Regulation of plasma fatty acid oxidation during low- and high-intensity exercise

4 authors, including:

Labros S Sidossis
Rutgers, The State University of New Jersey, USA - Harokopio University, Athen…

236 PUBLICATIONS **7,363** CITATIONS

Amalia Gastaldelli
Italian National Research Council

338 PUBLICATIONS **14,299** CITATIONS

Some of the authors of this publication are also working on these related projects:

Fatty acid oxidation in blood cells, specifically in monocytes, circulating acylcarnitines and low-grade systemic inflammation. View project

EYZHN project View project

Regulation of plasma fatty acid oxidation during low- and high-intensity exercise

LABROS S. SIDOSSIS, AMALIA GASTALDELLI, SAMUEL KLEIN, AND ROBERT R. WOLFE

Metabolism Unit, Shriners Burns Institute, and the Departments of Anesthesiology, Internal Medicine, Surgery, and Biochemistry, University of Texas Medical Branch, Galveston, Texas 77550

Sidossis, Labros S., Amalia Gastaldelli, Samuel Klein, and Robert R. Wolfe. Regulation of plasma fatty acid oxidation during low- and high-intensity exercise. *Am. J. Physiol.* 272 (*Endocrinol. Metab.* 35): E1065–E1070, 1997.—In the present study we examined the hypothesis that fatty acid oxidation is less during high-intensity exercise than during moderate-intensity exercise because of inhibition of long-chain fatty acid entry into the mitochondria. Six volunteers exercised at 40% peak oxygen consumption ($\dot{V}O_{2\,peak}$) for 60 min and at 80% $\dot{V}O_{2\,peak}$ for 30 min on two different occasions. [1-^{13}C]oleate, a long-chain fatty acid, and [1-^{14}C]octanoate, a medium-chain fatty acid, were infused for the duration of the studies. Lipids and heparin were infused during exercise at 80% $\dot{V}O_{2\,peak}$ to prevent the expected decrease in plasma free fatty acid (FFA) concentration. Plasma oleate and total FFA availability were similar in the two experiments. Oleate oxidation decreased from 2.8 ± 0.6 (40% $\dot{V}O_{2\,peak}$) to $1.8 \pm 0.2 \,\mu mol \cdot kg^{-1} \cdot min^{-1}$ (80% $\dot{V}O_{2\,peak}$, $P < 0.05$), whereas octanoate oxidation increased from $1.0e^{-05} \pm 1.0e^{-06}$ (40% $\dot{V}O_{2\,peak}$) to $1.3e^{-05} \pm 5.1e^{-06} \,\mu mol \cdot kg^{-1} \cdot min^{-1}$ (80% $\dot{V}O_{2\,peak}$, $P < 0.05$). Furthermore, the percentage of oleate uptake oxidized decreased from $67.7 \pm 2.8\%$ (40% $\dot{V}O_{2\,peak}$) to $51.8 \pm 4.6\%$ (80% $\dot{V}O_{2\,peak}$, $P < 0.05$), whereas the percentage of octanoate oxidized was similar during exercise at 40 and 80% $\dot{V}O_{2\,peak}$ (84.8 ± 2.7 vs. $89.3 \pm 2.7\%$, respectively). Our data suggest that, in addition to suboptimal FFA availability, fatty acid oxidation is likely limited during high-intensity exercise because of direct inhibition of long-chain fatty acid entry into mitochondria.

mitochondria; malonyl-coenzyme A; carnitine palmitoyltransferase; medium-chain fatty acids

FATTY ACID RELEASE into plasma and oxidation decrease as exercise intensity increases from 65 to 85% of peak oxygen consumption ($\dot{V}O_{2\,peak}$) (12). However, the fall in plasma free fatty acid (FFA) availability cannot fully explain the decrease in fatty acid oxidation because, when plasma FFA concentration was raised to 2 mM during exercise at 85% $\dot{V}O_{2\,peak}$, it remained lower than during exercise at 65% $\dot{V}O_{2\,peak}$, although fatty acid oxidation increased (12, 13). We have recently presented data supporting the hypothesis that the intracellular glucose availability determines the nature of substrate oxidation in human volunteers at rest (18). Specifically, we have shown that an increase in glycolytic flux, by infusing insulin and glucose, decreases fat oxidation by inhibiting fatty acid entry into the mitochondria (17). This concept has been previously tested in isolated rat hearts, in which it was shown that increased glucose-derived acetyl-CoA levels activated acetyl-CoA carboxylase, resulting in an increase in malonyl-CoA inhibition of fatty acid oxidation (14). In the present study, we tested the hypothesis that fatty acid oxidation de-

creases during high-intensity exercise because of inhibition of long-chain fatty acid (LCFA) entry into the mitochondria, possibly mediated by the increase in glycolytic flux during high-intensity exercise.

The mechanism by which glycolytic flux may regulate fatty acid oxidation is not well understood. A potential mechanism has been proposed from work in rat livers (7). Activated LCFA must bind to carnitine, a reaction catalyzed by the enzyme carnitine palmitoyltransferase I (CPT-I), to enter into the mitochondrial matrix (5). The product of this reaction, fatty acylcarnitine, is transported across the inner mitochondrial membrane via the carnitine-acylcarnitine translocase system (11). Findings from in vitro studies suggest that increased pyruvate availability increases malonyl-CoA formation (7–9), which inhibits CPT-I (7), thereby decreasing fatty acid oxidation. We have recently demonstrated that this mechanism may operate in human volunteers at rest, based on the observation that accelerated glycolytic flux inhibited LCFA but not CPT-I-independent medium-chain fatty acid (MCFA) oxidation (17).

The mechanism(s) regulating fatty acid oxidation during exercise might be different from that at rest. Unlike that in the resting state (17), the ratio of intramitochondrial acetyl-CoA to nonesterified CoA increases as exercise intensity increases (4), which could decrease fatty acid β-oxidation via feedback inhibition of 3-ketoacyl-CoA thiolase (15). If this is the case, then both LCFA and MCFA oxidation would be affected because both undergo β-oxidation once they have entered the mitochondria.

The aim of the present study was to test the hypothesis that inhibition of LCFA entry into mitochondria is, at least in part, responsible for the decrease in fatty acid oxidation during high-intensity exercise. We compared LCFA and MCFA oxidation during exercise at 40 and 80% $\dot{V}O_{2\,peak}$ in human volunteers. Isotopic tracers, [1-^{13}C]oleate and [1-^{14}C]octanoate, were infused to determine LCFA and MCFA oxidation, respectively. Unlike oleate, which requires CPT-I for oxidation, octanoate can freely diffuse across the inner mitochondrial membrane (5). Therefore, if inhibition of LCFA entry into the mitochondria decreases fatty acid oxidation during exercise at 80% $\dot{V}O_{2\,peak}$, oleate, but not octanoate, oxidation should decrease in the transition from low- to high-intensity exercise.

METHODS

Volunteers

Six male volunteers (age 30 ± 3 yr, weight 74 ± 8 kg, height 173.8 ± 4.0 cm, $\dot{V}O_{2\,peak}$ 34.1 ± 3.2 ml $\cdot kg^{-1} \cdot min^{-1}$) partici-

pated in this study. All volunteers were healthy, as indicated by comprehensive history, physical examination, and standard blood and urine tests, and consented to participate in this study, which was approved by the Institutional Review Board and the General Clinical Research Center of the University of Texas Medical Branch at Galveston.

Experimental Design

All experiments were performed in the morning, after the volunteers had fasted overnight (i.e., 12 h). Teflon catheters were placed percutaneously into an antecubital vein for isotope infusion and into a contralateral dorsal hand vein, which was heated for sampling of arterialized venous blood. After blood and breath samples were obtained for the determination of background enrichments, one of the following two experimental protocols was performed.

Protocol 1. The volunteers performed exercise either at 40% $\dot{V}o_{2peak}$ for 60 min (Fig. 1, *upper panel*) or at 80% $\dot{V}o_{2peak}$ for 30 min (Fig. 1, *lower panel*). The order of the trials was randomized and ~1 wk separated the two trials. At the beginning of exercise, an infusion of [1-14C]octanoate (ICN Radiochemicals, Irvine CA; prime = 16.0 nCi/kg, constant infusion = 1.0 $nCi \cdot kg^{-1} \cdot min^{-1}$) and [1-13C]oleate (99% enriched, MSD Isotopes, Montreal, Canada; 0.15 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), MCFAs and LCFAs, respectively, were started and continued for the duration of the exercise. The bicarbonate pools were primed via bolus infusions of NaH14CO3 (30 nCi/kg) and NaH13CO3 (5.5 $\mu mol/kg$) at the beginning of the study. During the 80% $\dot{V}o_{2peak}$ trial, a lipid emulsion (Intralipid 20%, Kabi, Clayton, NC; 0.5 $ml \cdot kg^{-1} \cdot h^{-1}$) containing LCFA [linoleic (50%), oleic (26%), palmitic (10%), linolenic (9%), and stearic (3.5%)] was infused together with heparin (Elkins Sinn, Cherry Hill, NJ; bolus of 7.0 U/kg; continuous infusion of 7.0 $U \cdot kg^{-1} \cdot h^{-1}$). Lipids were given during exercise at 80% $\dot{V}o_{2peak}$ to prevent the expected decline in plasma FFA concentration observed during strenuous exercise (12). It was not necessary to infuse octanoate during exercise at 80% $\dot{V}o_{2peak}$, since there is virtually no octanoate in plasma, and thus the specific activity (SA) of plasma octanoate should be the same as the SA of the octanoate infusate in both groups.

Protocol 2. On separate occasions the volunteers repeated *protocol 1* (Fig. 1) without isotope infusion. Only breath samples were collected during these tests and were used to quantify changes in breath carbon dioxide (CO_2) carbon enrichment due to the shift in substrate mix observed in the transition from rest to exercise (23).

Blood samples were obtained before tracer infusion and every 5 min during the infusion for determination of plasma oleate enrichment and plasma triacylglycerols (TG), FFA, insulin, lactate, and glucose concentration. Breath samples for determination of $^{13}CO_2$ enrichment and $^{14}CO_2$ SA were collected before tracer infusion and every 5 min during the study. Indirect calorimetry was performed during the last 10 min of each study for estimation of $\dot{V}o_2$ and carbon dioxide production ($\dot{V}co_2$).

Assays

Expired air for measurement of $^{14}CO_2$ SA was collected in 3-liter anesthesia bags, and CO_2 was trapped by bubbling through a 1:4:9 solution of phenolphthalein (0.1% solution; Fisher Scientific, Fair Lawn, NJ), benzethonium hydroxide (1.0 M solution; Sigma Chemical, St Louis, MO), and absolute ethanol to trap exactly 1 mmol of CO_2. Scintillation fluid (10 ml of toluene, 0.04% 2,5-diphenyloxazole; Sigma Chemical, St. Louis, MO) was added immediately in the vial, and $^{14}CO_2$ SA (disintegrations $\cdot$ min$^{-1} \cdot$ mmol^{-1}) was determined using a liquid scintillation counter. Ten milliliters of expired air were injected into evacuated tubes for determination of the $^{13}CO_2$-to-$^{12}CO_2$ ratio. Briefly, CO_2 was isolated from the breath samples before analysis by isotope ratio mass spectrometry by passage through a water trap, followed by condensation in a liquid nitrogen trap to allow other gases to be evacuated. The $^{13}CO_2$-to-$^{12}CO_2$ ratio was then determined with a SIRA VG Isotech triple-collector isotope ratio mass spectrometer (Cheshire, UK). The ratio is reported in units of tracer-to-tracee ratio (TTR), which is defined as: $TTR = (^{13}C/^{12}C)sa - (^{13}C/^{12}C)ref - [(^{13}C/^{12}C)bk - (^{13}C/^{12}C)ref]$, where sa is sample, ref is reference gas, and bk is baseline sample.

Blood samples (6 ml) were collected into prechilled tubes containing 120 μl of 0.2 M ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, and plasma was immediately separated by centrifugation and frozen until further processing. This procedure prevents in vitro lipolysis of TG in plasma from volunteers who were given heparin (Sidossis and Wolfe, unpublished observation). Plasma oleate enrichment was determined by following previously described procedures (22). Briefly, FFA were extracted from plasma, isolated by thin-layer chromatography, and converted to their methyl esters. The isotopic enrichment of oleate was determined by gas chromatography-mass spectrometry (Hewlett-Packard 5992) by selectively monitoring ions' mass-to-charge ratios of 296 and 297.

Plasma glucose and lactate concentrations were measured on a glucose-lactate analyzer (2300 STAT; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin concentration was determined using a radioimmunoassay method (Incstar, Stillwater, MN). Plasma TG concentration was measured enzymatically (RA-500; Technicon Instruments, Terrytown, NY). Plasma oleate and total FFA concentrations were determined by gas chromatography (Hewlett-Packard, 5890) with the use of heptadecanoic acid as an internal standard.

Calculations

Carbohydrate and total fatty acid oxidation rates were calculated from the indirect calorimetry data using stoichiometric equations (3). $\dot{V}o_2$ and $\dot{V}co_2$ values were the average over the last 10 min of each study. Fatty acid oxidation was determined by converting the rate of TG oxidation
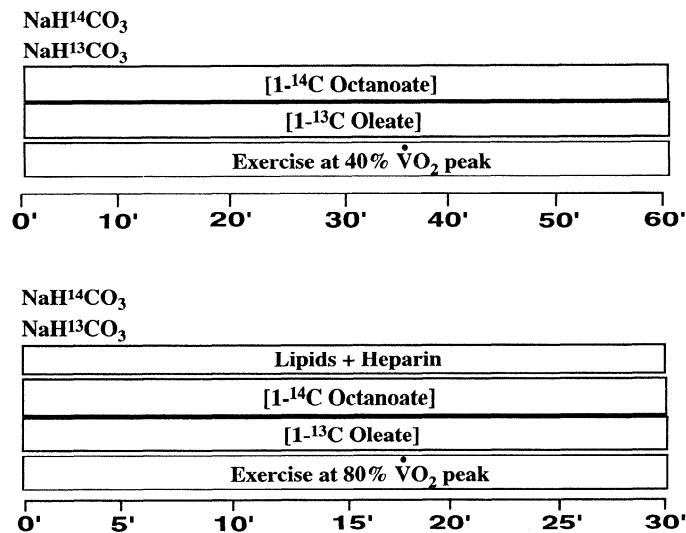
NaH14CO3
NaH13CO3

| [1-14C Octanoate] |
| [1-13C Oleate] |
| Exercise at 40% $\dot{V}O_2$ peak |

| 0' | 10' | 20' | 30' | 40' | 50' | 60' |

NaH14CO3
NaH13CO3

| Lipids + Heparin |
| [1-14C Octanoate] |
| [1-13C Oleate] |
| Exercise at 80% $\dot{V}O_2$ peak |

| 0' | 5' | 10' | 15' | 20' | 25' | 30' |

Fig. 1. Schematic representation of study design. *Upper panel*: exercise at 40% peak oxygen consumption ($\dot{V}o_{2peak}$). *Lower panel*: exercise at 80% $\dot{V}o_{2peak}$.
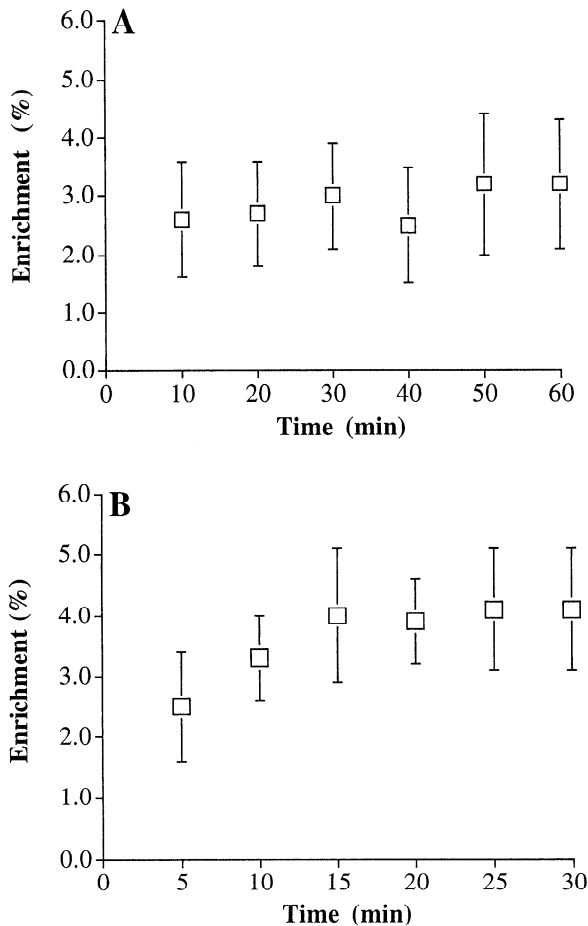
Fig. 2. Plasma oleate carbon enrichment during exercise at 40 (A) and 80% $\dot{V}O_{2peak}$ (B). Values are means ± SE for 6 volunteers.

$(g \cdot kg^{-1} \cdot min^{-1})$ to its molar equivalent, assuming the average molecular mass of TG to be 860 g/mol (3), and multiplying the molar rate of TG oxidation by three because each molecule contains three moles of fatty acids.

The rate of appearance (R_a) of oleate in plasma was calculated as: $R_a = F/E_o$, where F is oleate tracer infusion rate and E_o is oleate carbon enrichment in plasma (the mole fraction of C-13 oleate) averaged over the last 10 min of each study. The steady-state equation was used in the calculation of R_a, since our subjects reached an isotopic plateau in plasma oleate carbon enrichment during both exercise intensities (Fig. 2). FFA R_a was derived by dividing the R_a of oleate by the fractional contribution of oleate to the total FFA concentration, as determined by gas chromatography. The values for R_a at 80% $\dot{V}O_{2peak}$ are the result of both endogenous and exogenous (i.e., infused lipids) sources.

The rates of $^{13}CO_2$ and $^{14}CO_2$ excretion, resulting from the oxidation of [1-^{13}C]oleate and [1-^{14}C]octanoate, respectively, were calculated as: labeled CO_2 excretion = $\dot{V}CO_2 \times ECO_2$, where ECO_2 is breath CO_2 carbon enrichment averaged over the last 10 min of each study. In some untrained individuals exercising at high intensities, breath $\dot{V}CO_2$ overestimates cellular CO_2 production, as indicated by the fact that the respiratory exchange ratio exceeds one. Thus, when the respiratory exchange ratio exceeded one, we used an esti-mated $\dot{V}CO_2$, calculated by assuming a respiratory exchange ratio of one. When the estimated $\dot{V}CO_2$ is used, "labeled CO_2 excretion," although still an overestimation, is a more accu-rate representation of the true cellular CO_2 production.

The absolute rates of [1-^{13}C]oleate and [1-^{14}C]octanoate oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) were then calculated as: sub-strate oxidation = labeled CO_2 excretion/$E_o \times$ ar, where ar is the acetate correction factor estimated from Ref. 16. The SA of the octanoate infusion mixture was used as the octanoate enrichment in plasma, since there is virtually no octanoate in plasma. Plasma fatty acid oxidation was determined by dividing the R_a of oleate by the fractional contribution of oleate to the total FFA concentration, as determined by gas chromatography.

For the determination of breath carbon enrichment during exercise in *protocol 1*, the values for breath carbon enrich-ment obtained during exercise in *protocol 2* (i.e., without tracer infusion) were used as the background value (see experimental *protocol 2*).

Statistical Analysis

Differences between plasma oleate, TG, FFA, oleate, glu-cose, insulin, and lactate concentrations, and the calculated rates of fatty acid kinetics and oxidation during exercise at 40 and 80% $\dot{V}O_{2peak}$ (*protocol 1*), were identified using a paired *t*-test. Statistical significance was considered present if $P < 0.05$. Values are presented as means ± SE.

RESULTS

The volunteers exercised at 38 ± 2% $\dot{V}O_{2peak}$ and at 81 ± 2% $\dot{V}O_{2peak}$ during the "40% $\dot{V}O_{2peak}$" and at "80% $\dot{V}O_{2peak}$" trials, respectively. Plasma substrate and hor-mone concentrations are presented in Table 1 as the average of the last 10 min of both exercise intensities. Plasma oleate, TG, insulin, and glucose concentrations were similar in the two trials. Plasma FFA concentra-tion was maintained constant via infusion of lipids and heparin during exercise at 80% $\dot{V}O_{2peak}$ (Fig. 3). Plasma lactate concentration increased from 1.2 mM during exercise at 40% $\dot{V}O_{2peak}$ to 6.4 mM during exercise at 80% $\dot{V}O_{2peak}$ ($P < 0.05$; Fig. 4). Plasma oleate enrich-ment reached an apparent plateau within 15 min from the beginning of the exercise during both exercise intensities (Fig. 2).

Oleate and total plasma FFA oxidation significantly decreased during exercise at 80% $\dot{V}O_{2peak}$ (Table 2). In contrast, octanoate oxidation increased significantly during exercise at 80% $\dot{V}O_{2peak}$ (Table 2). The percent-age of oleate tracer that was taken up by cells and oxidized decreased from 67.7 ± 2.8% during exercise at 40% $\dot{V}O_{2peak}$ to 51.8 ± 4.6% during exercise at 80% $\dot{V}O_{2peak}$ ($P < 0.05$; Fig. 5), whereas the percentage of octanoate oxidized did not change significantly (84.8 ±

Table 1. *Substrate and hormone concentrations during exercise*

	40% $\dot{V}O_{2peak}$	80% $\dot{V}O_{2peak}$
Triacylglycerol, mmol/l	1.5±0.3	1.6±0.2
FFA, mmol/l	0.40±0.11	0.37±0.04
Oleate, mmol/l	0.16±0.05	0.14±0.02
Glucose, mmol/l	5.0±0.1	5.1±0.1
Lactate, mmol/l	1.2±0.1	6.4±0.4*
Insulin, μU/ml	22.0±6.1	25.6±6.7

Values are means ± SE for 6 volunteers. $\dot{V}O_{2peak}$, peak oxygen consumption; FFA, free fatty acid. *$P < 0.05$ vs. exercise at 40% $\dot{V}O_{2peak}$.
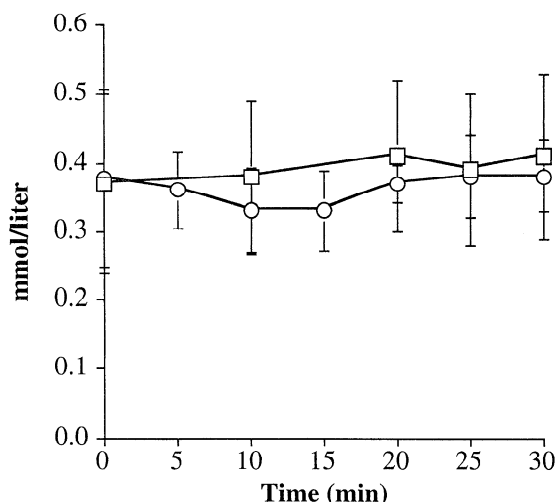
Fig. 3. Plasma free fatty acid concentration during final 30 min of exercise at 40 (□) and 80% $\dot{V}O_{2peak}$ (○). Values are means ± SE for 6 volunteers.

Table 2. *Fatty acid kinetics and oxidation during exercise*

	40% $\dot{V}O_{2peak}$	80% $\dot{V}O_{2peak}$
Oleate R_a	3.8 ± 0.8	4.5 ± 0.9
FFA R_a	11.6 ± 2.2	12.4 ± 2.6
Oleate oxidation	2.8 ± 0.6	$1.8 \pm 0.2^*$
Octanoate oxidation	$1.0e^{-05} + 1.0e^{-06}$	$1.3e^{-05} + 5.1e^{-06}$ *
Plasma fatty acid oxidation	8.3 ± 1.5	$4.9 \pm 1.0^*$
Total fatty acid oxidation	8.8 ± 1.1	$0.1 \pm 1.1†$

Values are means ± SE for 6 volunteers and are in μmol kg^{-1}·min^{-1}; R_a, rate of appearance. $^*P < 0.05$ vs. exercise at 40% $\dot{V}O_{2peak}$; †difference between plasma fatty acid oxidation (tracer methodology) and total fatty acid oxidation (indirect calorimetry) suggests that indirect calorimetry significantly underestimates fatty acid oxidation during high-intensity exercise in untrained men.

2.7 and 89.3 ± 2.7% during exercise at 40 and 80% $\dot{V}O_{2peak}$, respectively; Fig. 5). Total fatty acid oxidation, calculated from indirect calorimetry, decreased from 8.8 ± 1.1 (40% $\dot{V}O_{2peak}$) to 0.1 ± 1.1 μmol·kg^{-1}·min^{-1} (80% $\dot{V}O_{2peak}$). The difference observed between plasma fatty acid oxidation (tracer methodology) and total fatty acid oxidation (indirect calorimetry) questions the validity of indirect calorimetry in estimating substrate oxidation rates during exercise at 80% $\dot{V}O_{2peak}$ in untrained or moderately fit volunteers. Furthermore, because the $\dot{V}CO_2$ values are also used to estimate plasma fatty acid oxidation (tracer methodology), the calculated oleate and plasma fatty acid oxidation during exercise at 80% $\dot{V}O_{2peak}$ may represent overestimates of the true values. Nonetheless, the comparison of oleate and octanoate remains valid in this possible circumstance, because each value would be overestimated to the same extent.

When exercise was performed with no isotope infusion (*protocol 2*), breath carbon enrichment decreased
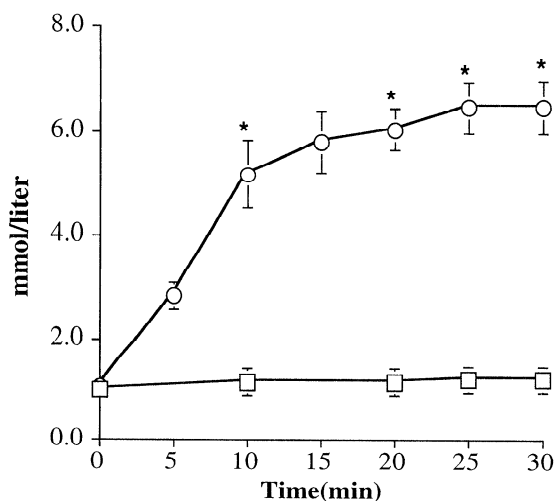
from baseline by $6.75e^{-07} \pm 3.8e^{-06}$ during exercise at 40% $\dot{V}O_{2peak}$, whereas it increased by $1.01e^{-05} \pm 6.0e^{-06}$ during exercise at 80% $\dot{V}O_{2peak}$. These changes reflect alterations in fuel utilization during exercise (23).

DISCUSSION

We have recently suggested that glycolytic flux may regulate fat oxidation at rest by determining the rate of LCFA entry into the mitochondria (17). In the present study, we examined the hypothesis that fatty acid oxidation decreases during high-intensity exercise because of inhibition of LCFA entry into the mitochondria, possibly mediated by the increase in glycolytic flux during high-intensity exercise. Oleate oxidation significantly decreased, whereas octanoate oxidation increased, during exercise at 80% compared with 40% $\dot{V}O_{2peak}$. Furthermore, the percentage of oleate uptake that was oxidized significantly decreased during exercise at 80% $\dot{V}O_{2peak}$, whereas the percentage of octanoate oxidized did not change. These data suggest that fatty acid oxidation during high-intensity exercise is likely limited by inhibition of LCFA entry into mitochondria.

Oleate and octanoate oxidation values were compared on the basis of the assumption that the only difference in the oxidation of these fatty acids is the need for oleate to utilize the CPT-I for transport across the inner mitochondrial membrane. McGarry and Foster (6) tested this hypothesis by comparing relative

Fig. 4. Plasma lactate concentration during final 30 min of exercise at 40 (□) and 80% $\dot{V}O_{2peak}$ (○). Values are means ± SE for 6 volunteers; $^*P < 0.05$ vs. exercise at 40% $\dot{V}O_{2peak}$.
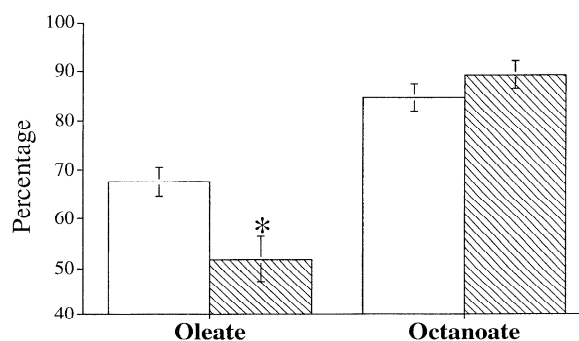

Fig. 5. Percentage of oleate and octanoate tracer uptake that was oxidized during exercise at 40 (open bars) and 80% $\dot{V}O_{2peak}$ (hatched bars). Means ± SE for 6 volunteers; $^*P < 0.05$. vs. exercise at 40% $\dot{V}O_{2peak}$.

rates of oxidation of (−)-octanoylcarnitine, octanoate, and oleate in perfused rat livers. Their findings support the above assumption.

Although it is known that both oleate and octanoate can be oxidized by most tissues in the body (2, 19, 20), the exact proportionality of their oxidation in different tissues is uncertain. However, for the comparison of oleate and octanoate oxidation to be a valid model for inhibition of LCFA into the mitochondria, it is not necessary that both substrates be proportionally oxidized in all tissues. Nevertheless, the observed increase in MCFA oxidation during high-intensity exercise most probably occurred in skeletal muscle, the major site of LCFA oxidation during exercise. This is because splanchnic MCFA oxidation is expected to decrease rather than increase during high-intensity exercise due to a decrease in splanchnic blood flow (1, 21) and the absence of any increased metabolic activity. Thus the observed changes in oleate and octanoate oxidation during exercise at 80% $\dot{V}O_{2\,peak}$ most probably represent changes occurring primarily in the skeletal muscles of the volunteers.

The rate of FFA appearance decreases as exercise intensity increases over 65% $\dot{V}O_{2\,peak}$, which results in a decrease in plasma FFA concentration (12). To maintain constant FFA supply to the cells and to keep the isotopic enrichment of oleate precursor similar during exercise at 40 and 80% $\dot{V}O_{2\,peak}$, we infused lipids and heparin during exercise at 80% $\dot{V}O_{2\,peak}$. Heparin was given to release lipoprotein lipase from the capillary endothelial surfaces and thus facilitate the breakdown of TG in the circulation. There was no need to infuse octanoate during exercise at 80% $\dot{V}O_{2\,peak}$, since there is virtually no octanoate in plasma, and thus no changes in plasma octanoate concentration and SA were expected.

The relative concentration of fatty acids in plasma may affect their kinetics and oxidation. To prevent this effect, we maintained a constant concentration of oleate during the two exercise intensities to have a valid comparison for oleate oxidation during exercise at 40 vs. 80% $\dot{V}O_{2\,peak}$. On the other hand, oleate and octanoate concentrations were significantly different, which raises the possibility that the observed differences in their kinetics and oxidation may have been due to differences in their relative concentrations. To compare the two fatty acids at similar concentrations we could have either decreased the oleate concentration or increased octanoate concentration. The situation of an extremely low oleate concentration, although potentially interesting, would be difficult to test experimentally because of the lack of effective means of reducing the oleate levels to that extent. For this reason, we increased the octanoate concentration in two experiments via infusion of unlabeled octanoate. Because the oxidation of octanoate is not dependent on the kinetics of CPT-I, the nature of response of octanoate oxidation to exercise would be expected to be unaffected by concentration, within a reasonable range. In these studies the percentage of octanoate tracer oxidized increased from 85.0 to 92.9%, and octanoate

tracer oxidation increased from $1.1e^{-05}$ to $1.3e^{-05}$ $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during exercise at 80% compared with 40% $\dot{V}O_{2\,peak}$. These data suggest that the observed differences between octanoate and oleate tracer oxidation were not due to differences in their relative concentrations. Because the two experiments in which unlabeled octanoate was infused confirmed our expectation that octanoate oxidation would be unaffected by concentration, we did not perform any further experiments manipulating the octanoate oxidation.

The acetyl-CoA-to-CoA ratio increases significantly during exercise at 80 vs. 40% $\dot{V}O_{2\,peak}$ (L. S. Sidossis, G. D. Lopaschuk, and R. R. Wolfe, unpublished observation). An increase in the acetyl-CoA-to-CoA ratio could, theoretically, decrease β-oxidation via feedback inhibition of 3-ketoacyl-CoA thiolase (15). However, the results of the present study do not support this hypothesis because, unlike oleate, octanoate oxidation significantly increased during exercise at 80% $\dot{V}O_{2\,peak}$. This would not be possible if β-oxidation were inhibited.

Accelerated glycolytic flux could decrease fatty acid oxidation by inhibiting CPT-I activity (17). During intense muscular activity, such as cycling at 80% $\dot{V}O_{2\,peak}$ in the present study, glycogenolysis and glycolysis are greatly stimulated, resulting in high rates of pyruvate and acetyl-CoA formation. Increased carbon flow through acetyl-CoA carboxylase could inhibit CPT-I activity and thus fatty acid entry into the mitochondria, probably via increased malonyl-CoA concentration. Impairment of the availability of substrate for β-oxidation would therefore limit fatty acid-derived acetyl-CoA formation and oxidation.

Although the exact site(s) of inhibition of fatty acid oxidation during exercise at 80% $\dot{V}O_{2\,peak}$ cannot be proved from the present study, we can, however, exclude some of the potentially limiting steps. Thus it does not seem likely that fatty acid oxidation was inhibited due to suboptimal plasma FFA availability, since the rate of FFA appearance and plasma FFA concentration were made similar in the two trials via infusion of lipids during exercise at 80% $\dot{V}O_{2\,peak}$. The role of cytosolic fatty acid binding proteins in fatty acid oxidation has not been fully elucidated (10), but the fact that they are abundantly expressed in cells makes it seem unlikely that they could be limiting in fatty acid oxidation. The only other sites where fatty acid regulation could occur are the activation step, catalyzed by the enzyme acyl-CoA synthetase (or thiokinase), and the esterification to carnitine, a reaction catalyzed by CPT-I. On the basis of the observation that, unlike octanoate, oleate oxidation significantly decreased during exercise at 80% $\dot{V}O_{2\,peak}$, we conclude that, in addition to suboptimal FFA availability, fatty acid oxidation is likely limited during high-intensity exercise by inhibition of LCFA entry into mitochondria.

REFERENCES

1. **De Boer, A., C. Kluft, J. M. Kroon, F. J. Kasper, H. C. Schoemaker, J. Pruis, D. D. Breimer, P. A. Soons, J. J. Emeis, and A. F. Cohen.** Liver blood flow as a major determinant of the clearance of recombinant human tissue-type plasminogen activator. *Thromb. Haemostasis* 67: 83–87, 1992.

2. **Even, P. C., A. Decrouy, and A. Chinet.** Defective regulation of energy metabolism in mdx-mouse skeletal muscles. *Biochem. J.* 304: 649–654, 1994.

3. **Frayn, K. H.** Calculation of substrate oxidation rates in vivo from gaseous exchange. *J. Appl. Physiol.* 55: 628–634, 1983.

4. **Friolet, R., H. Hoppeler, and S. Krahenbuhl.** Relationship between the coenzyme A and the carnitine pools in human skeletal muscle at rest and after exhaustive exercise under normoxic and acutely hypoxic conditions. *J. Clin. Invest.* 94: 1490–1495, 1994.

5. **Fritz, I. B.** Action of carnitine on long chain fatty acid oxidation by liver. *Am. J. Physiol.* 197: 297–304, 1959.

6. **McGarry, J. D., and D. W. Foster.** The metabolism of (−)-octanoylcarnitine in perfused livers from fed and fasted rats: evidence for a possible regulatory role of carnitine acyltransferase in the control of ketogenesis. *J. Biol. Chem.* 249: 7984–7990, 1974.

7. **McGarry, J. D., G. P. Mannaerts, and D. W. Foster.** A possible role for malonyl-CoA in the regulation of hepatic acid oxidation and ketogenesis. *J. Clin. Invest.* 60: 265–270, 1977.

8. **McGarry, J. D., S. E. Milles, C. S. Long, and D. W. Foster.** Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. *Biochem. J.* 214: 21–28, 1983.

9. **McGarry, J. D., K. F. Woeltje, M. Kuwajima, and D. W. Foster.** Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. *Diabetes Metab. Rev.* 5: 271–284, 1989.

10. **Ockner, R. K., R. M. Kaikaus, and N. M. Bass.** Fatty acid binding proteins: recent concepts of regulation and function. *Prog. Clin. Biol. Res.* 375: 189–204, 1992.

11. **Pande, S. V.** A mitochondrial carnitine acyltransferase translocase system. *Proc. Natl. Acad. Sci. USA* 72: 883–887, 1975.

12. **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.

13. **Romijn, J. A., E. F. Coyle, L. S. Sidossis, X. J. Zhang, and R. R. Wolfe.** Relationship between fatty acid delivery and fatty acid oxidation during strenuous exercise. *J. Appl. Physiol.* 79: 1939–1945, 1995.

14. **Saddik, M., J. Gamble, L. A. Witters, and G. D. Lopaschuk.** Acetyl-CoA carboxylase regulation of fatty acid oxidation in the heart. *J. Biol. Chem.* 268: 25836–25845, 1993.

15. **Schulz, H.** Regulation of fatty acid oxidation in heart. *J. Nutr.* 124: 165–171, 1994.

16. **Sidossis, L. S., A. R. Coggan, A. Gastaldelli, and R. R. Wolfe.** A new correction factor for use in tracer estimations of plasma fatty acid oxidation. *Am. J. Physiol.* 269 (*Endocrinol. Metab.* 32): E649–E656, 1995.

17. **Sidossis, L. S., C. A. Stuart, G. I. Shulman, G. D. Lopaschuk, and R. R. Wolfe.** Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. *J. Clin. Invest.* 98: 2244–2250, 1996.

18. **Sidossis, L. S., and R. R. Wolfe.** Glucose and insulin-induced inhibition of fatty acid oxidation: the glucose-fatty acid cycle reversed. *Am. J. Physiol.* 270 (*Endocrinol. Metab.* 33): E733–E738, 1996.

19. **Van Kempen, T. A., and J. Odle.** Carnitine affects octanoate oxidation to carbon dioxide and dicarboxylic acids in colostrum-deprived piglets: in vivo analysis of mechanisms involved based on CoA- and carnitine-ester profiles. *J. Nutr.* 125: 238–250, 1995.

20. **Veerkamp, J. H., H. T. van Moerkerk, and J. A. Bakkeren.** An accurate and sensitive assay of [^{14}C]octanoate oxidation and its application on tissue homogenates and fibroblasts. *Biochim. Biophys. Acta* 876: 133–137, 1986.

21. **Wasserman, D. H., D. B. Lacy, and D. P. Bracy.** Relationship between arterial and portal vein immunoreactive glucagon during exercise. *J. Appl. Physiol.* 75: 724–729, 1993.

22. **Wolfe, R. R.** *Radioactive and Stable Isotope Tracers in Biomedicine.* New York: Wiley-Liss, 1992, p. 422–424.

23. **Wolfe, R. R., J. H. Shaw, E. R. Nadel, and M. H. Wolfe.** Effect of substrate intake and physiological state on background ^{13}CO$_2$ enrichment. *J. Appl. Physiol.* 56: 230–234, 1984.