Fat Metabolism During High-Intensity Exercise in Endurance-Trained and Untrained Men

Andrew R. Coggan, Comasia A. Raguso, Amalia Gastaldelli, Labros S. Sidossis, and Catherine W. Yeckel

To determine whether trained individuals rely more on fat than untrained persons during high-intensity exercise, six endurance-trained men and six untrained men were studied during 30 minutes of exercise at 75% to 80% maximal oxygen consumption ($\dot{V}O_2$max). The rates of appearance (R_a) and disappearance (R_d) of glycerol and free fatty acids (FFAs) were determined using [1,1,2,3,3-^{2}H]glycerol and [1-^{13}C]palmitate, respectively, whereas the overall rate of fatty acid oxidation was determined using indirect calorimetry. During exercise, the whole-body rate of lipolysis (ie, glycerol R_a) was higher in the trained group (7.1 ± 1.2 *v* 4.5 ± 0.7 μmol · min^{-1} · kg^{-1}, $P < .05$), as was the R_a ($\approx R_d$) of FFA (9.0 ± 0.9 *v* 5.0 ± 1.0 μmol · min^{-1} · kg^{-1}, $P < .001$). FFA utilization was higher in trained subjects even when expressed as a percentage of total energy expenditure (10% ± 1% *v* 7% ± 1%, $P < .05$). However, this difference in plasma FFA flux could not account for all of the difference in fatty acid oxidation between trained and untrained subjects (20.8 ± 3.3 *v* 7.9 ± 1.6 μmol · min^{-1} · kg^{-1}, or 23% ± 3% *v* 13% ± 2% of total energy expenditure, both $P < .05$). Thus, the oxidation of fatty acids derived from some other source also must have been greater in the trained men. We conclude that trained athletes use more fat than untrained individuals even during intense exercise performed at the same percentage of $\dot{V}O_2$max. The additional fatty acids appear to be derived from both adipose tissue and, presumably, intramuscular triglyceride stores.

IT HAS LONG BEEN KNOWN that endurance training alters the mix of substrates oxidized during prolonged exercise. Sixty years ago, for example, Christensen and Hansen[1] observed that training reduced the respiratory exchange ratio (RER) during moderate-intensity exercise, indicative of a decrease in carbohydrate oxidation and a corresponding increase in fat oxidation. More recent studies have demonstrated a slower decline in muscle glycogen content[2] and a lower rate of glucose utilization[3] during exercise after training. By postponing the depletion of the body's limited muscle and liver glycogen stores, this substrate shift from carbohydrate to fat is thought to play an important role in the enhanced capacity for prolonged exercise that results from endurance training.

Although it is generally accepted that training increases the use of fat as a fuel during exercise, the precise source of the additional fatty acids oxidized in the trained state remains somewhat uncertain. The results of early cross-sectional studies of dogs[4] and humans[5,6] using ^{14}C-labeled palmitate led to the belief that trained individuals rely more on plasma-borne free fatty acids (FFAs) during exercise (cf Terjung and Kaciuba-Uscilko[7]). Consistent with this interpretation, Turcotte et al[8] and Kiens et al[9] have reported that training enhances muscle FFA uptake during the later stages of prolonged, single-leg knee extensor exercise. However, when exercise is performed with a large muscle mass (eg, cycling) at the same absolute intensity after training versus before training, the plasma FFA concentration is usually lower in the trained state.[10,11] This decline in FFA availability is due to a slower rate of adipose tissue lipolysis in the trained state,[11] which apparently results from a reduction in sympathetic nervous system (SNS) activity. Consequently, training actually reduces the rate of FFA utilization under these conditions.[10,11] It has therefore been hypothesized that trained persons depend more on intramuscular triglycerides during large muscle mass exercise, a conclusion that is supported by the results of some[12,13] but not all[8,14] studies using the muscle biopsy technique.

Recently, Brooks and Mercier[15] have questioned the basic premise that training increases the reliance on fat during "hard" exercise (ie, at ≥65% of maximal oxygen uptake [$\dot{V}O_2$max]), arguing that the training-induced substrate shift is a phenomenon that occurs only at lower absolute and/or relative exercise intensities. This hypothesis is inconsistent with earlier data demonstrating that the RER and muscle glycogen utilization are lower in trained compared with untrained persons during exercise at 65% to 80% $\dot{V}O_2$max (cf Coggan and Williams[16]). Likewise, we have recently shown that whole-body glucose uptake is lower in trained versus untrained subjects during exercise at 80% $\dot{V}O_2$max.[17] Nevertheless, data directly demonstrating a higher rate of FFA or intramuscular triglyceride utilization by trained individuals during intense exercise are noticeably lacking. Recently, Klein et al[18] reported that whole-body lipolysis (ie, glycerol rate of appearance [R_a]) was higher in trained versus untrained men during exercise at 70% $\dot{V}O_2$max, but since FFA kinetics or fat oxidation were not measured, it is not known whether this higher rate of lipolysis was also accompanied by a higher rate of fatty acid utilization. Similarly, while Kanaley et al[19] recently found no difference in FFA turnover or fat oxidation between moderately trained runners and marathoners during exercise at 70% to 85% $\dot{V}O_2$max, the absence of an untrained control group in the study precludes any conclusions regarding the effects of endurance training.

We hypothesized that endurance-trained individuals would rely more on fat for energy during intense exercise even when the exercise is performed at the same relative (and therefore

From the Shriners Burns Institute and The University of Texas Medical Branch, Galveston, TX.

Submitted March 26, 1999; accepted May 12, 1999.

Supported by grants from the John Sealy Memorial Endowment Fund (2566-92), the Shriners Hospitals for Children (15849), and the National Institutes of Health (AG-14769, DK-46017, and GCRC-00073).

Address reprint requests to Andrew R. Coggan, PhD, Director, Biomedical Mass Spectrometry Facility, University of Maryland School of Medicine, Department of Medicine, Division of Gerontology, VA Medical Center (BT18GR), 10 N Greene St, Room 4B195, Baltimore, MD 21201.

0026-0495/00/4901-0030$10.00/0

higher absolute) intensity as in untrained persons, and that the additional fatty acids would be derived primarily from intramuscular triglyceride stores. To test these hypotheses, stable isotopic tracers and indirect calorimetry were used to quantify fat metabolism in trained and untrained men cycling for 30 minutes at 75% to 80% to $\dot{V}O_2$max.

SUBJECTS AND METHODS

Subjects

Six endurance-trained men and six untrained men participated in the study. The trained group included one runner, two triathletes, and three cyclists (one US Cycling Federation category 2 and two category 3). Physical characteristics of the subjects are shown in Table 1. All subjects were healthy as indicated by medical history, physical examination, and standard analyses of blood and urine chemistry. The study protocol was approved by the Institutional Review Board of The University of Texas Medical Branch at Galveston.

Experimental Protocol

Subjects were instructed to consume a mixed normal diet containing at least 250 g carbohydrate per day and to refrain from alcohol or caffeine use and exercise for 48 hours. They then reported to the General Clinical Research Center after fasting overnight (12 hours). An indwelling catheter was inserted in a retrograde direction in a dorsal vein of a heated hand for sampling arterialized venous blood. A second catheter was inserted in an antecubital or forearm vein of the contralateral arm for tracer infusion. After a blood sample was obtained for subsequent determination of background isotopic enrichment, a primed (1.5 μmol/kg) continuous (0.10 $\mu mol \cdot min^{-1} \cdot kg^{-1}$) infusion of [1,1,2,3,3-^{2}H]glycerol (99% enriched; Tracer Technologies, Somerville, MA) was started using a syringe pump. After 60 minutes of glycerol infusion, a continuous infusion (0.04 $\mu mol \cdot min^{-1} \cdot kg^{-1}$) of [1-^{13}C]palmitate (98% enriched; Tracer Technologies) complexed with human albumin (Baxter, Glendale, CA) was initiated using a second pump. The subject rested in a chair for 105 minutes after the start of glycerol tracer infusion and then sat quietly on the cycle ergometer (Monark 829E, Stockholm, Sweden) for an additional 15 minutes while blood was drawn for subsequent determination of baseline substrate concentrations and kinetics. The subject then began pedaling the ergometer at a power output intended to elicit approximately 75% $\dot{V}O_2$max. The rate of [1,1,2,3,3-^{2}H]glycerol infusion was increased at the onset of exercise and every minute thereafter to mimic a monoexponential function with an asymptote of 0.20 $\mu mol \cdot min^{-1} \cdot kg^{-1}$ at 30 minutes. This procedure improves non–steady-state estimates of glycerol kinetics by minimizing the rate of change in isotopic enrichment during exercise (Fig 1A) while also avoiding the artifact that results from a single step-change in the tracer infusion rate. To avoid biasing the data, the same tracer infusion protocol was used for both trained and untrained subjects. Because only minimal changes in FFA enrichment occur during cycling exercise of this intensity and duration[20,21] (Fig 1B), the rate of [1-^{13}C]palmitate infusion was not altered in either group during the study.

Table 1. Subject Characteristics

Characteristic	Untrained	Trained
Age (yr)	24 ± 1	27 ± 3
Height (cm)	178 ± 3	178 ± 2
Weight (kg)	73.8 ± 3.4	68.7 ± 1.5
$\dot{V}O_2$max		
L/min	2.99 ± 0.23	4.44 ± 0.17*
$mL \cdot min^{-1} \cdot kg^{-1}$	40.2 ± 1.9	64.6 ± 2.3*

NOTE. Values are the mean ± SE for 6 subjects per group.
*$P < .001$, trained *v* untrained.

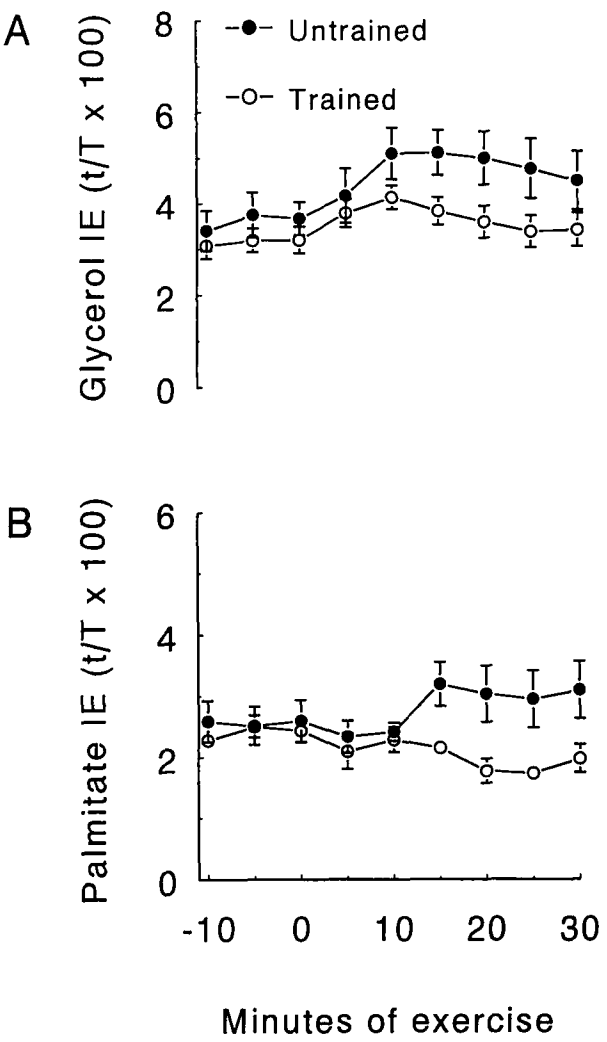

Fig 1. Plasma glycerol (A) and palmitate (B) isotopic enrichment ([IE] expressed as the ratio of tracer [t] to tracee [T]) in 6 endurance-trained men and 6 untrained men infused with [1,1,2,3,3-^{2}H]glycerol and [1-^{13}C]palmitate at rest and during 30 minutes of exercise at 75% to 80% $\dot{V}O_2$max.

Sample Collection and Analyses

Blood samples were obtained at -10, -5, 0, 5, 10, 15, 20, 25, and 30 minutes (where 0 minutes corresponds to the onset of exercise) and placed in tubes containing lithium heparin for subsequent measurement of glycerol and FFA concentrations and enrichments, or in tubes containing sodium fluoride/potassium oxalate for subsequent measurement of the lactate concentration. Additional blood samples were obtained at 0, 15, and 30 minutes and placed in tubes containing EDTA/aprotinin for subsequent measurement of the insulin concentration, or in tubes containing EGTA/reduced glutathione for later measurement of norepinephrine and epinephrine concentrations. All samples were stored on ice until the conclusion of the experiment and were then centrifuged to obtain plasma, which was frozen at −20°C (−70°C for hormone samples) until further processing.

The lactate concentration was measured using a lactate oxidase technique (2300A analyzer; Yellow Springs Instruments, Yellow Springs, OH). Glycerol concentrations were measured using an enzymatic colorimetric assay (RA-500 analyzer; Technicon, Tarrytown, NY). The

isotopic enrichment of glycerol was determined using previously described procedures.[21,22] Briefly, the plasma was deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and glycerol was isolated using cation- and anion-exchange resins (Dowex AG50W-X8 and AG1-X8; Bio-Rad Laboratories, Rockville Centre, NY). After lyophilization, the trimethylsilyl derivative of glycerol was prepared and the tracer to tracee ratio was determined using an electron-impact gas chromatograph–mass spectrometer ([GCMS] 5971 MSD; Hewlett-Packard, Fullerton, CA). Ions of *m/e* 205 and 208 were monitored.

FFA concentrations and enrichments were determined as previously described.[21,22] Briefly, FFAs were extracted from plasma using propanol: heptane:HCl, isolated from other lipids via thin-layer chromatography, and derivatized to their methyl esters. Palmitate and total FFA concentrations were measured by gas chromatography (Hewlett-Packard 5980 GC) using heptadecanoic acid as an internal standard. Isotopic enrichment of palmitate was determined by GCMS analysis of the methyl ester derivatives, monitoring ions of *m/e* 270 and 271.

A metabolic cart (Ametek OCM-2; Thermox Instruments, Pittsburgh, PA) was used to measure respiratory gas exchange (ie, oxygen uptake [$\dot{V}O_2$], CO_2 production [$\dot{V}CO_2$], etc.) for at least 10 minutes at rest and for 3- to 5-minute periods ending at 10, 20, and 30 minutes of exercise.

Calculations

The rates of appearance (R_a) and disappearance (R_d) of glycerol and palmitate were calculated using classic non–steady-state equations modified for use with stable isotopes.[21] The effective volume of distribution was assumed to be 230 mL/kg body weight for glycerol and 40 mL/kg body weight for palmitate.[21] FFA kinetics were calculated by dividing the palmitate R_a and R_d by the fractional contribution of palmitate to the total FFA concentration. To reduce the influence of random experimental error, enrichment and concentration data were smoothed with a spline function prior to the calculation of substrate kinetics.[21]

The overall rates of carbohydrate and triglyceride oxidation during exercise were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ as previously described.[21,22] Fat oxidation (in micromoles of fatty acid per minute per kilogram) was then determined by converting the rate of triglyceride oxidation (in grams per minute per kilogram) to its molar equivalent, assuming the average molecular weight of triglyceride to be 860 g/mol, and multiplying the molar rate of triglyceride oxidation by 3 because 1 mol triglyceride contains 3 mol fatty acids. These calculations were made using the average of the 20- and 30-minute values, since steady-state conditions with respect to the RER and lactate level prevailed at this time (see the Results).

Statistical Analyses

Data were analyzed using a two-way (group × time) ANOVA with the group as a between-subject factor and time as a within-subject factor. Statistical significance was defined as a P level of .05 or less. Where appropriate, significant differences identified by ANOVA were isolated using Student-Newman-Keul post hoc tests. All data are presented as the mean ± SE.

RESULTS

Respiratory Gas Exchange and Plasma Lactate Concentration

As intended, the relative intensity (ie, percent $\dot{V}O_2$max elicited) was identical in trained and untrained subjects after 10 minutes of exercise (Table 2). However, due to the trained subjects' higher $\dot{V}O_2$max, this required that they exercise at an approximately 50% higher $\dot{V}O_2$. In both groups, $\dot{V}O_2$ increased ($P < .05$) by about 1.5 mL · min^{-1} · kg^{-1} as exercise continued, which resulted in a tendency for the relative intensity to be slightly higher in untrained versus trained subjects after 20 and 30 minutes of exercise; however, this difference between groups was minor and not statistically significant. The RER increased ($P < .001$) from 0.81 to 0.82 at rest to almost 1.00 in untrained subjects and to greater than 0.9 in trained subjects (Table 2), which was accompanied by a rapid increase ($P < .001$) in the plasma lactate concentration (Fig 2). However, plasma lactate levels plateaued in both groups after 15 to 20 minutes of exercise, with this plateau in lactate accompanied by a decline and then a plateau in the RER. Both the RER and lactate concentration were significantly lower in the trained group versus the untrained group. As a result of their higher $\dot{V}O_2$ and lower RER, the calculated rate of fat oxidation was more than twice as high in trained subjects (20.8 ± 3.3 *v* 7.9 ± 1.6 μmol · min^{-1} · kg^{-1}, $P < .05$). This difference persisted even when fat oxidation was expressed as a percentage of total energy expenditure (23% ± 3% *v* 13% ± 2%, $P < .05$). On the other hand, carbohydrate oxidation was higher in the trained subjects when expressed in absolute terms (270 ± 10 *v* 200 ± 8 μmol · min^{-1} · kg^{-1}, $P < .01$) but lower when expressed as a percentage of total energy expenditure (76% ± 3% *v* 85% ± 2%, $P < .05$). (Protein oxidation was estimated to account for 1% to 2% of total energy expenditure in both groups.)

Plasma Glycerol and FFA Kinetics

At rest, the plasma glycerol concentration did not differ between the trained and untrained men (Fig 3A). Glycerol levels increased steadily ($P < .001$) during exercise in both groups; however, this increase was more rapid in trained subjects. Hence, during the last 15 minutes of exercise, glycerol concentrations were significantly higher ($P < .05$) in the trained group compared with the untrained group. This was due to a higher glycerol R_a ($P < .05$) in the trained subjects (Fig 3B).

Table 2. Respiratory Gas Exchange in Trained and Untrained Men During 30 Minutes of Cycling at 75% to 80% $\dot{V}O_2$max

Parameter	Group	Duration of Exercise (min)			
		0	10	20	30
$\dot{V}O_2$ (% of $\dot{V}O_2$max)	Untrained	14 ± 1	75 ± 2	79 ± 2	79 ± 2
	Trained	9 ± 1†	75 ± 2	76 ± 2	77 ± 2
$\dot{V}O_2$ (mL · min^{-1} · kg^{-1})	Untrained	5.4 ± 0.3	30.3 ± 1.3	32.0 ± 1.3	32.0 ± 1.3
	Trained	5.5 ± 0.2	47.7 ± 2.5†	48.7 ± 2.3†	49.3 ± 2.1†
RER	Untrained	0.82 ± 0.05	0.99 ± 0.01	0.95 ± 0.01	0.96 ± 0.01
	Trained	0.81 ± 0.03	0.94 ± 0.02*	0.93 ± 0.01*	0.92 ± 0.01*

NOTE. Values are the mean ± SE for 6 subjects per group.
*$P < .05$, †$P < .001$: trained *v* untrained.

The glycerol R_d also tended to be higher in the trained group (Fig 3C), but this difference only approached statistical significance ($P < .08$). There was no difference between groups in glycerol clearance (data not shown).

Plasma FFA concentrations were similar in the two groups at rest, and as expected at this intensity,[16,23] they initially decreased ($P < .001$) during exercise in both groups (Fig 4A). However, FFA levels rebounded slightly later during exercise in the trained subjects, whereas they plateaued in the untrained subjects. Consequently, FFA concentrations were higher ($P < .05$) in trained versus untrained men during the last 15 minutes of exercise. The FFA R_a and R_d demonstrated a roughly similar pattern, tending to decrease slightly during exercise in the untrained group but tending to increase gradually during exercise in the trained group (Fig 4B and C). The FFA R_a and R_d were therefore significantly higher ($P < .001$ and $P < .01$, respectively) in the trained versus untrained men during the last 20 minutes of exercise.

A higher rate of plasma FFA utilization therefore apparently contributed to the higher overall rate of fatty acid oxidation during exercise in the trained group. However, in neither group of subjects could the uptake of FFA from plasma account for all of the fatty acids oxidized during high-intensity exercise (Fig 5A). Thus, oxidation of fatty acids derived from some other source (presumably intramuscular triglycerides) also must have contributed to the energetic demands of exercise. This was particularly true in the trained subjects, in whom the difference between fatty acid oxidation and FFA R_d was approximately three times that found in the untrained subjects (Fig 5A). These differences between groups remained significant even when the FFA R_d and fatty acid oxidation were expressed as a percentage of total energy expenditure (Fig 5B).

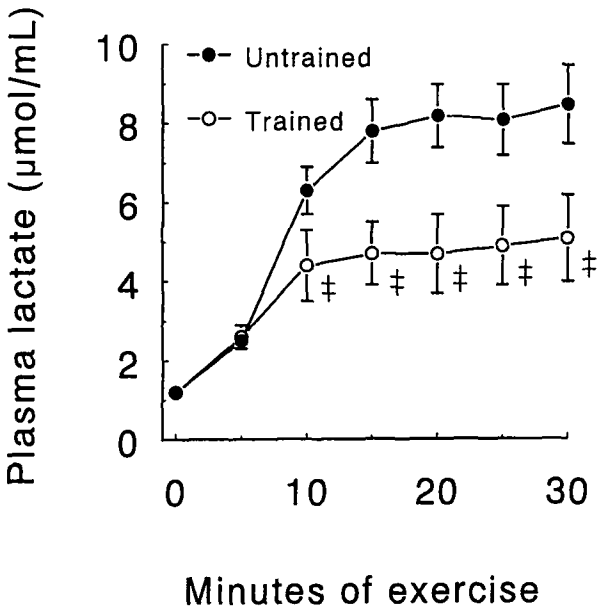

Fig 2. Plasma lactate concentration in 6 endurance-trained men and 6 untrained men infused at rest and during 30 minutes of exercise at 75% to 80% $\dot{V}O_2$max. Trained significantly lower *v* untrained, ‡$P <$ .001.

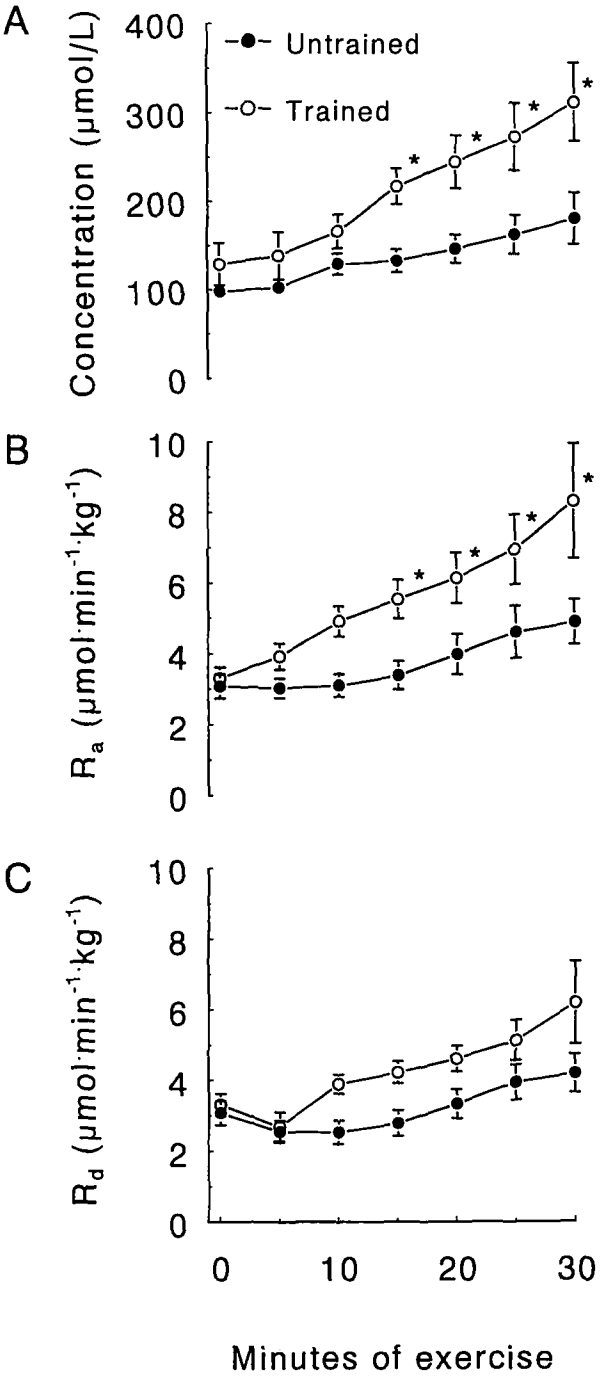

Fig 3. Plasma glycerol concentration (A), R_a (B), and R_d (C) in 6 endurance-trained men and 6 untrained men at rest and during 30 minutes of exercise at 75% to 80% $\dot{V}O_2$max. Trained significantly higher *v* untrained, *$P <$ .05.

Plasma Hormone Concentrations

Insulin concentrations tended to be lower in the trained subjects both at rest and during exercise, but this difference only approached significance ($P < .08$). Conversely, plasma norepinephrine levels tended to be higher in trained subjects during exercise, but this difference also was not significant. Plasma

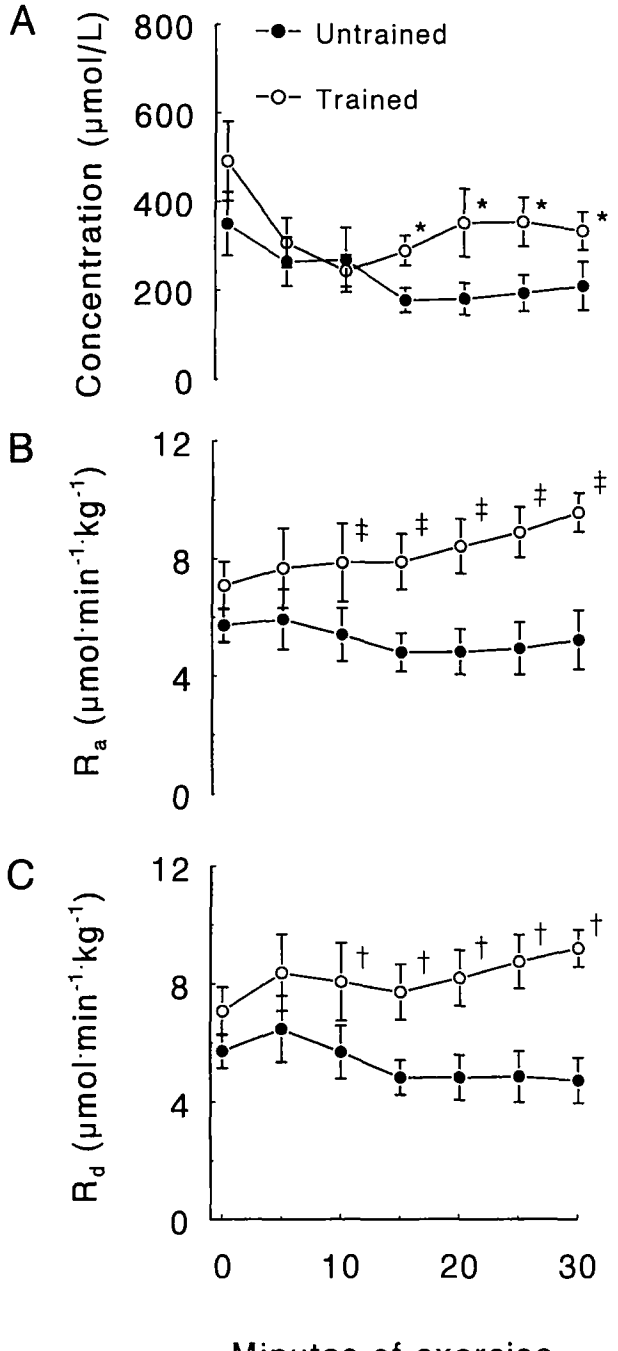

Fig 4. Plasma FFA concentration (A), R_a (B), and R_d (C) in 6 endurance-trained men and 6 untrained men at rest and during 30 minutes of exercise at 75% to 80% $\dot{V}O_2$max. Trained significantly higher *v* untrained, *$P < .05$, †$P < .01$, ‡$P < .001$.

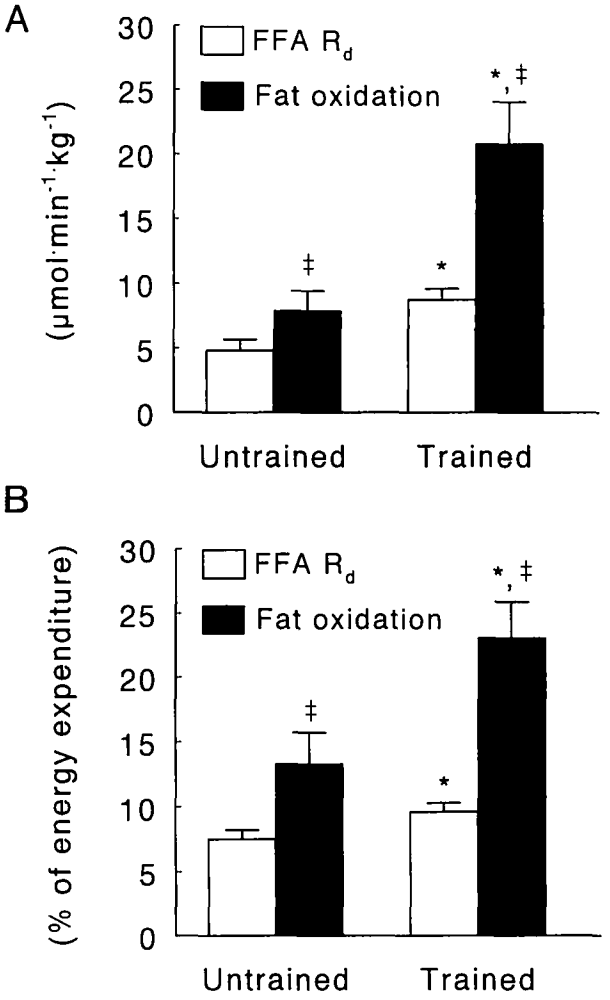

Fig 5. Mean FFA R_d in comparison to the mean rate of fatty acid oxidation in 6 endurance-trained men and 6 untrained men exercising at 75% to 80% $\dot{V}O_2$max. *FFA R_d of fatty acid oxidation significantly higher in trained *v* untrained, $P < .05$. ‡Fatty acid oxidation significantly higher *v* FFA R_d in the same group, $P < .001$.

epinephrine concentrations did not differ significantly between groups at rest or during exercise (Table 3).

DISCUSSION

The major purpose of the present study was to test the hypothesis that compared with untrained persons, trained individuals rely more on fat for energy during intense exercise even when exercising at the same relative (and therefore higher absolute) intensity as in the untrained state. Although there is considerable indirect support for this concept (cf Coggan and Williams[16]), as emphasized by Brooks and Mercier,[15] direct

Table 3. Plasma Hormone Concentrations in Trained and Untrained Men at Rest and During 30 Minutes of Cycling at 75% to 80% $\dot{V}O_2$max

Hormone	Group	Duration of Exercise (min) 0	15	30
Insulin (pmol/L)	Untrained	43 ± 7	34 ± 9	25 ± 6
	Trained	25 ± 2	16 ± 3	17 ± 2
Norepinephrine	Untrained	2.6 ± 0.5	8.2 ± 1.3	12.2 ± 2.6
(nmol/L)	Trained	2.0 ± 0.4	11.7 ± 1.3	25.1 ± 6.9
Epinephrine	Untrained	0.33 ± 0.05	0.93 ± 0.19	1.24 ± 0.24
(nmol/L)	Trained	0.42 ± 0.11	1.08 ± 0.19	1.84 ± 0.56

NOTE. Values are the mean ± SE for 6 subjects per group. A significant main effect for time ($P < .01$ to $.001$) was found for all 3 variables, but there were no significant group or group × time interaction effects.

evidence that trained subjects use more plasma FFAs or intramuscular triglycerides under such conditions has been lacking. However, we found that the FFA R_a and R_d were 60% to 80% higher in trained athletes compared with untrained men during exercise at 75% to 80% $\dot{V}O_2$max. This higher FFA flux was accompanied by a higher overall rate of fat oxidation as estimated from indirect calorimetry. These differences are unlikely to be simply due to the difference in the absolute metabolic rate between the two groups, because (1) above moderate workloads, FFA turnover and fat oxidation normally decrease, not increase, with increasing exercise intensity,[19-21] and (2) FFA uptake and fat oxidation were higher in trained men even when the data were expressed as a percentage of total energy expenditure. Thus, the present data provide strong evidence that trained subjects use more fatty acids than untrained individuals even during intense exercise performed at the same percentage of $\dot{V}O_2$max.

Despite the higher FFA R_d in trained versus untrained subjects, this could not account for all of the difference in fat oxidation between the two groups, even if it is assumed that 100% of the FFA R_d was oxidation. (In fact, during exercise, only 50% to 85% of the whole-body FFA R_d is oxidation[10,19,25,26]). Thus, a second important conclusion of the present study is that trained individuals also rely more heavily on non–adipose-tissue-derived fatty acids during intense exercise. The source of these additional fatty acids cannot be determined from the present data. However, it seems likely that they are obtained mostly from intramuscular triglyceride stores. The triglyceride content of human muscle is much greater than that of some other species such as the rat, and can serve as a significant source of energy during exercise.[27] Furthermore, previous studies of subjects performing moderate-intensity exercise at the same absolute power output before and after training have shown a greater utilization of intramuscular triglycerides during exercise in the trained state.[12,13] Although others[8,14] have been unable to confirm these observations, this may be simply due to the difficulty of accurately measuring small changes in muscle triglyceride levels using the biopsy method.[28] In the present study, for example, total fatty acid oxidation during the 30-minute exercise bout amounted to 42.6 $\pm$ 6.9 and 17.7 $\pm$ 3.9 mmol in the trained and untrained groups, respectively ($P < .01$). Even if all of these fatty acids were obtained from intramuscular stores (which is unlikely), when spread over about 10 kg (or more) of active muscle, this would correspond to a reduction in muscle triglyceride content of 10% or less.[27] Given the heterogeneous distribution of triglyceride within muscle,[28] detecting such a small change would obviously be difficult. However, reductions in intramuscular triglyceride content of 25% to 50% have been observed following more prolonged bouts of exercise.[12,13,27]

It is also possible, of course, that the trained subjects obtained more fatty acids from plasma-borne triglycerides during exercise. However, in general, the contribution of plasma triglycerides to energy metabolism during exercise is thought to be small.[23] Furthermore, Kiens and Lithell[24] found that training did not affect triglyceride uptake by the leg during prolonged knee extensor exercise.

The greater dependence on fat for energy in the trained men may be partially due to their higher rate of lipolysis during exercise, as implied by the higher glycerol R_a in this group. The present findings therefore confirm and extend the results of Klein et al,[18] who found that the glycerol R_a was higher in trained versus untrained men during exercise at 70% $\dot{V}O_2$max. The mechanism responsible for this higher glycerol R_a in trained versus untrained subjects during exercise at the same relative intensity is not known. However, it may be due to a greater SNS response in trained subjects under such conditions. In the present study, for example, plasma epinephrine and especially norepinephrine levels tended to be higher in trained men due to their much higher absolute metabolic rate when exercising at 75% to 80% $\dot{V}O_2$max. Although these differences only approached statistical significance ($P = .12$), they nevertheless may have resulted in a greater stimulation of adipose tissue and/or intramuscular lipolysis in the trained subjects, which would explain their higher glycerol (and FFA) R_a. Indeed, when catecholamine levels were similar in the two groups (ie, 15 minutes of exercise in trained men *v* 30 minutes of exercise in untrained men; Table 3), the glycerol R_a was also similar (Fig 3B), suggesting that the higher lipolytic rate in the trained men may be due to their greater neuroendocrine response. In contrast, when subjects are studied during prolonged exercise performed at the same absolute intensity before and after training, both SNS activation and the rate of fat mobilization are reduced,[10,11] as previously discussed. Of course, it is also possible that other factors (such as a difference in insulin concentration, which also tended to differ [$P = .08$] between trained and untrained subjects in the present study) account for these training-related differences in the lipolytic response.

Although the rate of lipolysis was higher in trained versus untrained subjects, this does not appear to be the primary factor responsible for the difference in fat oxidation between the two groups. First, lipolysis did not seem to be limiting in the untrained men, since their overall rate of fatty acid mobilization (which can be minimally estimated as 3 $\times$ glycerol R_a) always exceeded their rate of fatty acid oxidation. Second, while the rate of lipolysis was about 60% higher in the trained subjects, their rate of fat oxidation was more than twice that of the untrained group. In other words, the trained subjects must have oxidized a higher percentage of the fatty acids that were released via lipolysis. In keeping with this possibility, we recently found that although trained men oxidized a greater percentage of the oleate R_d during exercise than untrained men, the percentage of octanoate R_d oxidized was similar in the two groups.[25] Since oleate, a long-chain fatty acid, requires the carnitine transport system for entry into the mitochondria and octanoate, a medium-chain fatty acid, does not, these data suggest that training increases fat oxidation during exercise in part by enhancing mitochondrial fatty acid uptake. If so, this may be simply due to the greater total mitochondrial content and thus higher carnitine palmitoyl transferase I (CPT I) activity of trained muscle.[16] Additionally, it is possible that CPT I is less inhibited/more active during exercise in the trained state, as a result of a slower rate of carbohydrate utilization.[26] However, regardless of the exact cause, higher functional CPT I activity in the trained state would favor a more rapid uptake of fatty acids from the cytosol, thus minimizing their reesterification into intramuscular triglycerides. This mechanism, ie, a greater "pull" by the mitochondria, could therefore potentially explain

why the trained subjects of the present study apparently oxidized a larger fraction of fatty acids and seemingly relied more on intramuscular triglycerides than the untrained subjects.

A cross-sectional design was used in the present study, and it is therefore possible that the results reflect, at least in part, the influence of factors other than training per se (ie, genetics). However, the trained men of the present study, while highly fit, were not elite athletes, as evidenced by their $\dot{V}O_2$max (Table 1) and by the fact that only one was competitive on a regional level. Furthermore, previous longitudinal studies have shown that training increases fat oxidation even during intense exercise performed at the same percentage of $\dot{V}O_2$max in the trained and untrained states.[29,30] It therefore seems unlikely that genetic differences could fully account for the greater ability of trained subjects in the present study to use fat as a fuel during intense exercise.

The rate of glycerol tracer infusion was increased during exercise in an attempt to minimize variations in isotopic enrichment and thus the impact of the volume of distribution assumed in the Steele equation on the calculated R_a. This maneuver was only partially successful in that glycerol isotopic enrichment increased by 30% to 40% during the first 10 minutes of exercise in both groups (Fig 1). However, since the glycerol R_a increased by an even greater extent, especially in trained subjects (Fig 3), the changes in isotopic enrichment would be even larger if the tracer infusion rate were not increased during exercise. Moreover, because the same tracer infusion protocol was used in both groups, the lower enrichment in trained subjects means that the glycerol R_a must be higher in these men regardless of the model used to calculate it. However, using this approach does require a reasonable a priori estimate of the expected increase in the R_a, since an inappropriate increase in the rate of tracer infusion can lead to marked changes in isotopic enrichment and hence imprecision and even artifacts in the calculated R_a.

To summarize, we have shown that during intense exercise, trained individuals oxidize more fat than untrained subjects, even when the exercise is performed at the same percentage of $\dot{V}O_2$max. The additional fatty acids appear to be derived from both adipose tissue (supplied as plasma FFA) and, presumably, intramuscular triglyceride stores.

REFERENCES

1. Christensen EH, Hansen O: Respiratorscher Quotient und O_2-Aufnahme. Acta Physiol Scand 81:180-189, 1939
2. Karlsson J, Nordesjö L-O, Saltin B: Muscle glycogen utilization during exercise after physical training. Acta Physiol Scand 90:210-217, 1974
3. Coggan AR, Kohrt WM, Spina RJ, et al: Endurance training decreases plasma glucose turnover and oxidation during moderate intensity exercise in men. J Appl Physiol 68:990-996, 1990
4. Issekutz B, Miller HI, Paul P, et al: Aerobic work capacity and plasma FFA turnover. J Appl Physiol 20:293-296, 1965
5. Havel RJ, Naimark A, Borchgrevink CF: Turnover rate and oxidation of free fatty acids of blood plasma in man during exercise: Studies during continuous infusion of palmitate-1-C^{14}. J Clin Invest 42:1054-1063, 1963
6. Havel RJ, Carlson LA, Ekelund L-G, et al: Turnover rate and oxidation of different free fatty acids in man during exercise. J Appl Physiol 19:613-618, 1964
7. Terjung RL, Kaciuba-Uscilko H: Lipid metabolism during exercise: Influence of training. Diabetes Metab Rev 2:35-51, 1986
8. Turcotte LP, Richter EA, Kiens B: Increased plasma FFA uptake and oxidation during prolonged exercise in trained vs. untrained humans. Am J Physiol 262:E791-E799, 1992
9. Kiens B, Éssen-Gustavsson B, Christensen NJ, et al: Skeletal muscle substrate utilization during submaximal exercise in man: Effect of endurance training. J Physiol (Lond) 469:459-478, 1993
10. Martin WH III, Dalsky GP, Hurley BF, et al: Effect of endurance training on plasma FFA turnover and oxidation during exercise. Am J Physiol 265:E708-E714, 1993
11. Phillips SM, Green HJ, Tarnapolsky MA, et al: Effects of training duration on substrate turnover and oxidation during exercise. J Appl Physiol 81:2182-2191, 1996
12. Hurley BF, Nemeth PM, Martin WH III, et al: Muscle triglyceride utilization during exercise: Effect of training. J Appl Physiol 60:562-567, 1986
13. Phillips SM, Green HJ, Tarnapolsky MA, et al: Progressive effect of endurance training on metabolic adaptations in working skeletal muscle. Am J Physiol 270:E265-E272, 1996
14. Kiens B, Richter EA: Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. Am J Physiol 275:E332-E337, 1998
15. Brooks GA, Mercier J: Balance of carbohydrate and lipid utilization during exercise: The "crossover" concept. J Appl Physiol 76:2253-2261, 1994
16. Coggan AR, Williams BD: Metabolic adaptations to endurance training: Substrate metabolism during exercise, in Hargreaves M (ed): Exercise Metabolism. Champaign, IL, Human Kinetics, 1995, pp 177-210
17. Coggan AR, Raguso CA, Williams BD, et al: Glucose kinetics during high-intensity exercise in endurance-trained and untrained humans. J Appl Physiol 78:1203-1207, 1995
18. Klein S, Weber J-M, Coyle EF, et al: Effect of endurance training on glycerol kinetics during strenuous exercise in humans. Metabolism 45:357-361, 1996
19. Kanaley JA, Mottram CD, Scanlon PD, et al: Fatty acid kinetic responses to running above or below lactate threshold. J Appl Physiol 79:439-447, 1995
20. Jones NL, Heigenhauser GJF, Kuksis A, et al: Fat metabolism in heavy exercise. Clin Sci (Colch) 59:469-478, 1980
21. Romjin JA, Coyle EF, Sidossis LS, et al: Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. Am J Physiol 265:E380-E391, 1993
22. Sial S, Coggan AR, Carroll R, et al: Fat and carbohydrate metabolism during exercise in elderly and young subjects. Am J Physiol 271:E983-E989, 1996
23. Van der Vusse GJ, Reneman RS: Lipid metabolism in muscle, in Rowell LB, Shepherd JT (eds): Handbook of Physiology. New York, NY, Oxford, 1996, pp 952-994
24. Kiens B, Lithell H: Lipoprotein metabolism influenced by training-induced changes in human skeletal muscle. J Clin Invest 83:558-564, 1988
25. Sidossis LS, Coggan AR: Regulation of fatty acid oxidation in untrained versus trained men during exercise. Am J Physiol 274:E510-E515, 1998
26. Coyle EF, Jeukendrup AE, Wagenmakers AJM, et al: Fatty acid oxidation is directly regulated by carbohydrate metabolism during exercise. Am J Physiol 273:E268-E275, 1997
27. Gorski J: Muscle triglyceride metabolism during exercise. Can J Physiol Pharmacol 70:123-131, 1992
28. Wendling PS, Peters SJ, Heigenhauser GJF, et al: Variability of triacylglycerol content in human skeletal muscle biopsy samples. J Appl Physiol 81:1150-1155, 1996
29. Coyle EF, Martin WH III, Bloomfield SA, et al: Effects of detraining on responses to submaximal exercise. J Appl Physiol 59:853-859, 1983
30. Hurley BF, Hagberg JM, Martin WH III, et al: Effect of training on blood lactate levels during submaximal exercise. J Appl Physiol 56:1260-1264, 1984