# Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration

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**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.—Stable isotope tracers and indirect calorimetry were used to evaluate the regulation of endogenous fat and glucose metabolism in relation to exercise intensity and duration. Five trained subjects were studied during exercise intensities of 25, 65, and 85% of maximal oxygen consumption ($\dot{V}O_{2\,max}$). Plasma glucose tissue uptake and muscle glycogen oxidation increased in relation to exercise intensity. In contrast, peripheral lipolysis was stimulated maximally at the lowest exercise intensity, and fatty acid release into plasma decreased with increasing exercise intensity. Muscle triglyceride lipolysis was stimulated only at higher intensities. During 2 h of exercise at 65% $\dot{V}O_{2\,max}$ plasma-derived substrate oxidation progressively increased over time, whereas muscle glycogen and triglyceride oxidation decreased. In recovery from high-intensity exercise, although the rate of lipolysis immediately decreased, the rate of release of fatty acids into plasma increased, indicating release of fatty acids from previously hydrolyzed triglycerides. We conclude that, whereas carbohydrate availability is regulated directly in relation to exercise intensity, the regulation of lipid metabolism seems to be more complex.

mass spectrometry; stable isotopes; free fatty acids; muscle triglycerides; muscle glycogen

NUMEROUS STUDIES have addressed substrate metabolism during exercise, but the relation between exercise intensity and substrate mobilization from different endogenous stores has not been fully elucidated (27). Jones et al. (21) provided evidence that the rate of appearance of free fatty acids (FFA) is lower during moderate exercise intensity [70% of maximal $O_2$ consumption ($\dot{V}O_{2\,max}$)] than during low-intensity exercise (36% of $\dot{V}O_{2\,max}$; see Ref. 21). This paradoxical response in view of energy requirements may be related to alterations in adipose tissue lipolysis. However, whole body lipolytic rates have been measured only during low-intensity exercise (16, 37), and the effect of exercise intensity on adipose tissue lipolysis is unknown. It is also possible that lipolysis is stimulated during high-intensity exercise, but FFA are not released into the circulation because of either increased reesterification or entrapment within the adipocytes. Either of these possibilities could follow from vasoconstriction and thus deficient flow of albumin, which is necessary to bind to FFA for transport (2, 15, 28). Presently, however, the involvement of these mechanisms in the regulation of the release of FFA from the adipose tissue during different exercise intensities is unknown.

Fat oxidized by working muscle is derived not only from plasma FFA but also from muscle triglycerides. Utilization of muscle triglycerides may account for a considerable portion of energy requirements, especially in trained subjects (19, 20), but the rate of utilization of muscle triglycerides has not been quantified at different exercise intensities.

The aim of the present study was to determine the quantitative effects of exercise intensity and duration on endogenous FFA mobilization and utilization. We hypothesized that lipolysis within adipocytes is stimulated maximally during very-low-intensity exercise, since peripheral lipolysis is very sensitive to minimal sympathetic stimulation (4), whereas lipolysis of triglycerides within muscle and thus muscle triglyceride oxidation may be stimulated at higher exercise intensities. To put the data of FFA metabolism into the perspective of overall energy substrate metabolism, whole body carbohydrate metabolism was evaluated simultaneously.

Central to achieving the aim of the present study, it was necessary to quantify fat and carbohydrate oxidation during high-intensity exercise. Indirect calorimetry has previously been thought to be inaccurate due to excessive $CO_2$ production. Therefore, in a recent study, we described a new tracer method to quantify carbohydrate and fat oxidation rates independently from $CO_2$ production rates (26). Using this method, we validated indirect calorimetry during exercise intensities of 80–85% of $\dot{V}O_{2\,max}$ as a means for quantifying substrate oxidation rates in trained subjects. Because glucose and FFA taken up from the plasma (measured by the primed continuous infusion of [6,6-$^2H_2$]glucose and [$^2H_2$]palmitate) can be assumed to be completely oxidized during exercise (6, 17), the minimal contribution of muscle triglycerides and glycogen to energy production is represented by the difference between whole body substrate oxidation and plasma glucose and FFA uptake. From whole body lipolytic rates (measured by the primed continuous infusion of [$^2H_5$]glycerol) and the lipolytic rate of muscle triglycerides (derived from the rate of muscle triglyceride oxidation), the rate of lipolysis within the adipose tissue (peripheral lipolysis) is obtained. Thus this new approach allows for the first time the quantification of all essential aspects of lipid and carbohydrate kinetics and oxidation. We have applied these approaches in five trained subjects during three exercise intensities (25, 65, and 85% of $\dot{V}O_{2\,max}$).

## METHODS

### *Subjects*

Five endurance-trained cyclists (age 24 ± 2 yr, weight 75.2 ± 3.6 kg, height 1.78 ± 0.03 m, $\dot{V}O_{2\,max}$ 67 ± 3 ml·kg$^{-1}$·min$^{-1}$) volunteered for this study. All subjects were healthy, as indicated by medical history and physical examination. They were studied in the postabsorptive state after a 10- to 12-h fast. $\dot{V}O_{2\,max}$ was determined several weeks before the experimental trials while the subjects cycled on a stationary ergometer (Monark 819). $\dot{V}O_{2\,max}$ was determined during an incremental cycling protocol lasting 7–10 min. The study was approved by the institutional review boards of the University of Texas, Galveston and Austin.

### *Exercise Protocol*

The subjects were studied on three consecutive days in the postabsorptive state. On each day a different exercise intensity was performed (25, 65, or 85% of $\dot{V}O_{2\,max}$); the order of the intensities was randomized. On each occasion, stable isotopes were infused, and indirect calorimetry was used to determine oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) during a basal period of 2 h and during either 30 min (85% of $\dot{V}O_{2\,max}$) or 120 min of exercise (25 and 65% of $\dot{V}O_{2\,max}$) on a stationary ergometer (Monark 819). Immediately after exercise, the subjects lay down on a bed while the infusion of stable isotopes was continued for 1 h during recovery. Immediately after recovery from exercise, carbohydrate repletion was started by having the subjects drink a solution of cornstarch at frequent intervals. The amount of carbohydrate ingested each day (300–400 g) was approximately two times the maximal amount of glycogen oxidized during exercise.

### *Indirect Calorimetry*

Indirect calorimetry was performed at rest for at least 15 min continuously throughout the first 30 min of exercise (all 3 intensities of exercise) and for 5- to 10-min intervals every 15 min during the remainder of exercise (during exercise at 25 and 65% of $\dot{V}O_{2\,max}$). The values obtained from 20 to 30 min of exercise were used to calculate and compare substrate oxidation rates among the three levels of exercise because of the apparent steady state at that time. Values taken at 15-min intervals thereafter were used to assess the effect of exercise duration on substrate oxidation. The resting values were obtained after the subjects had been laying on a bed for at least 1 h. Inspired volumes of air were measured with a dry gas meter (Parkinson-Cowan CD-4). Aliquots of expired gas were sampled from a mixing chamber and analyzed for $O_2$ (Applied Electrochemistry S3A) and $CO_2$ (Beckman LB-2). Analog outputs from the gas analyzers and gas meter were directed to a laboratory computer for calculation of $\dot{V}O_2$ and $\dot{V}CO_2$.

### *Isotope Infusion*

Teflon catheters were placed percutaneously into an antecubital vein, and a sampling catheter was inserted in a dorsal hand vein of the contralateral arm. The heated hand technique was used to obtain arterialized blood samples (23). The subjects lay on a bed for 1 h after catheter placement. After a blood sample was drawn to determine background enrichment, primed constant infusions of [6,6-$^2H_2$]glucose (99% enriched; Merck, Rahway, NJ; 0.22 μmol·kg$^{-1}$·min$^{-1}$; prime 17.6 μmol/kg), [$^2H_5$]-glycerol (0.1 μmol·kg$^{-1}$·min$^{-1}$; prime 1.5 μmol/kg), and [$^2H_2$]palmitate (0.04 μmol·kg$^{-1}$·min$^{-1}$; no prime) were started using calibrated syringe pumps (Harvard Apparatus, Natick, MA). The exact infusion rate in each experiment was determined by measuring the concentrations in the infusates. The glycerol and palmitate tracers (both 99% enriched) were purchased from Tracer Technologies (Newton, MA). Palmitate was bound to albumin (Cutter Biological, Berkeley, CA) by following previously described procedures (36). After 2 h of infusion, with the subjects at rest, exercise was initiated, and the rate of isotope administration was doubled for palmitate and glucose and tripled for glycerol to minimize changes in substrate isotopic enrichment. During exercise at 85% of $\dot{V}O_{2\,max}$ the rate of infusion of [6,6-$^2H_2$]glucose was doubled and, after the first 10 min, tripled, compared with the infusion rate at rest. The isotope infusion was continued at resting rates during the 1st h of recovery to determine substrate kinetics.

### *Blood Sampling*

Blood was taken 105, 110, 115, and 120 min after the beginning of infusion to measure resting kinetics. During exercise at intensities of 25 and 65% of $\dot{V}O_{2\,max}$, blood was taken after 5, 15, 30, 45, 60, 75, 90, 105, and 120 min of exercise. During 30 min of exercise at 85% of $\dot{V}O_{2\,max}$, blood was drawn every 5 min. In all experiments, blood was drawn after 5, 15, 30, 45, and 60 min of recovery from exercise. All samples were placed in 10-ml vacutainers containing lithium heparin and placed on ice. Plasma was separated by centrifugation shortly after sampling and subsequently frozen until further processing.

### *Sample Analysis*

Plasma glucose concentration was measured using a glucose analyzer (Beckman Instruments) by use of the glucose oxidase method. The enrichment of [6,6-$^2H_2$]glucose in plasma was determined as previously described (35). Briefly, plasma was deproteinated with barium hydroxide and zinc sulfate solutions. Glucose was extracted by mixed-bed anion-cation exchange chromatography (AG-1-X8 and AG 50W-X8; Bio-Rad Laboratories, Richmond, CA) and reacted with acetic anhydride and pyridine to form the penta-acetate derivative. Isotopic enrichment was determined by gas chromatography-mass spectrometry (GC-MS model 5985B; Hewlett-Packard, Fullerton, CA) using electronic impact ionization, selectively monitoring ions at mass-to-charge ratio (*m/e*) 202, 201, and 200. Correction was made for the contribution of singly labeled molecules (*m/e* 201) to the apparent enrichment at *m/e* 202 (35).

FFA were extracted from plasma, isolated by thin-layer chromatography, and derivatized to their methyl esters. Palmitate and total FFA concentrations were determined by gas chromatography (Hewlett-Packard 5890) using heptadecanoic acid as an internal standard. Isotopic enrichment of palmitate was measured by GC-MS analysis of the methyl ester derivatives on a Hewlett-Packard 5992 (36). Ions of *m/e* 270 and 272 were selectively monitored. Isotopic enrichment of glycerol was determined by GC-MS (Hewlett-Packard 5985B) by following previously described procedures (38). Ions of *m/e* 205 and 208 were monitored. Plasma catecholamine concentrations were measured as previously described (11).

### *Calculations*

*Indirect calorimetry.* Carbohydrate and fat oxidation rates were calculated using stoichiometric equations (14). Nitrogen excretion rate was assumed to be 135 μg·kg$^{-1}$·min$^{-1}$. This average value was taken from the measured values determined in another study performed in our laboratory (3). A 30% error in this assumed value (which exceeds the total range of values in the previous study) would have no significant effect on the calculated values of fat and carbohydrate oxidation during exercise in the present study. Fatty acid oxidation was determined by converting the rate of triglyceride oxidation (g·kg$^{-1}$·min$^{-1}$) to its molar equivalent, assuming the average molecular weight

of triglyceride to be 860 g/mol (14) and multiplying the molar rate of triglyceride oxidation by three because each molecule contains three moles of fatty acids.

Rates of appearance ($R_a$) and, when appropriate, rates of disappearance ($R_d$ = tissue uptake) of glucose, glycerol, and palmitate at rest were calculated using the equation of Steele (33). This equation had to be modified for use of stable isotope since the amount of tracer infused is no longer negligible. The concentration (C) of the tracee at time ($t$) can be calculated from the measured concentration ($C_m$) and the enrichment (E; tracer-to-tracee ratio) at that time

$$C = C_m/(1 + E)$$

Therefore the working form of the Steele equation becomes

$$R_a = \frac{F - V_d \frac{C_m}{1+E}\frac{dE}{dt}}{E}$$

and

$$R_d = R_a - V_d \frac{\frac{dC_m}{dt}(1+E) - C_m\frac{dE}{dt}}{(1+E)^2}$$

where F is the infusion rate and $V_d$ is the effective volume of distribution. Values for $R_a$ were calculated at 20–30 min for comparisons among the three exercise intensities and at 15-min intervals thereafter for evaluation of the duration effect. During exercise and the first 30 min of recovery, kinetics were calculated as described above after first fitting enrichment and concentration data to curves using spline fitting (39). The effective $V_d$ was assumed to be 230 ml/kg for glycerol and 40 ml/kg for palmitate. The value for palmitate was chosen because acute changes in palmitate concentration are essentially restricted to plasma (being bound to albumin). The appropriate value for glucose is less clear, so data were computed for $V_d$ = 40, 100, and 165 ml/kg. The limitations of the Steele equation have been discussed extensively, but in situations in which changes in enrichment are small the value chosen for $V_d$ becomes inconsequential and thus the values calculated are reliable (35). 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, as determined by gas chromatography.

Glycerol $R_a$ was used as a reflection of lipolysis, with the assumption that all glycerol released in the process of lipolysis, whether in the adipose tissue or in muscle tissue, appears in the plasma. This is because glycerol reutilization requires phosphorylation via the enzyme glycerol kinase, and this occurs mainly in the liver (9, 24). Also, glycerol cannot be produced in the body, other than from lipolysis (38). Consequently, total FFA release equals three times glycerol $R_a$.

Given the rate of total lipolysis and total FFA release into plasma (and uptake) and the rate of total fat oxidation, it is possible to quantitatively distinguish lipolysis in adipose tissue (peripheral lipolysis) and intramuscular lipolysis. If all FFA taken up from the plasma during exercise can be assumed to be oxidized (17), then the following equation provides a minimal rate of intramuscular fatty acid oxidation

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

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

For every three fatty acids released from the intramuscular triglyceride pool, one glycerol will be released into plasma. Consequently, the minimum rate of release of glycerol from the intramuscular triglyceride pool (intramuscular lipolysis) will be calculated as follows

intramuscular lipolysis ($\mu$mol·kg$^{-1}$·min$^{-1}$)

$$= \frac{\text{intramuscular fatty acid oxidation } (\mu\text{mol FFA}\cdot\text{kg}^{-1}\cdot\text{min}^{-1})}{3\ \mu\text{mol FFA}/\mu\text{mol glycerol}}$$

The total rate of glycerol release is equal to the glycerol released from peripheral adipocytes and glycerol released from the intramuscular pool. Consequently, it is possible to calculate the rate of adipocyte (peripheral) lipolysis as follows

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

= total glycerol $R_a$ − intramuscular lipolysis ($\mu$mol·kg$^{-1}$·min$^{-1}$)

From the total rate of carbohydrate oxidation, obtained by indirect calorimetry, and the rate of tissue glucose uptake, reflected by glucose $R_d$, the minimum contribution of muscle glycogen stores to carbohydrate oxidation can be calculated assuming that 100% of plasma glucose uptake is oxidized during exercise (6). If <100% is oxidized, the calculated value for intramuscular glycogen oxidation will be underestimated. Therefore, the following equation provides the minimal rate of muscle glycogen oxidation

muscle glycogen oxidation

= total carbohydrate oxidation − glucose $R_d$

Thus, by this method, any glycogen breakdown that leads to lactate production rather than complete oxidation will not be included in the calculation of rate of oxidation. Glycogen breakdown therefore exceeds the calculated rate of glycogen oxidation by a percentage amount equal to the percent of plasma glucose converted to lactate, since the metabolic pathways of glycogen and glucose converge at glucose 6-phosphate and are the same thereafter.

### *Statistical Analysis*

The results are presented as means ± SE. The effect of time on the response within each exercise level was analyzed by a repeated-measures two-way analysis of variance (treatment × time). If necessary, the times were compared by Fishers least-significant difference test. The 30-min results of the three exercise intensities were compared by two-way analysis of variance for randomized block design, where the subjects are blocks and the three exercise levels are treatments. If necessary, the analysis of variance was followed by a multiple-comparison test to detect differences among groups. Statistical significance was set at $P < 0.05$.

## RESULTS

### *Basal State*

There were no differences in plasma concentrations of substrates and hormones, rates of appearance, or rates of carbohydrate and FFA oxidation at rest before the three intensities of exercise.

### *Exercise*

*Plasma concentrations.* After 30 min of exercise, there was a clear relationship between exercise intensity and plasma glucose concentrations. Plasma glucose concentrations were 77 ± 4, 98 ± 6, and 147 ± 1 mg/dl after 30 min of exercise at 25, 65, and 85% of $\dot{V}O_{2\,max}$, respectively ($P < 0.05$ for all values). During the subsequent 90 min of exercise (25 and 65% of $\dot{V}O_{2\,max}$), plasma glucose concentrations did not change (25% of $\dot{V}O_{2\,max}$) or decreased to

preexercise levels (70 ± 3 mg/dl; 65% of $\dot{V}O_{2\,max}$).

Plasma glycerol and FFA concentrations are shown in Fig. 1. Plasma glycerol concentrations increased during all exercise intensities. Glycerol concentrations after 30 min of exercise at 65 and 85% of $\dot{V}O_{2\,max}$ were significantly higher than those obtained during low-intensity exercise ($P < 0.05$). Glycerol concentrations were significantly higher after 120 min of exercise at 65% compared with exercise at 25% of $\dot{V}O_{2\,max}$ ($P < 0.01$).

Plasma FFA concentrations showed a progressive increase during exercise of 25% of $\dot{V}O_{2\,max}$ (rest: 0.86 ± 0.12; 30 min: 0.97 ± 0.18; 120 min: 1.17 mmol/l; $P < 0.02$). During 65% of $\dot{V}O_{2\,max}$, there was an initial decrease (rest: 0.86 ± 0.14; 5 min: 0.67 ± 0.13 mmol/l; $P < 0.05$), followed by a steady increase in plasma FFA concentrations (30 min: 0.90 ± 0.14; 120 min: 1.12 ± 0.12 mmol/l; not significant vs. 25% of $\dot{V}O_{2\,max}$). During exercise of 85% of $\dot{V}O_{2\,max}$, however, FFA levels were persistently decreased (rest: 0.99 ± 0.05; 5 min: 0.631 ± 0.06; 30 min: 0.52 ± 0.08 mmol/l; $P < 0.05$). In recovery from the high-intensity exercise, FFA concentrations transiently increased. Plasma lactate concentrations remained essentially at the resting concentration during exercise at 25% $\dot{V}O_{2\,max}$. Lactate concentration increased from the resting values of 1.04 ± 0.08 to 2.27 ± 0.29 mmol/l at 65% $\dot{V}O_{2\,max}$, and during exercise at 85% $\dot{V}O_{2\,max}$ the lactate concentration was 7.9 ± 0.9 mmol/l at 10 min and 9.0 ± 0.5 mmol/l at 30 min.

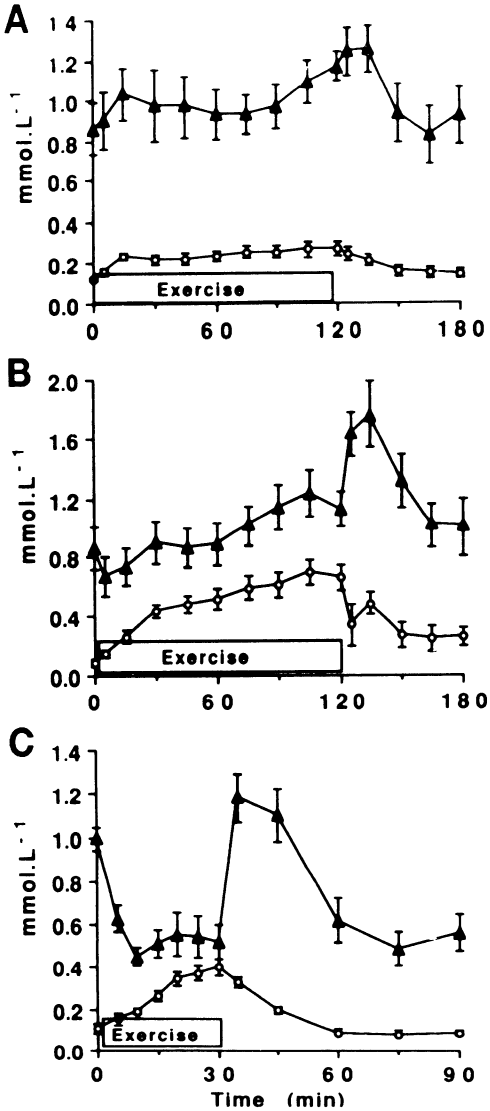


Fig. 1. Glycerol (○) and free fatty acid (FFA; ▲) concentrations (means ± SE) during exercise at 25% (*A*), 65% (*B*), and 85% (*C*) of maximal $O_2$ consumption ($\dot{V}O_{2\,max}$) and subsequent recovery in 5 trained subjects.

*Substrate Kinetics*

The infusion rates of the stable isotopes were increased at the start of exercise to minimize the effect of non-steady-state conditions of isotopic enrichment on the calculation of substrate kinetics (37). Because we did not know in advance the precise effects of exercise intensity on substrate kinetics (i.e., the goal of the study), we were only partially successful in maintaining isotopic steady state (Figs. 2–4). However, the enrichments around the 30 min of exercise were relatively stable for all tracers (Figs. 2–4), and it was at this time point that the three exercise intensities were compared. Consequently, the use of different pool fractions, the major confounding variable in the calculation of non-steady-state kinetics (35), did not appreciably alter the calculated $R_a$ for any substrate.

*Glucose.* The $R_a$ and $R_d$ of glucose increased significantly ($P < 0.02$) during all three exercise intensities roughly in proportion to exercise intensity (Fig. 5). The use of different pool fractions (40, 100, 165 ml/kg) had minimal effect on the calculated $R_a/R_d$ values of glucose, owing to the near-steady-state isotopic enrichment. The values with a pool fraction of 100 ml/kg were used in the calculations shown in Fig. 5.

*FFA.* FFA $R_a$ increased at the start of exercise and then remained stable during the 120 min of exercise at 25% $\dot{V}O_{2\,max}$, while increasing progressively throughout exercise at 65% $\dot{V}O_{2\,max}$ (Fig. 6). In contrast, FFA $R_a$ during exercise at 85% of $\dot{V}O_{2\,max}$ did not increase above resting values, despite the stimulation of lipolysis, as reflected by an increasing glycerol $R_a$ (Fig. 6). As a result, there was an inverse relationship between exercise intensity and FFA $R_a$ after 30 min of exercise (Table 1). During the 2nd h of exercise at 25 and 65% of $\dot{V}O_{2\,max}$, there was no significant difference in FFA $R_a$, although the average increase in FFA $R_a$ was slightly higher during exercise at 65% of $\dot{V}O_{2\,max}$ (Fig. 6).

Within 5 min of recovery from low and moderate exercise, FFA $R_a$ decreased. In contrast, immediately after stopping high-intensity exercise, there was a significant but transient increase in FFA $R_a$ compared with the values obtained at the end of exercise ($P < 0.02$; Fig. 6).

*Glycerol.* Glycerol $R_a$ increased significantly above resting levels during exercise at all three intensities ($P < 0.05$; Fig. 6). After 30 min of exercise, glycerol $R_a$ was significantly higher during exercise at 65 and 85% of $\dot{V}O_{2\,max}$ compared with low-intensity exercise. During recovery, there was an immediate decrease in glycerol $R_a$. This occurred also during recovery after high-intensity exercise (85% of $\dot{V}O_{2\,max}$), indicating that the transient increase in FFA $R_a$ immediately after exercise was not explained by an increase in lipolysis (Fig. 6). There was a

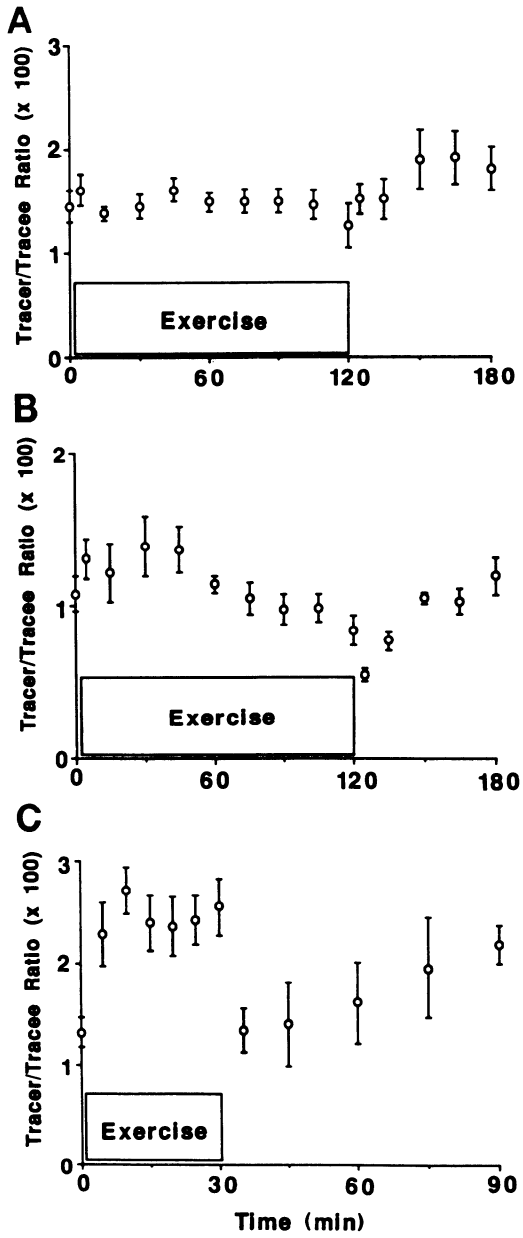


Fig. 2. Tracer-to-tracee ratios (means ± SE) of palmitate during infusion of [$^2H_2$]palmitate, measured at rest, during 3 levels of exercise [25% (*A*), 65% (*B*), and 85% (*C*) of $\dot{V}O_{2\,max}$], and during 1st h of recovery. Each value represents means ± SE of 5 trained subjects. Infusion rates during exercise were increased 2-fold above resting infusion rate and returned to resting infusion rate during recovery period.

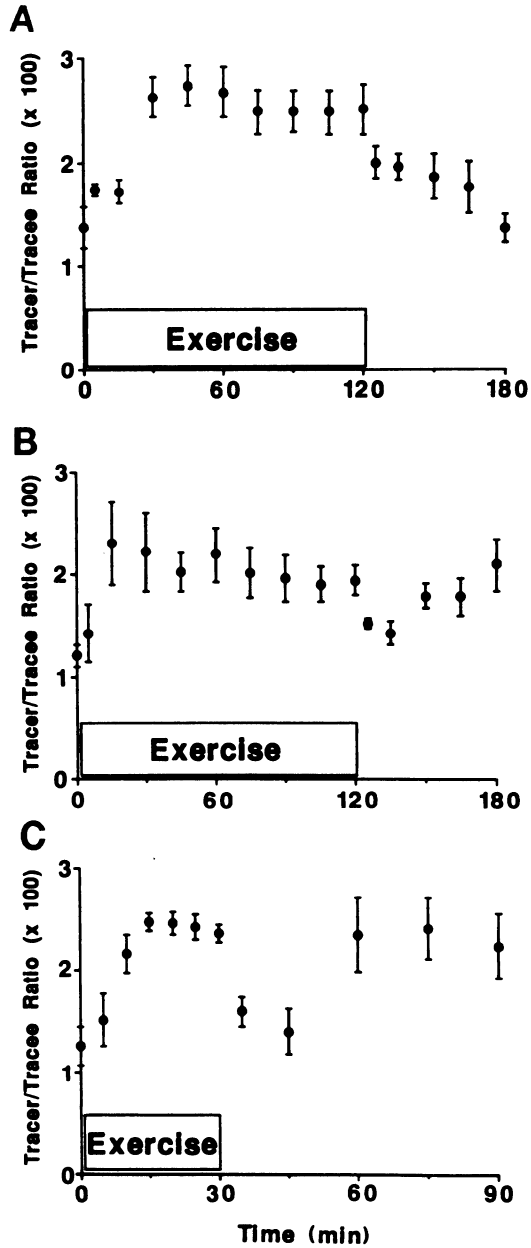


Fig. 3. Tracer-to-tracee ratios of glycerol during infusion of [$^2H_5$]glycerol, measured at rest, during 3 levels of exercise [25% (*A*), 65% (*B*), and 85% (*C*) of $\dot{V}O_{2\,max}$], and during 1st h of recovery. Each value represents means ± SE of 5 trained subjects. Infusion rates during exercise were increased 3-fold above resting infusion rate and returned to resting infusion rate during recovery period.

transient and significant increase in the ratio FFA $R_a$/glycerol $R_a$ from the end of exercise to the early phase of recovery (25% of $\dot{V}O_{2\,max}$ from 2.3 ± 0.1 at 2 h of exercise to 4.2 ± 0.5 in recovery; 65% of $\dot{V}O_{2\,max}$ from 2.3 ± 0.2 at 2 h of exercise to 5.1 ± 0.6 in recovery; 85% of $\dot{V}O_{2\,max}$ from 1.3 ± 0.2 at 30 min of exercise to 5.0 ± 1.4 in recovery).

The peripheral lipolytic response after 30 min of exercise was not significantly different at the three intensities (Table 1). In contrast, intramuscular lipolysis increased significantly at 65 and 85% compared with 25% $\dot{V}O_{2\,max}$. This was in accordance with a significant decrease in the ratio of FFA $R_a$/glycerol $R_a$ from 2.4 ± 0.1 (25% of $\dot{V}O_{2\,max}$) and 1.7 ± 0.1 (65% of $\dot{V}O_{2\,max}$) to 1.3 ± 0.3 (85% of $\dot{V}O_{2\,max}$; $P < 0.05$), because FFA derived from muscle triglycerides can be assumed to be oxidized without having passed through the plasma.

### *Substrate Oxidation Rates*

From 20 to 30 min of exercise, inspiratory volumes, $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory exchange ratios were constant in the three exercise protocols. The respiratory exchange ratios were significantly different from each other at 0.73 ± 0.01, 0.83 ± 0.02, and 0.91 ± 0.01 (25, 65, and 85% of $\dot{V}O_{2\,max}$, respectively; $P < 0.02$).

Carbohydrate oxidation rates calculated during 20–30 min of exercise at 25, 65, and 85% of $\dot{V}O_{2\,max}$ increased significantly with exercise intensity (Fig. 7). The values

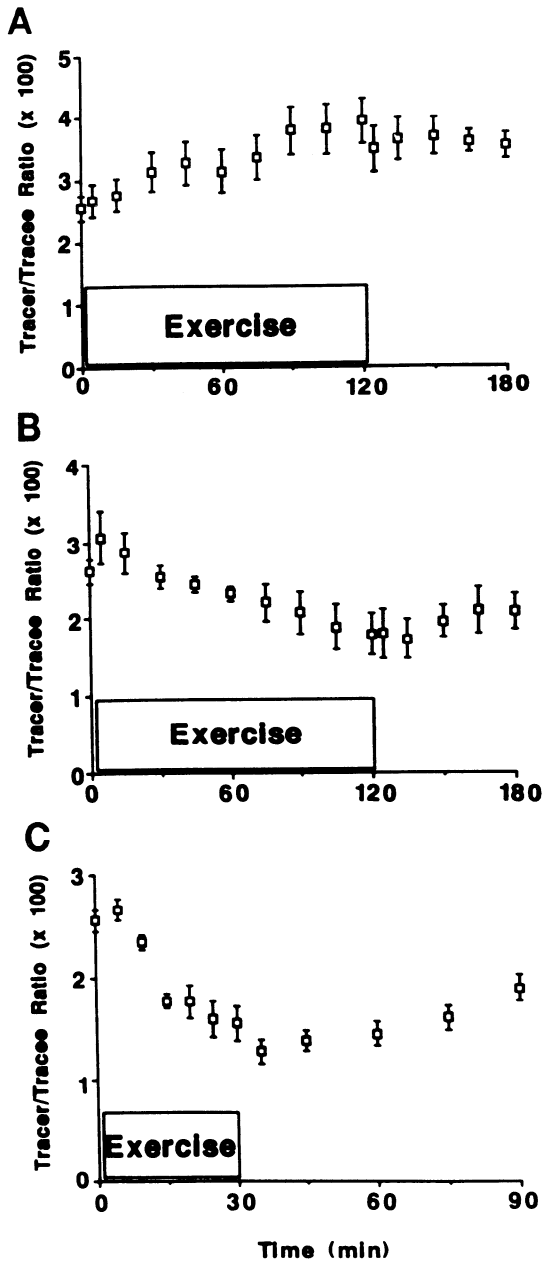


Fig. 4. Tracer-to-tracee ratios of glucose during infusion of [6,6-$^2H_2$]-glucose, measured at rest, during 3 levels of exercise [25% (*A*), 65% (*B*), and 85% (*C*) of $\dot{V}O_{2\,max}$], and during 1st h of recovery. Each value represents mean ± SE of 5 trained subjects. Infusion rates were increased 2-fold above resting rate with start of exercise and 3-fold during 10- to 30-min period at 85% $\dot{V}O_{2\,max}$. During recovery, infusion rate was returned to resting levels.

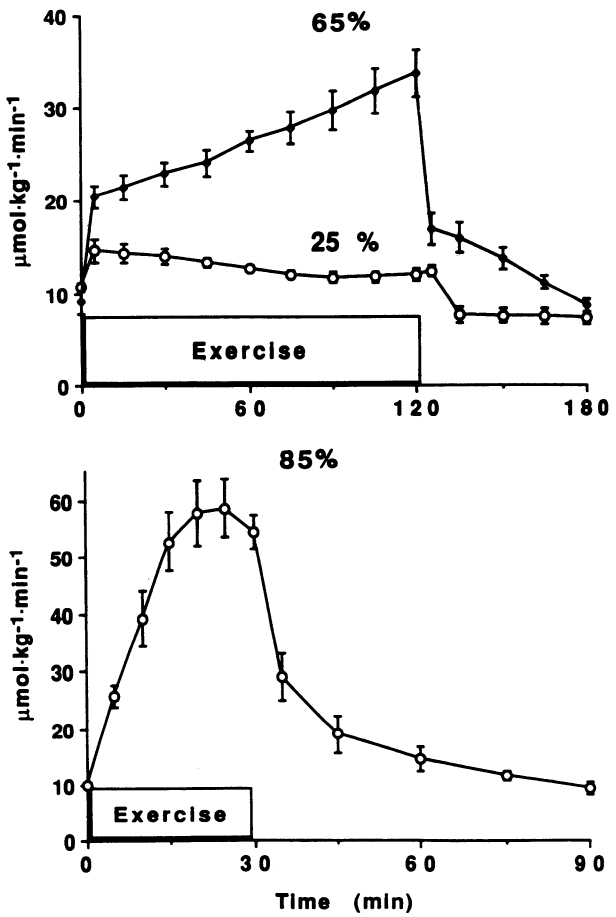


Fig. 5. Rates of appearance of glucose (means ± SE) during exercise at 25, 65, and 85% of $\dot{V}O_{2\,max}$ and subsequent recovery in 5 trained subjects calculated by non-steady-state equations with pool fraction of 100 ml/kg. Values obtained during different exercise intensities were significantly different ($P < 0.05$).

of total carbohydrate oxidation at 65 and 85% of $\dot{V}O_{2\,max}$ were significantly higher than the rate of glucose tissue uptake (the maximal amount of plasma glucose available for oxidation). This difference reflects the minimal contribution of muscle glycogen to carbohydrate oxidation, which also increased significantly with increasing exercise intensity (Fig. 7).

Fat oxidation rates were related differently to exercise intensity than carbohydrate oxidation rates (Fig. 7). Fat oxidation after 30 min of exercise was not different at 25 vs. 85% $\dot{V}O_{2\,max}$, despite a more than threefold increase in energy expenditure. At 65% of $\dot{V}O_{2\,max}$, fat oxidation rate was significantly higher than observed during exercise at 25 and 85% $\dot{V}O_{2\,max}$ (Fig. 7). During exercise at 65 and 85% $\dot{V}O_2$, whole body fat oxidation was significantly higher than tissue FFA uptake, with the difference representing the minimal contribution of muscle triglyceride stores to whole body fat oxidation (Fig. 7).

Figure 8 shows the maximal contributions of plasma glucose and plasma FFA and the minimal contributions of muscle triglycerides and muscle glycogen stores to energy expenditure after 30 min of exercise at 25, 65, and 85% of $\dot{V}O_{2\,max}$. The increase in caloric equivalents from glucose tissue uptake with increasing exercise intensities is largely counterbalanced by the relative decrease in plasma FFA uptake. As a consequence, the contribution of plasma substrates to overall energy production remained essentially constant at the different levels of exercise, meaning that, at higher exercise intensities, muscle substrate stores (particularly glycogen) become predominant (Fig. 8).

The effect of the duration of exercise on the metabolic responses was evaluated during exercise at 25 and 65% $\dot{V}O_{2\,max}$. At 25% $\dot{V}O_{2\,max}$, there was no significant alteration in the relative contribution of the various substrates to energy production over the 2-h exercise period (Fig. 9). On the other hand, during exercise at 65% $\dot{V}O_{2\,max}$, there was a progressive increase on the reliance on plasma FFA

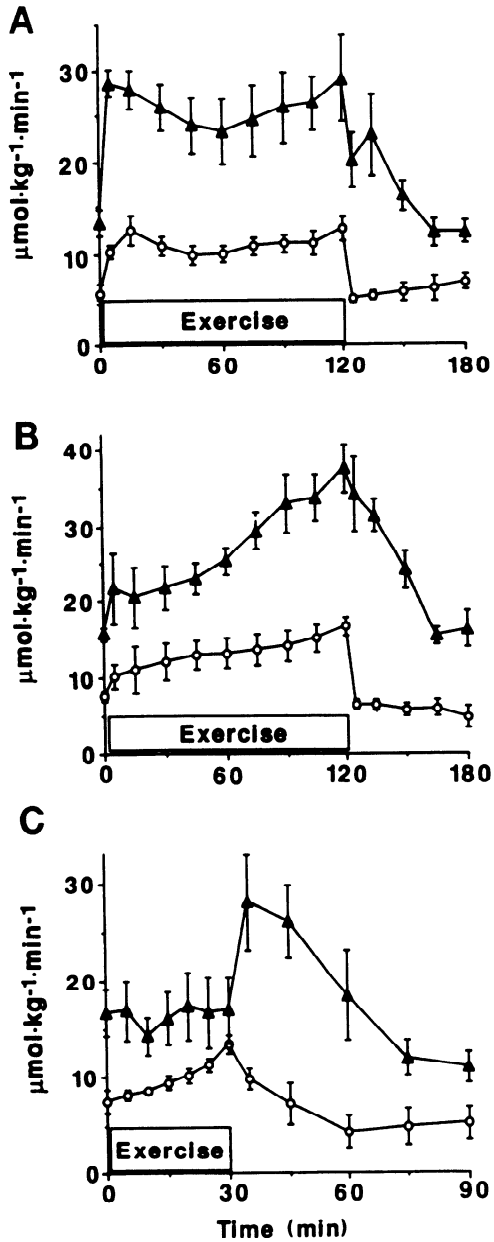


Fig. 6. Rates of appearance of glycerol (○) and FFA (▲; means ± SE) during exercise at 25% (*A*), 65% (*B*), and 85% (*C*) of $\dot{V}O_{2\,max}$ and subsequent recovery in 5 trained subjects. FFA rate of appearance ($R_a$) and glycerol $R_a$ increased during exercise ($P < 0.02$), with exception of high-intensity exercise, which revealed no change in FFA $R_a$. During recovery from high-intensity exercise, there was a transient increase of ~60% in FFA $R_a$ ($P < 0.01$), whereas glycerol $R_a$ decreased.

Table 1. *Effect of exercise intensity on fat metabolism, measured after 30 min of exercise in five endurance-trained athletes*

| Exercise Intensity, % of $\dot{V}O_{2\,max}$ | Glycerol $R_a$ | | | Plasma FFA $R_a$ | Total Fat Oxidation |
|---|---|---|---|---|---|
| | Whole body | Peripheral | Intramuscular | | |
| 25 | 10.9±1.0 | 9.8±1.6 | 1.1±0.7 | 25.8±2.6 | 26.8±1.5 |
| 65 | 13.6±1.9 | 6.9±0.6 | 6.7±1.2* | 22.8±2.7* | 42.8±3.7* |
| 85 | 13.4±0.9 | 8.5±2.5 | 4.9±1.9* | 17.0±3.4*† | 29.6±4.3† |

Values are means ± SE in $\mu mol \cdot kg^{-1} \cdot min^{-1}$. $R_a$, rate of appearance. * $P < 0.05$ vs. exercise at 25% of maximal $O_2$ consumption ($\dot{V}O_{2\,max}$). † $P < 0.05$ vs. exercise at 65% of $\dot{V}O_{2\,max}$.

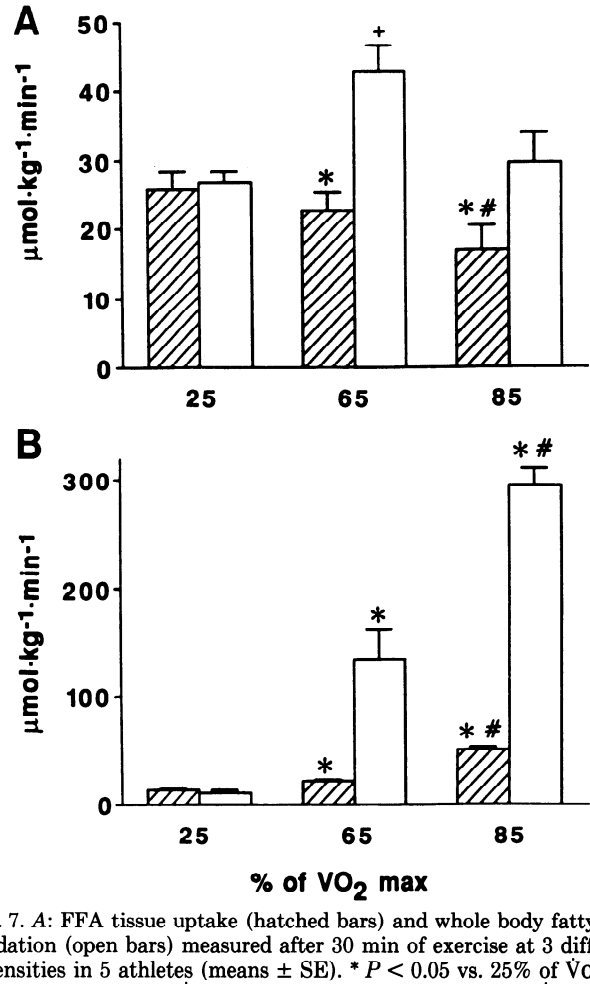


Fig. 7. *A*: FFA tissue uptake (hatched bars) and whole body fatty acid oxidation (open bars) measured after 30 min of exercise at 3 different intensities in 5 athletes (means ± SE). * $P < 0.05$ vs. 25% of $\dot{V}O_{2\,max}$; # $P < 0.05$ vs. 65% of $\dot{V}O_{2\,max}$; † $P < 0.05$ vs. 25 and 85% of $\dot{V}O_{2\,max}$. *B*: glucose tissue uptake (hatched bars) and whole body carbohydrate oxidation (open bars) measured after 30 min of exercise at 3 different intensities in 5 athletes (means ± SE). * $P < 0.05$ vs. 25% of $\dot{V}O_{2\,max}$; # $P < 0.05$ vs. 65% of $\dot{V}O_{2\,max}$.

and glucose as energy substrates. As a consequence the contribution of muscle substrate stores to energy production decreased (Fig. 9).

### *Plasma Catecholamine Concentrations*

After 30 min of low-intensity exercise, there was a slight but significant increase in plasma epinephrine and norepinephrine concentrations (Table 2). Plasma catecholamine concentrations increased in relation to exercise intensity. After 60 min of recovery the values were not significantly different from preexercise values.

## DISCUSSION

Although numerous studies have addressed the mobilization and utilization of carbohydrate at different exercise intensities, little quantitative data are available regarding lipid kinetics. In this study, we have used an isotopic tracer approach to quantify aspects of lipid kinetics not previously studied in exercise, including the distinction between lipolysis occurring in adipocytes (peripheral lipolysis) and intramuscular lipolysis. Peripheral

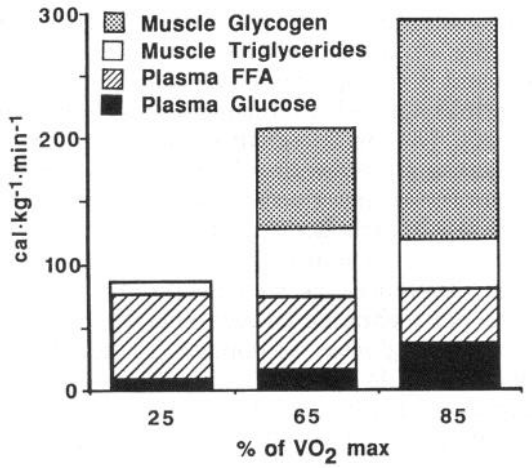


Fig. 8. Maximal contribution to energy expenditure derived from glucose and FFA taken up from blood and minimal contribution of muscle triglyceride and glycogen stores after 30 min of exercise, expressed as function of exercise intensity. Total amount of calories (cal) available from plasma does not change in relation to exercise intensity.

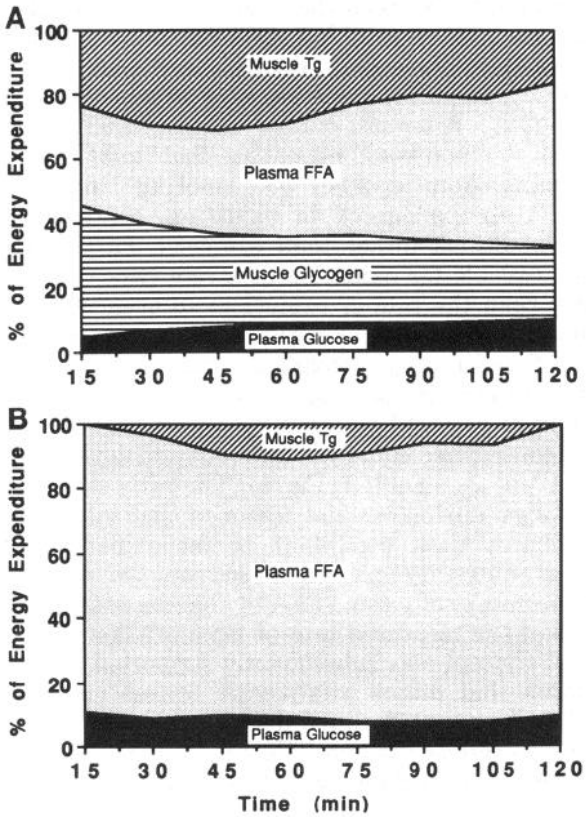


Fig. 9. Relative contribution of blood-borne and intramuscular substrates to energy production during 120 min of exercise at 25% $\dot{V}O_{2\,max}$ (*B*) and 65% (*A*) $\dot{V}O_{2\,max}$. Tg, triglycerides.

lipolysis was high during low-intensity exercise and did not increase further with more intense exercise. However, little lipolysis of muscle triglycerides occurred during low-intensity exercise (i.e., 25% $\dot{V}O_{2\,max}$) compared with exercise at the higher intensities. Despite high lipolytic rates, the $R_a$ of plasma FFA, and presumably oxidation, was highest during exercise at 25% $\dot{V}O_{2\,max}$, and FFA $R_a$ declined progressively as the exercise intensity increased to 65 and 85% $\dot{V}O_{2\,max}$. The progressive decline in plasma

Table 2. *Plasma concentrations of catecholamines at rest, during exercise, and after 60 min of recovery*

| Exercise Intensity, % $\dot{V}O_{2\,max}$ | Rest | Exercise, min | | Recovery (60 min) |
|---|---|---|---|---|
| | | 30 | 120 | |
| | | *Epinephrine* | | |
| 25 | 38±6 | 59±3* | 52±8* | 36±4 |
| 65 | 58±17 | 199±18*† | 239±28*† | 62±17 |
| 85 | 36±7 | 625±86*‡ | | 44±8 |
| | | *Norepinephrine* | | |
| 25 | 231±43 | 388±54* | 372±41* | 223±27 |
| 65 | 291±31 | 1,694±237*† | 1,851±286*† | 424±130 |
| 85 | 266±19 | 5,085±914*‡ | | 293±52 |

Values are means ± SE in mg/l. * $P < 0.05$ vs. rest. † $P < 0.05$ vs. exercise at 25% of $\dot{V}O_{2\,max}$. ‡ $P < 0.01$ vs. exercise at 25 and 65% of $\dot{V}O_{2\,max}$.

FFA turnover with increasing exercise intensity was offset by progressive increases in blood glucose turnover. Therefore the contribution of plasma substrates to caloric expenditure remained essentially constant over this wide range of exercise intensities. The changes in lipid metabolism occurred concurrently with changes in carbohydrate metabolism, consistent with what would have been predicted from previous work, namely that glucose tissue uptake and especially muscle glycogen utilization increased roughly exponentially in relation to exercise intensity.

### *Evaluation of Methods*

The use of stable isotope tracers for quantification of glucose and palmitate turnover is well established (35). Whole body oxidation rates of fat and carbohydrate were calculated by indirect calorimetry. This method relies on the assumption that $\dot{V}O_2$ and $\dot{V}CO_2$ accurately reflect tissue $O_2$ consumption and $CO_2$ production (14). There is little controversy with respect to $\dot{V}O_2$, of which there are no large stores in the body. However, at exercise intensities that cause hyperventilation, $\dot{V}CO_2$ may overestimate tissue $CO_2$ production (13). This can potentially result in the overestimation of the rate of carbohydrate oxidation and, concomitantly, an underestimation of fat oxidation (13). Therefore, before the present study, we conducted a preliminary study that developed a new breath $^{13}C$-to-$^{12}C$ ratio method for the calculation of carbohydrate and fat oxidation during exercise at low to high intensities that is completely independent of the measurement of $\dot{V}CO_2$ (26). This new method proved that carbohydrate and fat oxidation calculated by indirect calorimetry during exercise is valid, including during exercise at 85% $\dot{V}O_{2\,max}$ in trained subjects, as performed in the present study.

The rates of fat and carbohydrate oxidation in excess of the measured blood glucose and plasma FFA uptake are assumed to equal the oxidation of intramuscular triglyceride and glycogen, respectively. There is no reason to believe that there are major fates other than oxidation for glucose and FFA taken up by the tissues during exercise (6, 17). The maximal rate of FFA oxidation from peripheral lipolysis can thus be considered to be equal to plasma FFA uptake. The difference between that rate and total fat oxidation can be attributed largely to intramuscular

fat oxidation, although it is also possible that plasma triglycerides could contribute a small amount to total fat oxidation.

The general validity of this approach to quantifying the rate of intramuscular triglyceride oxidation is supported by the fact that the analogous approach with glucose metabolism yielded rates of muscle glycogen breakdown comparable to those derived from studies that used data obtained from biopsies (19). An advantage of the methods used in this paper is that muscle biopsies are not needed, and the calculated value of the rate of glycogen breakdown represents the average response of the body's musculature.

The rate of whole body lipolysis was assessed by measuring glycerol $R_a$. Glycerol appears in the blood only as the product of lipolysis and can be reutilized only in tissues that contain glycerol kinase, predominately the liver. In animals the activity of glycerol kinase in extrahepatic tissues is either absent or low (9, 24). However, there is currently no evidence as to whether there is sufficient glycerol kinase in muscle to allow a physiologically significant rate of glycerol oxidation. The recent paper by Elia et al. (10) raises the possibility that glycerol released from intramuscular lipolysis might be directly oxidized, thereby resulting in the underestimation of whole body lipolysis by our tracer technique. Elia et al. (10) observed a significant isotopic exchange of glycerol across forearm muscle tissue. However, from the data of Elia et al. (10), it remained unclear if this was only due to slow equilibration of enriched glycerol with the muscle pool of glycerol or if it was related to concomitant muscle glycerol utilization and production. The fat oxidation data in our study provide support for the contention that glycerol $R_a$ is an accurate reflection of whole body lipolysis. During exercise at 65% of $\dot{V}O_{2\,max}$, the intensity at which the highest fat oxidation rates were observed, the rate of three times glycerol $R_a$, reflecting the maximal availability of FFA derived from lipolysis, and fatty acid oxidation rates were in good agreement. If the assumption of the presence of a substantial activity of glycerol kinase in human muscle was true, glycerol derived from hydrolysis of muscle triglycerides would have been oxidized directly without passing through the plasma pool, thus causing whole body lipolysis to be consistently lower than FFA oxidation rates. From these considerations, we conclude that glycerol $R_a$ is a reliable reflection of the rate of lipolysis within adipose tissue and/or exercising muscle. Previously reported values for glycerol $R_a$ in exercising humans (16) used tritium as a tracer, which presented a serious problem at the time because of inadequate separation from glucose in the analytical procedure, thereby leading to a severalfold underestimation of the true value of glycerol $R_a$. The use of GC-MS in the present study allows for a reliable and distinct separation and identification and quantification of $[^2H_5]$glycerol enrichment (35).

*Low-intensity exercise (25% $\dot{V}O_{2\,max}$).* The results from our technique indicate that intramuscular triglycerides and glycogen did not contribute significantly to energy production during exercise at 25% $\dot{V}O_{2\,max}$ (Figs. 8, 9). Carbohydrate oxidation appeared to be met solely by blood glucose uptake, and thus muscle glycogen was not utilized (Fig. 8). This conclusion is consistent with the measurement (via biopsy) of changes in glycogen concentration within a muscle exercising at low intensities (29). Additionally, the measurement of the arteriovenous difference across an exercising limb during low-intensity exercise indicates that glucose uptake generally approximates the rate of carbohydrate oxidation, considering the error introduced when attempting to measure small arteriovenous differences as well as limb blood flow (5). Therefore, our technique for assessing the caloric contribution of blood glucose as opposed to muscle glycogen provides reasonable results.

*Moderate-intensity exercise (65% $\dot{V}O_{2\,max}$).* Exercise at 65% $\dot{V}O_{2\,max}$ in these subjects elicited maximal rates of fat oxidation of $>42$ $\mu mol \cdot kg^{-1} \cdot min^{-1}$. This high rate of fat oxidation is comparable to three times the $R_a$ of glycerol for the whole body (i.e., 13.6 $\mu$mol glycerol $\cdot$ $kg^{-1} \cdot min^{-1} \times 3$ $\mu$mol FFA/$\mu$mol glycerol = 40.8 $\mu$mol FFA $\cdot$ $kg^{-1} \cdot min^{-1}$) observed after 30 min of exercise. This close matching between the highest possible availability of FFA (i.e., $3 \times$ glycerol $R_a$) and total fat oxidation could indicate that whole body lipolysis and thus total FFA availability limits fat oxidation after 30 min of exercise at 65% $\dot{V}O_{2\,max}$. After 120 min of exercise at 65% $\dot{V}O_{2\,max}$, glycerol $R_a$ increased, indicating that total fatty acid availability from lipolysis (50 $\mu mol \cdot kg^{-1} \cdot min^{-1}$) was greater than the rate of fat oxidation. However, under most conditions (other than exercise), fatty acids are made available for oxidation at a rate two- to threefold greater than the rate of oxidation; so even when availability of FFA exceeds oxidation numerically, availability may still be limiting, perhaps because of transport limitations into the mitochondria (34).

Lipolysis in peripheral adipocytes and of intramuscular stores contributed equally to fat oxidation during exercise at 65% $\dot{V}O_{2\,max}$ (Table 1, Fig. 8). The early studies of fat metabolism during exercise reasoned that intramuscular triglycerides must contribute to fat oxidation during moderate-intensity exercise based on the observation that the uptake of plasma FFA by the exercising limb was less than the measured rate of fat oxidation (17). This general concept was subsequently supported by the observation that muscle triglyceride concentration (measured on biopsies) declined during prolonged moderate-intensity exercise (i.e., 55–75% $\dot{V}O_{2\,max}$). On the basis of assumptions regarding the amount of muscle mass recruited, it was estimated that intramuscular triglycerides accounted for a considerable percentage of the fat oxidized during moderate-intensity exercise (12, 19). However, no study has attempted to systematically quantify muscle triglyceride use during exercise at various intensities, probably due to the technical difficulty in measuring muscle triglyceride on small biopsy samples as well as recognition of the fact that various muscles differ markedly in triglyceride use (25, 32), making the extrapolation to whole body oxidation rates tenuous. However, our presently reported method for quantifying intramuscular triglyceride use is based on whole body measures and does not suffer these limitations, although it should be recognized that our calculation represents the minimal rate of

intramuscular triglyceride use because it assumes that all of the plasma FFA taken up from plasma are oxidized.

*High-intensity exercise (85% $\dot{V}O_{2\,max}$).* Plasma FFA concentration was much lower during exercise at 85% $\dot{V}O_{2\,max}$ than at lower intensities due to a marked reduction in FFA $R_a$ into the plasma (Table 1, Fig. 6). The decrease in FFA $R_a$ was not due primarily to an inhibition of whole body lipolysis or peripheral lipolysis, since glycerol $R_a$ was maintained. Jones et al. (21) have previously reported that FFA (specifically palmitate) release into plasma is reduced in untrained subjects during high-intensity exercise, but they did not quantify lipolysis; thus the reason for the decrease in FFA release was unclear. In our study, plasma FFA concentration increased dramatically during the minutes immediately after high-intensity exercise due to an increase in FFA $R_a$, despite a simultaneous reduction in lipolysis, as reflected by glycerol $R_a$. This relationship is probably explained by an entrapment of FFA within adipose tissue during exercise, with a release after exercise (18). Because FFA are hydrophobic, they can only be transported in the blood bound to albumin, which has only limited FFA carrier capacity (31). A reduction in adipose tissue blood flow could result in decreased FFA release from the adipose tissue. In contrast, glycerol is water soluble; therefore, its appearance in plasma is not blood flow dependent. There is some evidence that blood flow to adipose tissue, as well as skin, declines as exercise intensity increases, especially when it approaches 80–90% $\dot{V}O_{2\,max}$ (1, 30), and plasma catecholamines increase exponentially (22; Table 2). The entrapment of FFA during high-intensity exercise could therefore result from $\alpha$-adrenergic inhibition of adipose tissue blood flow or high FFA-to-albumin ratios in adipose tissue (2). This could be an example of hierarchy between different physiological functions (fat metabolism vs. circulation) modulated by a common regulator (catecholamines). Although this response is not advantageous in terms of availability of fatty acids, it does provide a greater delivery of oxygen to the active muscle. During intense exercise, oxygen availability is more likely to be a rate-limiting determinant of muscle function than fatty acid availability, since muscle glycogen breakdown can provide adequate energy substrates for a short time.

Fat oxidation declined as exercise intensity increased from 65 to 85% $\dot{V}O_{2\,max}$ along with the decline in FFA appearance in plasma and plasma FFA concentration. Much of this decline in fat oxidation was probably due to the effects of high-intensity exercise on stimulation of muscle glycogenolysis and glucose uptake (Fig. 8). However, it is possible that part of the reduction in fat oxidation during high-intensity exercise was due to a suboptimal availability of plasma FFA. There is some evidence during exercise that is intense enough to elicit a low concentration of plasma FFA that exogenous supplementation of FFA during exercise results in an increase in fat oxidation (8).

*Overall pattern of substrate availability and oxidation during exercise of increasing intensity.* An important finding of this study is that lipolysis in peripheral adipose tissue appears to be stimulated maximally during low-intensity exercise and that it does not increase when exercise intensity is increased and when plasma catecholamines increase dramatically (Tables 1 and 2). This suggests that lipolysis in peripheral adipose tissue, at least in endurance-trained men, is sensitive to the small increases in plasma catecholamines during low-intensity exercise. Lipolysis of intramuscular triglycerides, however, appears to be regulated differently in that it is not stimulated during low-intensity exercise. However, lipolysis of muscle triglycerides is stimulated when exercising at 65% $\dot{V}O_{2\,max}$, yet it does not appear to increase when exercise intensity is increased to 85% $\dot{V}O_{2\,max}$. This may reflect that the higher threshold for catecholamine stimulation is close to the maximal effective dose of catecholamines.

In this study the decline in plasma FFA $R_a$ with increasing exercising intensity was counterbalanced by increases in glucose $R_a$. Because the glucose and FFA taken up by the tissues during exercise are almost completely oxidized (6, 17), this indicates a progressive shift from plasma FFA oxidation to blood glucose oxidation with increasing exercise intensity. Although it is well known that blood glucose uptake and oxidation increase with exercise intensity (5), it has not been generally recognized that plasma FFA uptake and presumably oxidation decrease reciprocally so that the combined contribution of these plasma substrates to total energy expenditure remains constant through a wide range of exercise intensities (i.e., 25–85% $\dot{V}O_{2\,max}$; see Fig. 8).

### *Effect of Duration of Exercise on Metabolic Response*

The original goal of the study was to evaluate the effects of both exercise intensity and duration on the metabolic response. However, this was not possible at 85% $\dot{V}O_{2\,max}$, because this level of intensity could only be maintained for 30 min, and 20 min of exercise was required before a good isotopic steady state was achieved in all factors. Thus the response over time can only be evaluated at 25 and 65% $\dot{V}O_{2\,max}$. At 25% $\dot{V}O_{2\,max}$, a physiological steady state seemed to exist, as there were no significant changes from 30 min to 2 h in substrate availability and oxidation. At 65% $\dot{V}O_{2\,max}$, on the other hand, there was a progressive increase in plasma FFA and glucose availability over time. Because the rates of total fat and carbohydrate oxidation did not change over time, it can be assumed that there was a progressive decrease in the reliance on intramuscular triglycerides and glycogen as energy substrates.

There is evidence to suggest that muscle glycogen contribution to energy production decreases over time during exercise of moderate to high intensity (7). However, no prior information exists on the rate of intramuscular triglyceride utilization during exercise in humans. We presume the progressive stimulation of peripheral lipolysis, which resulted in increased plasma FFA concentration and uptake by the muscle cells and which minimized the use of intramuscular triglycerides, rather than the reverse, for several reasons. Most importantly, there is no known mechanism by which intramuscular triglyceride or fatty acid concentration can regulate peripheral lipolysis. Furthermore, plasma FFA concentration increased over time, so there was no substrate-feedback signal to the

adipose tissue that intramuscular fatty acid availability from local triglyceride hydrolysis was decreasing. These results support the notion that the breakdown of intramuscular triglyceride occurs to compensate for the sluggish response of FFA release at the onset of moderate exercise. In contrast to the situation during 65% $\dot{V}O_{2\,max}$ exercise, during 25% $\dot{V}O_{2\,max}$, availability of fatty acids derived from plasma were apparently adequate to meet substrate requirements for that intensity of exercise. Thus muscle triglyceride breakdown occurred to a minimal degree, despite the systemic stimulation of lipolysis reflected by the high rate of glycerol $R_a$. This implies different mechanisms of regulation of peripheral and muscle lipolysis, but, at this point, we have no indication of the specific factors regulating intramuscular lipolysis.

In summary, lipolysis in peripheral adipocytes appears to be high during low-intensity exercise (i.e., 25% $\dot{V}O_{2\,max}$) and does not increase with increasing exercise intensity despite large increases in circulating catecholamines. In contrast, little intramuscular lipolysis occurs during low-intensity exercise compared with moderate- and high-intensity exercise. Despite high lipolytic rates in peripheral adipose tissue, the rate of release of FFA into plasma declines progressively with increased exercise intensity to a point where plasma FFA concentration during exercise at 85% $\dot{V}O_{2\,max}$ is markedly suppressed. It is possible that this reduced availability of plasma FFA may contribute to part of the decline in fat oxidation observed when the intensity of exercise is increased from 65 to 85% $\dot{V}O_{2\,max}$. Finally, this progressive decline in plasma FFA turnover with increasing exercise intensity appears to be offset by progressively increasing blood glucose turnover; therefore the contribution of plasma substrates to caloric expenditure remains remarkably constant over this wide range of exercise intensities (i.e., 25–85% $\dot{V}O_{2\,max}$).

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REFERENCES

1. **Armstrong, R. B., M. D. Delp, E. F. Goljan, and M. H. Laughlin.** Distribution of blood flow in muscles of miniature swine during exercise. *J. Appl. Physiol.* 62: 1285–1298, 1987.
2. **Bulow, J., J. Madsen, A. Astrup, and N. J. Christensen.** Vasoconstrictor effect of high FFA/albumin ratios in adipose tissue in vivo. *Acta Physiol. Scand.* 125: 661–667, 1985.
3. **Carraro, F., C. A. Stuart, W. H. Hartl, J. Rosenblatt, and R. R. Wolfe.** Effect of exercise and recovery on muscle protein synthesis in human subjects. *Am. J. Physiol.* 259 (*Endocrinol. Metab.* 22): E470–E476, 1990.
4. **Clutter, W. E., D. M. Bier, S. D. Shah, and P. E. Cryer.** Epinephrine plasma metabolic clearance rates and physiologic thresholds for metabolic and hemodynamic action in man. *J. Clin. Invest.* 66: 94–101, 1980.
5. **Coggan, A. R.** Plasma glucose metabolism during exercise in humans. *Sports Medicine* 11: 102–124, 1991.
6. **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 exercise in men. *J. Appl. Physiol.* 68: 990–996, 1990.
7. **Coyle, E. F., A. R. Coggan, M. K. Hemmert, and J. L. Ivy.** Muscle glycogen utilization during prolonged strenuous exercise when fed carbohydrate. *J. Appl. Physiol.* 61: 165–172, 1986.
8. **Costill, D. L., E. Coyle, G. Dalsky, W. Evans, W. Fink, and D. Hoopes.** Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *J. Appl. Physiol.* 43: 695–699, 1977.
9. **Dixon, M., and E. L. Webb.** *Enzymes.* New York: Academic, 1979, p. 842–843.
10. **Elia, M., K. Khan, G. Galder, 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.
11. **Endert, D.** Determination of noradrenaline and adrenaline in plasma by radioenzymatic assay using high pressure liquid chromatography for the radiochemical products. *Clin. Chem. Acta* 96: 233–239, 1979.
12. **Essen, B.** Intramuscular substrate utilization during prolonged exercise. *Ann. NY Acad. Sci.* 301: 30–44, 1977.
13. **Ferranini, E.** The theoretical bases of indirect calorimetry: a review. *Metab. Clin. Exp.* 37: 287–301, 1988.
14. **Frayn, K. N.** Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* 55: 628–634, 1983.
15. **Hagenfeldt, L., and J. Wahren.** Turnover of free fatty acids during recovery from exercise. *J. Appl. Physiol.* 39: 247–250, 1975.
16. **Havel, R. J.** Some influences of the sympathetic nervous system and insulin on mobilization of fat from adipose tissue: studies on the turnover rates of free fatty acids and glycerol. *Ann. NY Acad. Sci.* 131: 91–101, 1965.
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. **Hodgetts, V., S. W. Coppack, K. N. Frayn, and D. R. Hockaday.** Factors controlling fat mobilization from human subcutaneous adipose tissue during exercise. *J. Appl. Physiol.* 71: 445–451, 1991.
19. **Hurley, B. F., P. M. Nemeth, W. H. Martin III, J. M. Hagberg, G. P. Dalsky, and J. O. Holloszy.** Muscle triglyceride utilization during exercise. *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. **Jones, N. L., G. J. F. Heigenhauser, A. Kuksis, C. G. Matsos, J. R. Sutton, and C. J. Toews.** Fat metabolism in heavy exercise. *Cli. Sci. Lond.* 59: 469–478, 1980.
22. **Mazzeo, R. S.** Catecholamine responses to acute and chronic exercise. *Med. Sci. Sports Exercise* 23: 839–845, 1991.
23. **McGuire, E. A. H., J. H. Helderman, J. D. Tobin, R. Andres, and M. Berman.** Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J. Appl. Physiol.* 41: 565–573, 1976.
24. **Newsholme, E. A., and K. Taylor.** Glycerol kinase activities in muscles from vertebrates and invertebrates. *Biochem. J.* 112: 465–474, 1969.
25. **Reitman, J., K. M. Baldwin, and J. O. Holloszy.** Intramuscular triglyceride utilization by red, white, and intermediate skeletal muscle and heart during exhausting exercise. *Proc. Soc. Exp. Biol. Med.* 142: 628–631, 1973.
26. **Romijn, J. A., E. F. Coyle, J. Hibbert, and R. R. Wolfe.** Comparison of indirect calorimetry and a new breath $^{13}C/^{12}C$ ratio method during strenuous exercise. *Am. J. Physiol.* 263 (*Endocrinol. Metab.* 26): E64–E71, 1992.
27. **Romijn, J. A., and R. R. Wolfe.** Effects of prolonged exercise on endogenous substrate supply and utilization. *Presp. Exerc. Sci. Sports Med.* 5: 207–234, 1992.
28. **Rosell, S.** Release of free fatty acids from subcutaneous adipose tissue in dogs following sympathetic nerve stimulation. *Acta Physiol. Scand.* 67: 343–351, 1966.

29. **Saltin, B., and J. Karlsson.** Muscle glycogen utilization during work of different intensities. In: *Muscle Metabolism During Exercise*, edited by B. Pernow and B. Saltin. New York: Plenum, 1971, p. 289-299.
30. **Smolander, J., J. Saalo, and O. Korhonen.** Effect of work load on cutaneous vascular response to exercise. *J. Appl. Physiol.* 71: 1614-1619, 1991.
31. **Spector, A. A.** Fatty acid binding to plasma albumin. *J. Lipid Res.* 16: 165-179, 1975.
32. **Spriet, L. L., G. J. F. Heigenhauser, and N. L. Jones.** Endogenous triglyceride utilization by rat skeletal muscle during tetanic stimulation. *J. Appl. Physiol.* 60: 410-415, 1986.
33. **Steele, R.** Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann. NY Acad. Med. Sci.* 82: 420-430, 1959.
34. **Winder, W. W., J. Arogyasami, R. J. Barton, I. M. Elayan, and P. R. Vehrs.** Muscle malonyl-CoA decreases during exercise. *J. Appl. Physiol.* 67: 2230-2233, 1989.
35. **Wolfe, R. R.** Radioactive and stable isotope tracers in biomedicine. *Principles and Practice of Kinetic Analysis.* New York: Wiley-Liss, 1992.
36. **Wolfe, R. R., J. E. Evans, C. J. Mullany, and J. F. Burke.** Measurement of plasma free fatty acid turnover and oxidation using 1-$^{13}$C-palmitic acid. *Biomed. Mass. Spectrom.* 7: 168-171, 1980.
37. **Wolfe, R. R., S. Klein, F. Carraro, and J. M. Weber.** Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *Am. J. Physiol.* 258 (*Endocrinol. Metab.* 21): E382-E389, 1990.
38. **Wolfe, R. R., and E. J. Peters.** Lipolytic response to glucose infusion in human subjects. *Am. J. Physiol.* 252 (*Endocrinol. Metab.* 15): E189-E196, 1987.
39. **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.