resolved from TMS-heptadecanoate (elution time = 4.3 min), which was used as the internal standard to determine palmitate concentration (37).

GC-MS

Enrichment of each derivative was measured by injection of 1 µl of each sample into a GC oven (model 5890, Hewlett-Packard, Fullerton, CA). The capillary column was a DB-5 fused silica capillary column (30 m × 0.32 mm ID, 0.25-µm film thickness; J & W Scientific, Folsom, CA). Mass analysis was performed using a VG Trio-2 mass spectrometer (VG Masslab, Cheshire, UK) operating in EI^+ mode. Data were processed and analyzed using MassLynx software (Fisons, Montreal, PQ, Canada).

Selected ion mass-to-charge ratio (*m/z*) was recorded at 205 and 208 atomic mass units (amu) and 205 and 207 amu (with an appropriate correction for enrichment at 206 amu) to determine glycerol and glucose enrichment, respectively. Selected ion monitoring of *m/z* 313 and 315 was used to measure palmitate enrichment. In addition, *m/z* 327 was monitored to determine the relative abundance of TMS-heptadecanoate.

Changes in enrichment for each of the tracers are shown in Fig. 1. For glucose, enrichment was stable at rest and increased during exercise because of the increase in infusion rate (Fig. 1*A*). Calculation of rate of glucose production (R_a) by use of a variety of pool sizes (250 and 50 ml/kg) had no significant effect on glucose R_a at any time during exercise. Glycerol enrichment, as with glucose, was constant before exercise and increased with the increase in infusion rate at the onset of exercise (Fig. 1*B*). Palmitate enrichment also increased at the onset of exercise in all training states and then remained relatively stable throughout the exercise period (Fig. 1*C*).

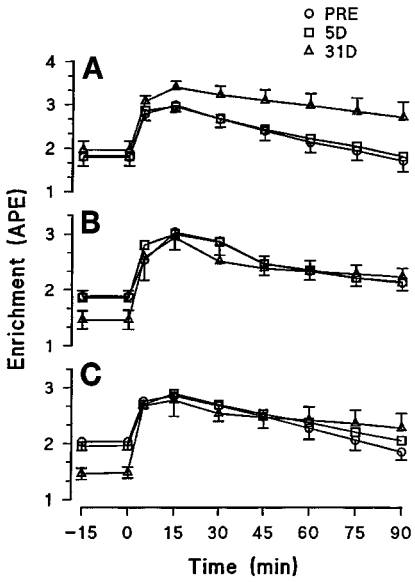

Fig. 1. Effect of exercise and training on normalized enrichment of plasma glucose (*A*), glycerol (*B*), and palmitate (*C*). Infusion rates were increased to 3 times resting for glucose and glycerol and 2 times resting for palmitate. Values are means ± SE (n = 7). PRE, pretraining; 5D, after 5 days of training; 31D, after 31 days of training; APE, atom percent excess.

Calculations

Indirect calorimetry. CHO and fat oxidation rates, as well as energy expenditure during exercise, were calculated using stoichiometric equations and appropriate caloric equivalents (10), with the assumption that the nitrogen excretion rate was 135 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (31). Errors in the estimate of nitrogen excretion of 30% (or greater) have very little effect on estimates of CHO and fat oxidation (31). Fatty acid oxidation was determined by converting triglyceride oxidation ($g^{-1} \cdot min^{-1}$) to the molar equivalent by assuming that the average molar mass of triglyceride is 860 g/mol (10) and multiplying the molar rate of triglyceride oxidation by three because each molecule contains three moles of fatty acids (31).

Isotope kinetics. Rates of appearance (R_a) and disappearance (R_d) of glucose, glycerol, and palmitate were calculated using the steady-state tracer dilution equation at rest (3). Because the enrichment and concentration change during exercise, it was necessary to calculate substrate R_a and R_d according to the equation of Steele (35). The Steele equation was modified, according to Romijn et al. (31), for use of stable isotopes, because the amount of tracer infused is no longer negligible (37).

The effective volumes of distribution were assumed to be 230 ml/kg for glycerol and 40 ml/kg for palmitate (31). The appropriate volume of distribution for glucose was assumed to be 100 ml/kg (6, 23, 31); however, R_a was also calculated using a volume of distribution for glucose of 50 and 250 ml/kg [the lower and upper possible boundaries for the glucose distribution space (37)]. The R_a of FFA was calculated by dividing the R_a of palmitate by the fractional contribution of palmitate to the total FFA concentration.

By using glycerol R_a as a reflection of lipolysis (25, 31), three times glycerol R_a should estimate total FFA release. If one assumes that all the FFA that are taken up from plasma during exercise are oxidized (17, 33), then the following series of equations described by Romijn et al. (31) yields the minimal rate of intramuscular fatty acid oxidation (*Eq. 1*), the rate of intramuscular lipolysis (*Eq. 2*), and the rate of peripheral (adipocyte) lipolysis (*Eq. 3*)

intramuscular fatty acid oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)

$$= \text{total fatty acid oxidation } (\mu mol \text{ FFA} \cdot kg^{-1} \cdot min^{-1}) - \text{FFA } R_d \quad (1)$$

If we assume that every three fatty acids released into the intramuscular pool results in the release of one glycerol, then

$$\text{intramuscular lipolysis } (\mu mol \cdot kg^{-1} \cdot min^{-1}) = \frac{\text{intramuscular fatty acid oxidation (from } Eq.\ 1; \mu mol \text{ FFA} \cdot kg^{-1} \cdot min^{-1})}{3\ \mu mol \text{ FFA}/\mu mol \text{ glycerol}} \quad (2)$$

Because the total rate of glycerol release is equal to the glycerol released from peripheral adipocytes and the intramuscular pool, then the rate of adipocyte lipolysis can be calculated as follows

peripheral lipolysis ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)

$$= \text{total glycerol } R_a - \text{intramuscular lipolysis } (\mu mol \cdot kg^{-1} \cdot min^{-1}) \quad (3)$$

It is also possible to estimate the minimal rate of muscle glycogen oxidation by use of the calculated rate of whole body CHO oxidation from O_2 consumption and CO_2 production and assuming that 100% of glucose R_d is oxidized during exercise (3, 4, 31). If <100% of plasma glucose uptake is oxidized, then the minimal rate of glycogen oxidation will be underestimated (31)

muscle glycogen oxidation

$$= \text{total carbohydrate oxidation} - \text{glucose } R_d \quad (4)$$

Using this method, one cannot account for the breakdown of glycogen that leads to lactate formation that is not completely oxidized. In addition, <100% of glucose R_d would be oxidized (3) and would be reflected in an underestimation of the calculated glycogen oxidation.

Statistical Analysis

Data were analyzed using a two-way repeated-measures analysis of variance. Where appropriate a one-way analysis of variance was used to determine the effect of training measurements that were not repeated during exercise. When a significant main effect was found, significant pairwise differences were performed using a Student-Newman-Keuls test. Significance was set at $P < 0.05$. Data are presented as means ± SE.

RESULTS

Resting Kinetics

Resting turnover of glycerol and FFA were calculated using enrichments at −15 and 0 min and the steady-state tracer dilution equation (3). Turnover of all substrates at rest was not affected at 5D. However, at 31D there were increases in the R_a of glycerol and FFA compared with both other times. This result was also reflected in a higher resting concentration of glycerol and FFA (Table 1). Compared with PRE, resting fat oxidation was increased ~11% at 31D.

Exercise

Lactate. Exercise resulted in increases in blood lactate concentration above resting at all training times (Fig. 2). Blood lactate concentrations were progressively reduced ($P < 0.05$) with increasing training duration at 5, 15, and 30 min. At 31D, blood lactate was lower ($P < 0.05$) than PRE at all times during the exercise bout (Fig. 2).

Glucose. Exercise resulted in an increase in glucose concentration at 5 min ($P < 0.05$) in all training conditions. This increase in glucose was transient, however, and the concentration at 15 min was not significantly different from the resting concentration regardless of training times (Fig. 3*A*). Blood glucose concentration was lower during exercise at 75 and 90 min ($P < 0.05$) during the PRE ride than at 5D and 31D at 60 min compared with 31D ($P < 0.05$). At 5D,

Table 1. *Effect of training on resting lipid kinetics*

	PRE	5D	31D
Glycerol, mM	0.062 ± 0.01	0.066 ± 0.01	0.076 ± 0.02*†
FFA, mM	0.57 ± 0.02	0.61 ± 0.02	0.66 ± 0.01*†
Glycerol R_a, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	1.7 ± 0.05	1.8 ± 0.05	2.7 ± 0.14*†
FFA R_a, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	4.7 ± 0.1	4.8 ± 0.1	6.8 ± 0.5*†
FFA R_a/glycerol R_a	2.8 ± 0.11	2.6 ± 0.07	2.5 ± 0.22
Fat oxidation, $\mu mol \cdot kg^{-1} \cdot min^{-1}$	4.7 ± 0.2	4.9 ± 0.3	5.2 ± 0.3*

Values are means ± SE ($n = 7$). FFA, free fatty acids; R_a, rate of appearance; R_d, rate of disappearance; PRE, pretraining; 5D, after 5 days of training; 31D, after 31 days of training. *Significantly different from PRE ($P < 0.05$). †Significantly different from 5D ($P < 0.05$).

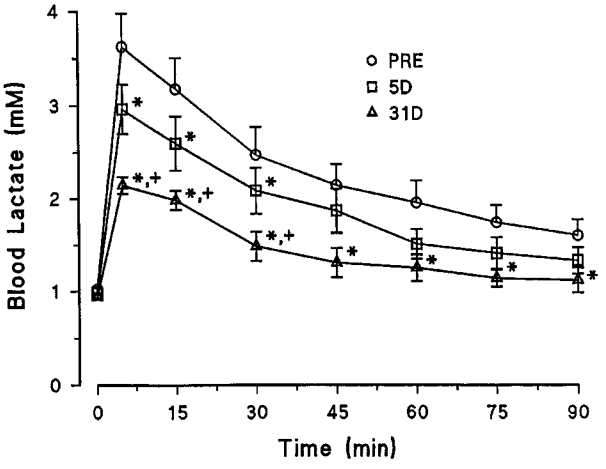

Fig. 2. Effects of exercise and training on blood lactate concentration. Values are means ± SE ($n = 7$). *Significantly different from PRE ($P < 0.05$). + Significantly different from 5D ($P < 0.05$).

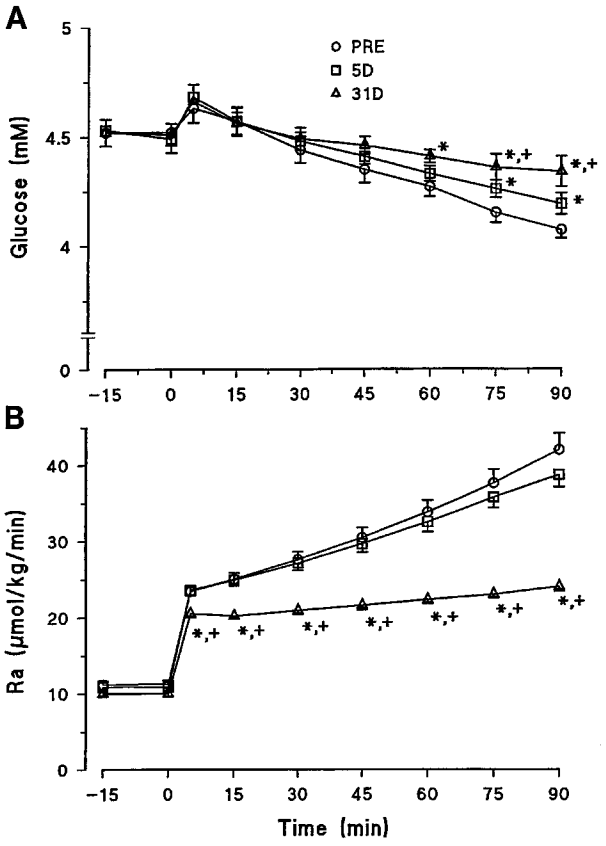

Fig. 3. Effect of exercise and training on blood glucose concentration (*A*) and glucose production (R_a, *B*). Values are means ± SE ($n = 7$). * Significantly different from PRE ($P < 0.05$). + Significantly different from 5D ($P < 0.05$).

compared with 31D, blood glucose was lower at 75 and 90 min of exercise (Fig. 3*A*). In contrast to 31D where no change in plasma glucose concentration occurred throughout the exercise period, glucose was depressed with exercise at 90 min at 5D compared with 31D. Comparable increases in glucose R_a (130 ± 2%) were observed in the rest-to-exercise transition for the PRE and 5D conditions (Fig. 3*B*). However, at 31D the increase in glucose R_a during the rest-to-exercise transition was reduced ($P < 0.01$). Glucose R_a rose more rapidly during PRE and at 5D than at 31D (Fig. 3*B*). The mean reduction in glucose R_a at 31D during exercise was ~30% compared with PRE and 5D. Glucose R_a was significantly reduced at all time points at 31D compared with PRE and 5D (Fig. 3).

Changes in glucose R_d paralleled those in glucose R_a. Mean glucose R_d during exercise was 31.8 ± 2.7 and 30.7 ± 2.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ for PRE and 5D, respectively ($P > 0.8$). At 31D, mean glucose R_d (21.7 ± 1.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was reduced ($P < 0.01$) at all exercise time points compared with PRE and 5D. The rate of irreversible clearance (metabolic clearance rate) of glucose was also attenuated at all time points during exercise after training at 31D.

Glycerol. Exercise resulted in increases ($P < 0.05$) in blood glycerol above resting levels at all training times (Fig. 4*A*). By 15 min, blood glycerol concentration was higher for the 31D condition. At 90 min, blood glycerol concentration increased to a greater extent during both the PRE and 5D rides (Fig. 4*A*). Glycerol R_a was elevated during exercise at 90 min in PRE and at 5D compared with 31D (Fig. 4*B*). The physiological significance of these small changes in glycerol concentration remains questionable.

FFA. FFA concentration decreased significantly at the onset of exercise in all training conditions but recovered to resting levels by 15 min at PRE and 5D and by 45 min at 31D (Fig. 5*A*). At PRE and 5D, FFA concentration was higher than at rest at all exercise times beyond 30 min (Fig. 5*A*). The 31D protocol resulted in a higher resting FFA concentration than observed in the other conditions. With exercise at 31D, FFA levels were not higher than those at rest regardless of the exercise duration. In addition, FFA concentration was higher at all times beyond 15 min at PRE and 5D than at 31D (Fig. 5*A*). Calculated FFA R_a was higher at 60, 75, and 90 min during PRE than at 31D. At 75 and 90 min of exercise, FFA R_a was higher at 5D than at 31D (Fig. 5*B*). However, FFA R_a was greater at 31D than at PRE and 5D at 15 min of exercise (Fig. 5*B*). FFA R_d was taken as a measure of plasma FFA oxidation. The fractional contribution of palmitate to the total FFA concentration was not affected by training or time ($P > 0.75$).

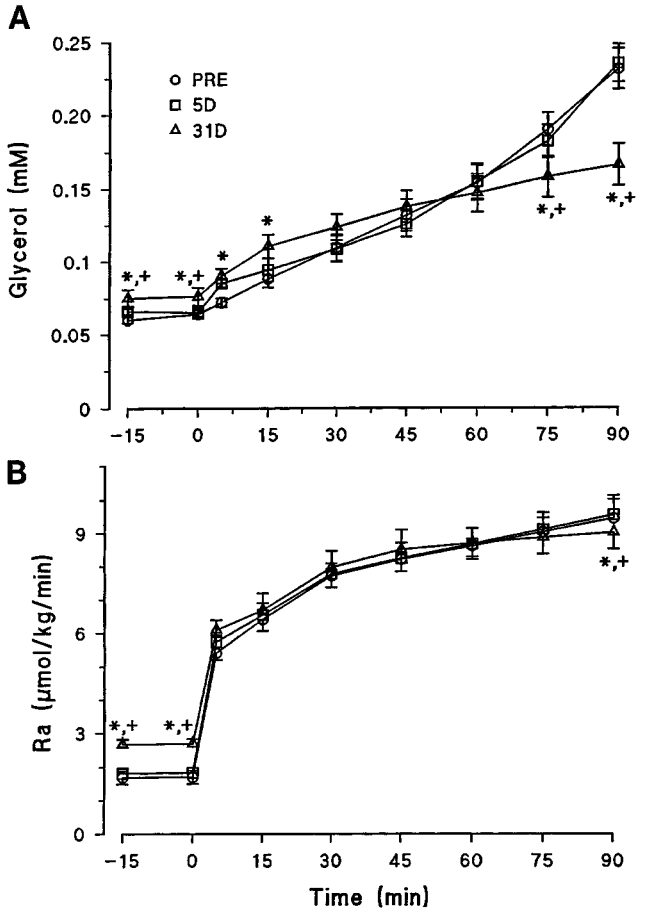

Fig. 4. Effect of exercise and training on blood glycerol concentration (*A*) and glycerol production (R_a, *B*). Values are means ± SE ($n = 7$). * Significantly different from PRE ($P < 0.05$). + Significantly different from 5D ($P < 0.05$).

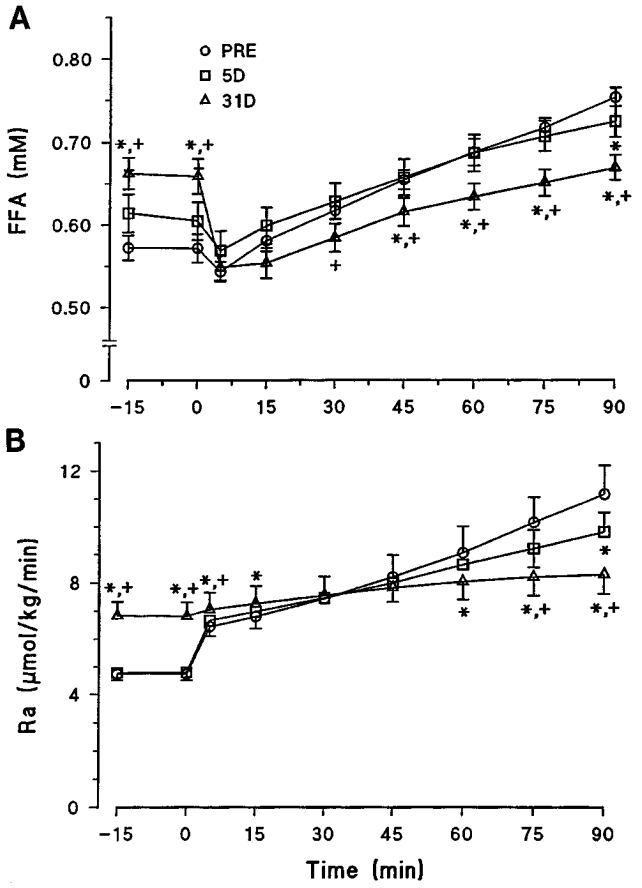

Fig. 5. Effect of exercise and training on serum free fatty acid (FFA) concentration (*A*) and FFA production (R_a, *B*). Values are means ± SE. *Significantly different from PRE ($P < 0.05$). ⁺Significantly different from 5D ($P < 0.05$).

Catecholamines. The plasma concentrations of epinephrine and norepinephrine during the exercise protocol are shown in Fig. 6. During exercise, plasma epinephrine concentration rose ~4.6-fold at PRE. The increase in epinephrine was attenuated at 5D (~3.9-fold) and attenuated further at 31D (~2.4-fold). Hence, epinephrine concentration was reduced ($P < 0.001$) at 5D at 60 and 90 min and was further reduced at 31D at all times. Plasma norepinephrine concentrations were increased during the exercise bout at all times compared with rest in all training conditions (Fig. 6*B*; $P < 0.001$). Norepinephrine concentrations were reduced at 60 and 90 min of exercise at 5D and were reduced further at 31D at all times during exercise (Fig. 6*B*). There was no effect of training on resting plasma epinephrine or norepinephrine concentrations (Fig. 6).

Lipolysis. Training resulted in an increased rate of intramuscular lipolysis at 31D at all times during exercise compared with all other conditions (Table 2). An increased rate of intramuscular lipolysis was also observed at 45 and 75 min of exercise at 5D compared with PRE (Table 2). Rates of peripheral lipolysis during exercise were greater ($P < 0.05$) at PRE and 5D at all times. Additionally, peripheral lipolysis was lower at 5D at 75 min than at PRE (Table 2).

Substrate oxidation. Total whole body CHO oxidation (glucose R_d + glycogen oxidation) was greater at PRE than at 5D ($P < 0.05$) and at 31D ($P < 0.001$). This was due to a mean decrease in the rate of glycogen oxidation at 5D compared with that at PRE, which was further potentiated at 31D (Table 3). Training also resulted in an increase in mean total fat oxidation at 5D compared with PRE ($P < 0.05$), with fat oxidation from IMTG forming a greater ($P < 0.05$) proportion of the total fat oxidation (Table 3). Mean total fat oxidation increased by ~70% at 31D compared with 5D. This increase was due predominantly to an increase in IMTG oxidation, which was greater at 31D at all time points during exercise than at PRE and 5D (Table 3).

DISCUSSION

The findings of this investigation show that training induces a time-dependent decrease in whole body CHO oxidation and an increase in whole body fat oxidation. These observations have been made previously (3, 18, 22, 23). However, we report here that training results in a shift from peripheral lipolysis and plasma FFA oxidation toward an increase in intramuscular lipolysis and IMTG oxidation during exercise. Confirming our earlier results (28) and those of others (3, 4, 6, 23), we also show that training resulted in a reduction in whole

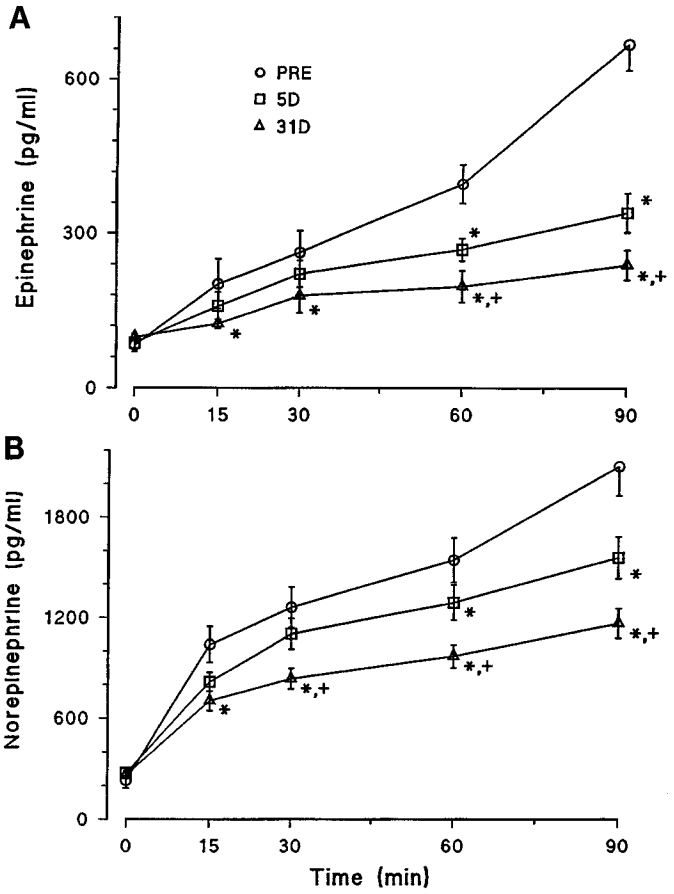

Fig. 6. Effect of exercise and training on plasma concentration of epinephrine (*A*) and norepinephrine (*B*). *Significantly different from PRE ($P < 0.05$). ⁺Significantly different from 5D ($P < 0.05$).

Table 2. *Effect of training on calculated rates of peripheral and intramuscular lipolysis during exercise*

	Time, min					
	15	30	45	60	75	90
Peripheral lipolysis, μmol·kg^{-1}·min^{-1}						
PRE	9.0 ± 0.3	10.8 ± 0.4	11.6 ± 0.3	11.9 ± 0.3	12.6 ± 0.5	12.6 ± 0.5
5D	9.0 ± 0.4	10.4 ± 0.3	10.9 ± 0.4	11.3 ± 0.5	11.8 ± 0.5*	12.2 ± 0.6
31D	6.8 ± 0.9*†	8.6 ± 0.9*†	8.8 ± 0.8*†	8.9 ± 0.9*†	9.0 ± 0.9*†	8.4 ± 0.9*†
Intramuscular lipolysis, μmol·kg^{-1}·min^{-1}						
PRE	0.6 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	1.0 ± 0.3	1.0 ± 0.4	1.5 ± 0.5
5D	0.8 ± 0.2	1.2 ± 0.2	1.5 ± 0.2*	1.7 ± 0.3	1.9 ± 0.3*	2.1 ± 0.6
31D	3.2 ± 0.6*†	3.3 ± 0.5*†	3.5 ± 0.4*†	4.1 ± 0.6*†	4.2 ± 0.5*†	5.1 ± 0.7*†

Values are means ± SE ($n = 7$). Estimates of peripheral and intramuscular lipolysis were made according to equations and assumptions outlined in METHODS. See Table 1 footnote for definition of abbreviations. * Significantly different from PRE ($P < 0.05$). † Significantly different from 5D ($P < 0.05$).

body glucose R_a and R_d. Inasmuch as glucose R_d reflects glucose oxidation, these data indicate that the rate of glucose oxidation was depressed with training.

Model Assumptions

Romijn and co-workers (31) described a series of calculations that allow the derivation of minimal rates of substrate oxidation and also the partitioning of whole body lipolysis into peripheral (adipocyte) lipolysis or intramuscular lipolysis. We have used the same equations and assumptions in the current study. Some discussion and evaluation of these assumptions, particularly as they apply to the current study, are warranted. The assumption that glycerol R_a reflects whole body lipolysis comes from the findings that glycerol is produced by no process other than lipolysis and that glycerol kinase is present in significant quantities only in the liver (25, 31). However, recent observations by Elia and co-workers (9) suggest that muscle may metabolize glycerol to an extent that makes the measurement of glycerol R_a unreliable as an index of lipolysis. It was acknowledged by the authors, however, that their results may have been due to a slow equilibration of enriched glycerol with the intramuscular pool of glycerol (9). Several other observations point to the fact that glycerol R_a is a reliable reflection of lipolysis. In the present study three times the rate of glycerol R_a, representing the highest rate of fat availability, was always greater than the calculated rate of fat oxidation. If a substantial portion of glycerol were metabolized in the muscle, as suggested by Elia and co-workers (9), then one would expect that glycerol R_a would consistently underestimate fat oxidation, which was not the case. Partial triglyceride hydrolysis would also result in an underestimation of lipolysis; however, the extent to which this occurs "in vivo" is unknown. Glycerol R_a has been shown to be responsive to a number of lipolytic agents such as epinephrine and fasting (36, 37). Furthermore, the results from this study and others (36, 37) indicate that the ratio of FFA to glycerol R_a (Table 1) is not significantly different from the theoretical value of 3 (36, 37). One would not expect to obtain a value of 3 for this ratio if there were a continual uptake and "utilization" of glycerol by muscle (9). From these observations, it would appear that glycerol R_a is a reliable reflection of the rate of lipolysis within adipose tissue and/or exercising muscle.

A somewhat tenuous assumption made by Romijn et al. (31) and by us was that 100% of FFA R_d is oxidized. A variety of studies have estimated FFA oxidation by measuring the enrichment (or specific activity) in breath after infusion of carbon isotope-labeled FFA (20, 22, 33, 34). However, retention of labeled CO_2 is complicated by shifts in bicarbonate pool buffering (22, 34) and isotopic exchange reactions within the tricarboxylic acid cycle (34). However, whole body estimates of FFA oxidation range from 50% (22) to ~77% (34) when complete label retention is taken into account. In

Table 3. *Effect of training on calculated minimal whole body rates of glycogen and estimated intramuscular triglyceride oxidation*

	Time, min					
	15	30	45	60	75	90
Glycogen oxidation, μmol·kg^{-1}·min^{-1}						
PRE	121.3 ± 12.8	115.5 ± 11.0	110.1 ± 10.4	102.7 ± 9.5	95.8 ± 8.0	84.0 ± 8.5
5D	120.6 ± 11.4	109.8 ± 11.5	101.2 ± 9.8	96.3 ± 9.3	91.3 ± 10.7	79.8 ± 9.4
31D	93.8 ± 8.6	85.1 ± 6.5	80.7 ± 6.7	73.6 ± 5.1	67.7 ± 5.5	57.8 ± 3.9
IMTG oxidation, μmol·kg^{-1}·min^{-1}						
PRE	1.9 ± 0.3	2.3 ± 0.5	2.2 ± 0.6	2.9 ± 0.7	2.9 ± 0.9	4.5 ± 0.8
5D	2.4 ± 0.5	3.6 ± 0.5	4.4 ± 0.6*	5.0 ± 0.9	5.6 ± 0.8*	6.3 ± 0.7
31D	9.5 ± 1.6*†	9.9 ± 1.5*†	10.4 ± 1.3*†	12.2 ± 1.7*†	12.7 ± 1.6*†	15.2 ± 2.0*†

Values are means ± SE ($n = 7$). Estimates of minimal rates of oxidation were made according to equations and assumptions outlined in METHODS. IMTG, intramuscular triglyceride; see Table 1 footnote for definition of other abbreviations. * Significantly different from PRE ($P < 0.05$). † Significantly different from 5D ($P < 0.05$). Main effects for time and training were observed for glycogen oxidation ($P < 0.001$).

addition, the fractional extraction and oxidation (as a percentage of overall flux) are unchanged after training (20, 22). The result is that the percentage of FFA oxidized is the same regardless of training status. Hence, it appears that the current estimate of the amount of FFA oxidized is an overestimate and consequently results in an underestimation of the contribution of triglyceride oxidation from other sources. We and others (31) have labeled these other sources of triglyceride oxidation as intramuscular. It is possible that there may have been a contribution of other triglyceride stores that we did not take into account. However, we believe that, because the exercising legs account for >80% of the overall release of CO_2 during prolonged cycling, it is unlikely that oxidation of triglycerides from "other" sources would have been significant (17, 20, 22).

Fat Turnover and Oxidation

The increase in IMTG oxidation with training that we have estimated here has been shown previously using measures of IMTG stores (19) and by measuring muscle FFA uptake (20). Our findings support those of Martin et al. (22), who showed that training resulted in a reduction in plasma FFA R_a and R_d during exercise. Hence, the increase in fat oxidation induced by training would appear, by difference, to have originated from intramuscular sources (22). However, it appears that IMTG oxidation is most prominent late in exercise. In addition, independent confirmation of this finding comes from muscle tissue samples obtained during the current study, in which we measured IMTG concentrations before and after exercise and found that training resulted in greater depletion (oxidation) of IMTG at 31D (29). This response may also have been mediated by the increase in resting concentration of IMTG that we observed with training (29).

The training-induced reduction in plasma FFA R_a that occurred late in exercise was, not surprisingly, accompanied by a corresponding drop in the calculated rate of peripheral lipolysis. As in earlier studies (3, 23), we observed that training resulted in a reduction in the circulating levels of norepinephrine and epinephrine during exercise. Presumably, the reduction in both of these hormones, which are potent stimulators of lipolysis (30, 36, 38), is linked to the training-induced reduction in peripheral lipolysis and plasma FFA R_a that we and others have reported (22). However, given the reduction in adrenergic drive after training, it is paradoxical how training results in an increase in IMTG oxidation and yet reduces peripheral lipolysis during exercise (22). The enzyme thought to be responsible for IMTG hydrolysis, hormone-sensitive lipase, is responsive to adrenergic stimulation via increased adenosine 3′,5′-cyclic monophosphate (26). Although training has been shown to result in an increase in lipoprotein lipase activity, evidence for a direct effect of training on hormone-sensitive lipase activity is lacking (26).

Despite the fact that total fat oxidation increased after training, we observed a small reduction in the amount of total lipolysis during the exercise. This finding may seem somewhat inconsistent with the notion of increased fat oxidation after training. However, the highest rate of FFA availability from lipolysis (i.e., 3 times glycerol R_a) that we observed throughout exercise, at all training time points, was always greater than the rate of total fat oxidation. This indicates that lipolysis was not rate limiting at any time point during exercise and, hence, in these subjects was in excess of that required. This finding suggests that factors other than FFA availability, such as the enzymatic capacity to transport and oxidize fat, in the mitochondria (24, 25) may limit the rate of fat oxidation in untrained and even moderately trained individuals. Support for this finding comes from the observation of Romijn and colleagues (31), who showed that at ~65% $\dot{V}O_{2peak}$ highly trained cyclists ($\dot{V}O_{2peak}$ > 60 $ml \cdot kg^{-1} \cdot min^{-1}$) were able to sustain rates of fat oxidation of >40 $\mu mol \cdot kg^{-1} \cdot min^{-1}$.

Our finding of an increase in resting lipid kinetics and triglyceride recycling after training confirms the results of Romijn et al. (32), who showed that trained athletes had greater rates of lipolysis and triglyceride cycling at rest than their sedentary counterparts. Similar observations have been made after training in elderly persons (30). The mechanism behind this increase in resting lipid kinetics is somewhat obscure. The present results were not likely influenced by weight loss (as a result of food restriction), because the subjects did not lose weight during the course of the study. Additionally, analysis of dietary records showed that, whereas subjects increased their total caloric intake after the initiation of training, the macronutrient composition of their diets, which has been shown to affect whole body fuel metabolism (30, 32), did not change (data not shown). We did not observe any training-induced changes in resting catecholamine concentration, in contrast to the results of Poehlman et al. (30), who showed that the increased resting fat oxidation after training was associated with an increase in norepinephrine concentration and R_a. The mechanism(s) of an increase in resting lipid kinetics awaits further investigation.

CHO Turnover and Oxidation

Evidence supporting a reduced CHO oxidation after training comes from the finding of a reduction in glycogen depletion (cf. Ref. 18). In addition, training has been shown to result in a reduction in glucose R_d after as little as 10 days of training (23, 28) and also with longer training programs (3, 4, 6). Moreover, many studies have reported that training results in reductions in RER, which directly supports a decrease in CHO oxidation and an increased oxidation of fat (18). The reduction in glucose R_d that occurs with training has recently been shown to be due, for the most part, to a reduction in hepatic glycogenolysis (6). The measured rates of hepatic gluconeogensis were also reduced after training (6). The present results show that at 5D there was a small decrease in total CHO oxidation and a corresponding increase in fat oxidation. This was likely

a result of simultaneous reductions in glucose R_d and muscle glycogen oxidation.

We (28) and others (6, 23) have previously commented on the inability of training-induced hormonal changes in the concentrations of epinephrine, insulin, and glucagon to explain the reduction of glucose R_a with training. Because there is a hierarchy of hormones that regulate hepatic glycogenolysis and glucose metabolism (7), with epinephrine, insulin, and glucagon being the prime mediators, it would be expected that changes in some or all of these hormones might somehow mediate the reduction in glucose R_a (3, 4, 6, 23, 28). The changes we observed in the plasma epinephrine concentration could explain some of the reduction in glucose R_a at 31D; however, the differences in R_a occurred even when the blood concentrations of epinephrine were not significantly different between the training times. There is general agreement that hepatic glycogenolysis and gluconeogenesis are also autoregulated (6, 21) and respond to changes in glucose utilization, despite feedforward signals that might stimulate both processes (6, 21). On the basis of current findings, it is possible that a reduction in glucose R_d after training acted as a feedback signal to the liver and reduced hepatic glycogenolysis, although what mediates the training-induced reduction in glucose R_d is unclear (5, 23, 28). In this study we did not measure the concentrations of insulin or glucagon; however, these hormones have been measured before and after training by others (6, 23), and changes in these hormones are also unable to explain changes in glucose R_a (6, 23, 28).

We report here that endurance training resulted in a greater increase in fat oxidation during moderate-intensity prolonged exercise. This increase in fat oxidation was progressive for training duration, with small increases observed as early as 5 days of training. Oxidation of fats from intramuscular stores appears to be an increasingly important source of fat in the trained state, particularly late in exercise. However, oxidation of plasma FFA was increased in the trained state early in the exercise. We have also shown that training resulted in an expected reduction in CHO oxidation. This reduction was small early in the training protocol but was markedly increased with prolonged training. The reduction in CHO oxidation was due for the most part to reductions in muscle glycogen oxidation; however, decreases in plasma glucose turnover could account for ~32% of the overall reduction in CHO oxidation after prolonged training.

The authors thank George Obminsky for assistance in administering the laboratory protocols, Jeff Pickersgill for expert assistance in the gas chromatography-mass spectrometry analysis, and S. Fraser for capable contribution to the preparation of the manuscript.

This work was supported by the Natural Science and Research Council and the Medical Research Council of Canada. G. J. F. Heigenhauser is a career investigator with the Heart and Stroke Foundation (Ontario, Canada).

Address for reprint requests: H. J. Green, Dept. of Kinesiology, University of Waterloo, Waterloo, ON N2L 3G1, Canada (E-mail: green@healthy.uwaterloo.ca).

Received 19 March 1996; accepted in final form 16 July 1996.

REFERENCES

1. **Brooks, G. A., and J. Mercier.** Balance of carbohydrate and lipid utilization during exercise: the "crossover concept." *J. Appl. Physiol.* 76: 2253–2261, 1994.
2. **Cadefau, J., H. J. Green, R. Cussó, M. Ball-Burnett, and G. Jamieson.** Coupling of muscle phosphorylation potential to glycolysis during submaximal exercise of varying intensity following short-term training. *J. Appl. Physiol.* 76: 2586–2593, 1994.
3. **Coggan, A. R., W. M. Kohrt, R. J. Spina, D. M. Bier, and J. O. Holloszy.** Endurance training decreases plasma glucose turnover and oxidation during moderate-intensity exercise in men. *J. Appl. Physiol.* 68: 990–996, 1990.
4. **Coggan, A. R., C. A. Raguso, B. D. Williams, L. S. Sidossis, and A. Gastaldelli.** Glucose kinetics during high-intensity exercise in endurance trained and untrained humans. *J. Appl. Physiol.* 78: 1203–1207, 1995.
5. **Coggan, A. R., R. J. Spina, W. M. Kohrt, D. M. Bier, and J. O. Holloszy.** Effect of prolonged exercise on muscle citrate concentration before and after endurance training in men. *Am. J. Physiol.* 264 (*Endocrinol. Metab.* 27): E215–E220, 1993.
6. **Coggan, A. R., S. C. Swanson, L. A. Mendenhall, D. L. Habash, and C. L. Kien.** Effect of endurance training on hepatic glycogenolysis and gluconeogenesis during prolonged exercise in men. *Am. J. Physiol.* 268 (*Endocrinol. Metab.* 31): E375–E383, 1995.
7. **Cryer, P. E.** Glucose counterregulation: prevention and correction for hypoglycemia in humans. *Am. J. Physiol.* 264 (*Endocrinol. Metab.* 27): E149–E155, 1993.
8. **Da Silva, G. A., A. El-Manshawi, G. J. F. Heigenhauser, and N. L. Jones.** Measurement of mixed venous carbon dioxide pressure by rebreathing during exercise. *Respir. Physiol.* 59: 379–392, 1985.
9. **Elia, M., K. Kahn, G. Calder, and A. Kurpad.** Glycerol exchange across the human forearm assessed by a combination of tracer and arteriovenous exchange techniques. *Clin. Sci. Lond.* 84: 99–104, 1993.
10. **Frayn, K. N.** Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* 55: 628–634, 1983.
11. **Galbo, H.** Exercise physiology: humoral function. *Sport Sci. Rev.* 1: 65–93, 1992.
12. **Green, H., R. Helyar, M. Ball-Burnett, N. Kowalchuk, S. Symon, and B. Farrance.** Metabolic adaptations to training precede changes in muscle mitochondrial capacity. *J. Appl. Physiol.* 72: 484–491, 1992.
13. **Green, H. J., L. L. Jones, M. E. Houston, M. E. Ball-Burnett, and B. W. Farrance.** Muscle energetics during prolonged cycling after exercise hypervolemia. *J. Appl. Physiol.* 66: 622–631, 1989.
14. **Green, H. J., S. Jones, M. Ball-Burnett, B. Farrance, and D. Ranney.** Adaptations in muscle metabolism to prolonged exercise and training. *J. Appl. Physiol.* 78: 138–145, 1995.
15. **Green, H. J., S. Jones, M. Ball-Burnett, and I. Fraser.** Early adaptations in blood substrates, metabolites, and hormones to prolonged exercise training in man. *Can. J. Physiol. Pharmacol.* 69: 1222–1229, 1991.
16. **Green, H. J., S. Jones, M. E. Ball-Burnett, D. Smith, J. Livesey, and B. W. Farrance.** Early muscular and metabolic adaptations to prolonged exercise training in man. *J. Appl. Physiol.* 70: 2032–2038, 1991.
17. **Havel, R. J., B. Pernow, and N. L. Jones.** Uptake and release of free fatty acids and other metabolites in the legs of exercising men. *J. Appl. Physiol.* 23: 90–99, 1967.
18. **Holloszy, J. O., and E. F. Coyle.** Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. *J. Appl. Physiol.* 56: 831–838, 1984.
19. **Hurley, B. F., P. M. Nemeth, W. H. Martin III, J. M. Hagberg, and G. P. Dalsky.** Muscle triglyceride utilization during exercise: effect of training. *J. Appl. Physiol.* 60: 562–567, 1986.
20. **Jansson, E., and L. Kaijser.** Substrate utilization and enzymes in skeletal muscle of extremely endurance-trained men. *J. Appl. Physiol.* 62: 999–1005, 1987.
21. **Jenssen, T. N. Nurjhan, A. Consoli, and J. E. Gerich.** Failure of substrate-induced gluconeogenesis to increase overall glucose appearance in normal humans. Demonstration of hepatic auto-

regulation without a change in plasma glucose concentration. *J. Clin. Invest.* 86: 489–497, 1990.

22. **Martin, W. H., III, G. P. Dalsky, B. F. Hurley, D. E. Mathews, D. M. Bier, J. M. Hagberg, M. A. Rogers, D. S. King, and J. O. Holloszy.** Effect of endurance training on free fatty acid turnover and oxidation during exercise. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E708–E714, 1994.
23. **Mendenhall, L. A., S. C. Swanson, D. L. Hobosh, and A. R. Coggan.** Ten days of exercise training reduces glucose production and utilization during moderate-intensity exercise. *Am. J. Physiol.* 266 (*Endocrinol. Metab.* 29): E136–E143, 1994.
24. **Molé, P. A., L. B. Oscai, and J. O. Holloszy.** Adaptation of muscle to exercise. Increase in levels of palmitoyl CoA synthetase, carnitine palmitoyltransferase, and palmitoyl CoA dehydrogenase, and in the capacity to oxidize fatty acids. *J. Clin. Invest.* 50: 2323–2330, 1971.
25. **Newsholme, E. A., and K. Taylor.** Glycerol kinase activities in muscles from vertebrates and invertebrates. *Biochem. J.* 112: 465–474, 1969.
26. **Oscai, L. B., D. A. Essig, and W. K. Palmer.** Lipase regulation of muscle triglyceride hydrolysis. *J. Appl. Physiol.* 69: 1571–1577, 1990.
27. **Phillips, S. M., H. J. Green, M. J. MacDonald, and R. L. Hughson.** Progressive effect of endurance training on $\dot{V}O_2$ kinetics at the onset of submaximal exercise. *J. Appl. Physiol.* 79: 1914–1920, 1995.
28. **Phillips, S. M., H. J. Green, M. A. Tarnopolsky, and S. M. Grant.** Decreased glucose turnover following short-term training is unaccompanied by changes in muscle oxidation potential. *Am. J. Physiol.* 269 (*Endocrinol. Metab.* 32): E222–E230, 1995.
29. **Phillips, S. M., H. J. Green, M. A. Tarnopolsky, G. J. F. Heigenhauser, and S. M. Grant.** Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. *Am. J. Physiol.* 270 (*Endocrinol. Metab.* 33): E265–E272, 1996.
30. **Poehlman, E. T., A. W. Gardner, P. J. Arciero, M. I. Goran, and J. Calles-Escandon.** Effects of endurance training on total fat oxidation in elderly persons. *J. Appl. Physiol.* 76: 2281–2287, 1994.
31. **Romijn, J. A., E. F. Coyle, L. S. Sidossis, A. Gastaldelli, J. F. Horowitz, E. Endert, and R. R. Wolfe.** Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E380–E391, 1993.
32. **Romijn, J. A., S. Klein, E. F. Coyle, L. S. Sidossis, and R. R. Wolfe.** Strenuous endurance training increases lipolysis and triglyceride-fatty acid cycling at rest. *J. Appl. Physiol.* 75: 108–113, 1993.
33. **Sidossis, L. S., A. R. Coggan, A. Gastaldelli, and R. R. Wolfe.** Pathway of plasma FFA oxidation in human subjects: implications for tracer studies. *J. Clin. Invest.* 95: 278–284, 1995.
34. **Sidossis, L. S., A. R. Coggan, A. Gastaldelli, and R. R. Wolfe.** A new correction factor for use in tracer estimations of plasma fatty acid oxidation. *Am. J. Physiol.* 269 (*Endocrinol. Metab.* 32): E649–E656, 1995.
35. **Steele, R.** Influence of glucose loading and injected insulin on hepatic glucose output. *Ann. NY Acad. Sci.* 82: 420–430, 1959.
36. **Townsend, R. R., S. Klein, and R. R. Rolfe.** Changes in lipolytic sensitivity following repeated epinephrine infusion in humans. *Am. J. Physiol.* 266 (*Endocrinol. Metab.* 29): E155–E160, 1994.
37. **Wolfe, R. R.** *Radioactive and Stable Isotope Tracers in Biomedicine.* New York: Wiley-Liss, 1992, p. 119–164.
38. **Wolfe, R. R., E. J. Peters, S. Klein, O. B. Holland, J. Rosenblatt, and H. Gary.** Effect of short-term fasting on lipolytic responsiveness in normal and obese human subjects. *Am. J. Physiol.* 252 (*Endocrinol. Metab.* 15): E189–E196, 1987.