Increase in Fat Oxidation on a High-Fat Diet Is Accompanied by an Increase in Triglyceride-Derived Fatty Acid Oxidation

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The aim of this study is to investigate the mechanism behind the slow increase in fat oxidation on a high-fat diet. Therefore, we determined 24-h substrate oxidation using respiration chambers and the rate of appearance and oxidation of plasma-derived fatty acids in seven healthy nonobese men (age 23 ± 2 years, height 1.85 ± 0.03 m, weight 70.4 ± 2.3 kg, % body fat 13 ± 1). Before testing, they consumed a low-fat diet (30% fat, 55% carbohydrate) at home for 3 days. Measurements were performed after 1 day consumption of either a low-fat diet (LF), a high-fat diet (HF1, 60% fat, 25% carbohydrate), or a high-fat diet preceded by a glycogen-lowering exercise test (HF1+EX), and after 7 days on a high-fat diet (HF7). After an overnight fast, an infusion of [U-^{13}C]palmitate (0.00806 μmol · min^{-1} · kg^{-1}) was started and continued for 2 h at rest followed by 1 h of exercise at 50% of maximal power output (W_{max}). Whole-body fat oxidation was measured using indirect calorimetry, and plasma-derived fatty acid oxidation was evaluated by measuring breath $^{13}CO_2$ enrichment and corrected with the acetate recovery factor. Twenty-four–hour fat oxidation gradually increased on the high-fat diet. Both at rest and during exercise, there was no change in rate of appearance of fatty acids and plasma-derived fatty acid oxidation. Triglyceride-derived fatty acid oxidation tended to be higher after 7 days of high-fat diet at rest ($P < 0.07$). This difference was significant during exercise ($P < 0.05$). In conclusion, the results from this study suggest that triglyceride-derived fatty acid oxidation (VLDL or intramuscular triglycerides) plays a role in the increase in fat oxidation on a high-fat diet, but plasma-derived fatty acids remain the major source for fat oxidation. *Diabetes* 49:640–646, 2000

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CPT-I, carnitine palmitoyltransferase-I; FFA, free fatty acid; FQ, food quotient; GC, gas chromatography; HF, high-fat diet; HF1+EX, high-fat diet with prior glycogen-lowering exercise; IRMS, isotope ratio mass spectrometry; LF, low-fat diet; LPL, lipoprotein lipase; R_a, rate of appearance; R_d, rate of disappearance; RQ, respiratory quotient; TCA, tricarboxylic acid; TTR, tracer/tracee ratio; W_{max}, maximal power output.

The prevalence of obesity and diabetes in Westernized societies is still increasing. This increase has often been attributed to the increase in fat intake and decrease in physical activity. It has repeatedly been shown that on a high-fat diet, fat oxidation is not rapidly adjusted to fat intake, resulting in positive fat balance (1–3). We recently demonstrated that after a switch from a low-fat to a high-fat diet, fat oxidation only gradually increased and equaled fat intake after 7 days (4). Because carbohydrate and fat metabolism are closely interrelated, we hypothesized that on the high-fat diet, a gradual decrease in muscle glucose oxidation caused by a reduced blood glucose availability and decreasing glycogen stores were responsible for the increase in fat oxidation. Indeed, we recently showed that fat oxidation could be raised rapidly on a high-fat diet when glycogen stores were lowered by exhaustive exercise (5,6).

The mechanism by which consumption of a high-fat diet and/or decrease in glycogen stores leads to an increase in fat oxidation is not clear. Insulin might play a role, because insulin is known to be an important inhibitor of lipolysis (7). On a high-fat diet, decreasing insulin levels might lead to less inhibition of lipolysis, resulting in higher free fatty acid (FFA) availability and increased plasma FFA oxidation. However, it has recently been suggested that not FFA availability, but glucose availability determines the mix of substrates being oxidized (8,9). In rats, exercise-induced glycogen depletion did not result in increased plasma FFA uptake and oxidation, but it increased uptake and oxidation of plasma glucose as long as blood glucose concentration was maintained (9). When total carbohydrate availability decreased, an increase in intramuscular triglyceride oxidation was observed (10). These results might explain that on a high-fat diet (which equals lower exogenous carbohydrate availability), a decrease in glycogen stores is necessary before fat oxidation will increase and suggest that the increase in fat oxidation would be derived from triglycerides. Finally, it has been shown that high-fat feeding increases lipoprotein lipase (LPL) activity in the muscle capillary bed, possibly allowing for increased VLDL triglyceride oxidation (11).

Therefore, the aims of the present study were *1*) to investigate the mechanism behind the gradually increasing fat oxidation on a high-fat diet and *2*) to identify the different fat sources contributing to this increased fat oxidation. The 24-h substrate oxidation and substrate balances were measured to

TABLE 1
Subject characteristics

Age (years)	23.1 ± 1.8
Height (m)	1.85 ± 0.03
Weight (kg)	70.4 ± 2.3
Body fat (%)	13 ± 1
BMI (kg/m^2)	20.6 ± 0.6
W_{max} (W)	267 ± 22
W_{max}/kg (W)	3.8 ± 0.2

Data are means ± SE.

indirectly assess changes in glycogen stores in seven healthy nonobese men on a low-fat and 7 days of a high-fat diet. FFA availability, plasma FFA oxidation, triglyceride-derived fatty acid oxidation, and total fat oxidation in rest and during exercise were measured using stable isotopes techniques. To further examine the role of the glycogen stores in the increase in fat oxidation on a high-fat diet, measurements were also performed with subjects on a high-fat diet preceded by a bout of glycogen-lowering exercise.

RESEARCH DESIGN AND METHODS

Subjects. The characteristics of the seven healthy nonobese male volunteers are presented in Table 1. The nature and risks of the experimental procedure were explained to the subjects, and all subjects gave their written informed consent. The study was approved by the Medical-Ethical Committee of Maastricht University.

Experimental design. The experimental design is presented in Fig. 1. The experiment consisted of three treatments: high-fat diet (HF), low-fat diet (LF), and high-fat diet with prior glycogen-lowering exercise (HF1+EX). Before all treatments, subjects consumed a low-fat diet for 3 days at home (days 1–3).

HF1+EX and LF treatment. HF1+EX and LF treatments were performed twice using different tracers—once with [U-^{13}C]palmitate infusion and once with [1,2-^{13}C]acetate infusion to determine the acetate recovery factor (12) under the different experimental circumstances. On the third day on the low-fat diet, subjects came to the laboratory. In the HF1+EX treatment, subjects came to the laboratory at 1500 to perform a glycogen depletion test on a cycle ergometer. After completing this test, subjects entered a respiration chamber for a 36-h stay. During the stay in the respiration chamber, they were given a high-fat diet. In the LF treatment, subjects came to the laboratory at 1800 for a 36-h stay in the respiration chamber without performing a glycogen depletion test, and they consumed a low-fat diet. On the morning of day 5, subjects left the respiration chamber at 0800 and subsequently underwent an isotope infusion test with either [U-^{13}C]palmitate infusion or [1,2-^{13}C]acetate infusion. After this, the HF1+EX and LF treatments were ended.

HF treatment. The HF treatment consisted of four 36-h periods in the respiration chamber, starting on the evening of days 3, 5, 7, and 9. During this whole period, subjects consumed a high-fat diet. On the morning of day 5 (HF1) and day 11 (HF7), after leaving the respiration chamber, subjects underwent a [U-^{13}C]palmitate infusion test. On the morning of day 9, they underwent a [1,2-^{13}C]acetate infusion test. The calculated acetate recovery factor on day 9 was used to correct plasma FFA oxidation on day 5 (HF1) and day 11 (HF7) of the HF treatment.

Isotope infusion test. In the morning at 0800, after leaving the respiration chamber, subjects underwent an isotope infusion test. Teflon catheters were inserted in an antecubital vein for isotope infusion and retrogradely into a contralateral dorsal hand vein for sampling of arterialized venous blood. After placement of the catheters, subjects rested on a bed. To obtain arterialized venous blood, the cannulated hand was placed in a hot box where air was circulated at 60°C. After 30 min, baseline oxygen consumption and carbon dioxide production was measured, and breath and blood samples were collected. Immediately thereafter, subjects were given an intravenous dose of 0.085 mg/kg of $NaH^{13}CO_3$ to prime the bicarbonate pool. Then, at t = 0, a constant intravenous infusion of either [U-^{13}C]palmitate (0.00806 µmol · min^{-1} · kg^{-1}) or [1,2-^{13}C]acetate (0.0645 µmol · min^{-1} · kg^{-1}) was started and continued for 120 min at rest. With these infusion rates, the amount of ^{13}C infused during palmitate and acetate infusion are similar. After the 120 min at rest, subjects cycled at 50% of maximal power output (W_{max}) for 1 h, and the infusion rate of palmitate or acetate was doubled. The concentration of palmitate in the infusate was measured for each experiment, to determine the exact infusion rate, with analytical gas chromatography (GC) using heptadecanoic acid as internal standard (see SAMPLEANALYSIS). The palmitate tracer (60 mg of potassium salt of [U-^{13}C]palmitate, 99% enriched; Cambridge Isotope Laboratories, Andover, MA) was dissolved in heated sterile water and passed through a 0.2-µm filter into a 5% warm human serum albumin to make a 0.670 mmol/l solution. The acetate concentration was measured in each infusate with an enzymatic method (Boehringer Mannheim, Mannheim, Germany). The acetate tracer (sodium salt of [1,2-^{13}C]acetate, 99% enriched; Cambridge Isotope Laboratories) was dissolved in 0.9% saline. The chemical and isotopic purity (99%) of palmitate and acetate tracers were checked by ^{1}H and ^{13}C nuclear magnetic resonance and GC/mass spectrometry. Blood samples and breath samples were taken at t = 0, 90, 100, 110, and 120 at rest and t = 150, 160, 170, and 180 during exercise. At rest, V_{O_2} and V_{CO_2} were measured continuously during the first 90 min using open circuit spirometry (Oxycon-β; Mijnhardt, Bunnik, the Netherlands). During exercise, V_{O_2} and V_{CO_2} were measured immediately before the measurement of breath $^{13}CO_2$ enrichment.

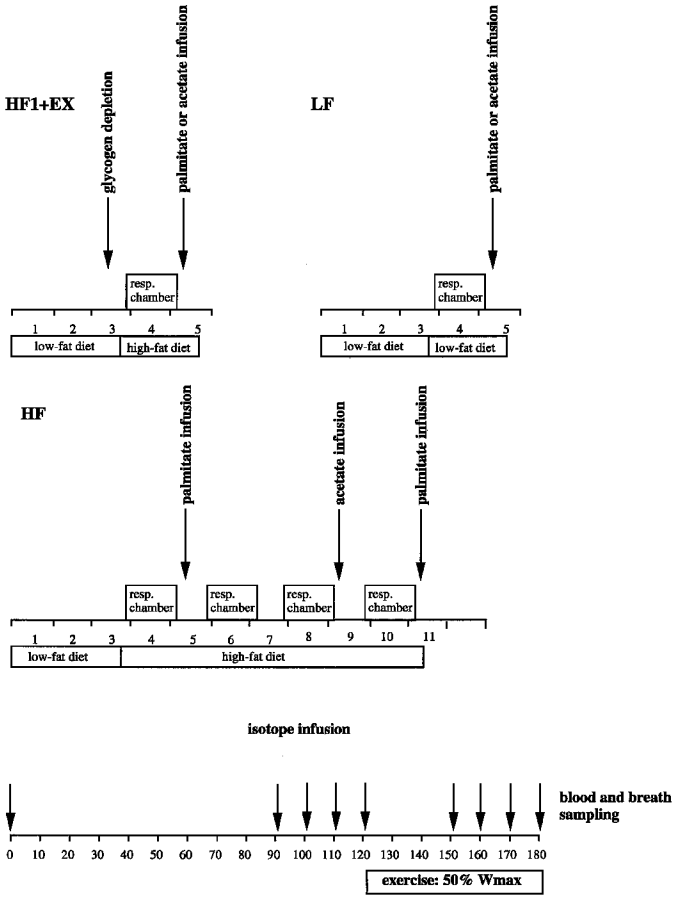

FIG. 1. Experimental design of the study. resp. chamber, respiration chamber.

Diets. The low-fat diets consisted of 30% energy as fat, 55% energy as carbohydrate, and 15% energy as protein. The high-fat diets consisted of 60% energy as fat, 25% energy as carbohydrate, and 15% energy as protein. Before the experiment, subjects filled out a 3-day food intake record to estimate habitual dietary intake. Metabolizable energy intake and macronutrient composition of the diet was calculated using the Dutch food composition table (13). On days 1 and 2 and the first part of day 3, a low-fat diet for consumption at home was provided. Subjects were given a fixed amount of food (based on their food intake record) and ad libitum access to low-fat snacks. On the evening of day 3, subjects consumed their dinner and evening snack (either low- or high-fat) in the respiration chamber. In the LF and HF treatment, energy intake for dinner and evening snack was fixed at 35% and 10% of estimated daily energy expenditure (1.7 · basic metabolic rate based on the Harris and Benedict equations [14]). In the HF1+EX treatment, the evening snack had an energy content equal to energy expended during the exercise test. On day 4, subjects were given an amount of energy equal to 1.55 times the sleeping metabolic rate, as measured during the preceding night. In a previous study, we showed that with a comparable activity protocol

used in the chamber, a physical activity index of 1.58 was reached (4). Subjects were asked not to consume any products with a high abundance of ^{13}C (carbohydrates derived from C4 plants like corn and sugar cane) 1 week before and during the entire experimental period.

Procedures

Body composition. After an overnight fast, body density was determined by underwater weighing in the fasted state. Body weight was measured with a digital balance accurate to 0.01 kg (type E1200; Sauter, Albstadtl-Ebingen, Germany). Lung volume was measured simultaneously with the helium dilution technique using a spirometer (Volugraph 2000; Mijnhardt). Body fat percentage was calculated using the equations of Siri (15). Fat-free mass in kilograms was calculated by subtracting fat mass from total body mass.

Sample analysis. Oxygen saturation (Hemoximeter OSM2, Copenhagen, Denmark) was determined immediately after sampling in heparinized blood and was used to check arterialization. Fifteen milliliters of arterialized venous blood was sampled in tubes containing EDTA to prevent clotting and immediately centrifuged at 3,000 rpm (1,000*g*) for 10 min at 4°C. Plasma was immediately frozen in liquid nitrogen and stored at –80°C until further analysis. Plasma substrates were determined using the hexokinase method (Roche, Basel, Switzerland) for glucose, the Wako NEFA C testkit (Wako Chemicals, Neuss, Germany) for FFAs, the glycerolkinase-lipase method (Boehringer Mannheim) for glycerol and triglycerides, and the ultrasensitive human insulin radioimmunoassay kit (Linco Research, St. Charles, MO) for plasma insulin.

Breath samples were analyzed for $^{13}C/^{12}C$ ratio using a GC-isotope ratio mass spectrometry (GC-IRMS) system (Finnigan MAT 252, Bremen, Germany). For determination of plasma palmitate, FFAs were extracted from plasma, isolated by thin-layer chromatography, and derivatized to their methyl esters. Palmitate concentration was determined on an analytical GC with flame ionization detection using heptadecanoic acid as internal standard, and on average it comprised 23 ± 4% of total FFA. Isotope tracer/tracee ratio (TTR) of palmitate was determined using GC-combustion-IRMS (Finnigan MAT 252) and corrected for the extra methyl group in its derivative.

Calculations. ^{13}C enrichment of breath CO_2 and plasma metabolites is given in TTR. TTR was defined as

$$(^{13}C/^{12}C)_{sa} - (^{13}C/^{12}C)_{bk}$$

in which sa represents sample and bk represents background.

Total carbohydrate and fat oxidation were calculated using stoichiometric equations (16)

$$\text{total fat oxidation (g/min)} = 1.695\ V_{O_2} - 1.701\ V_{CO_2}$$

$$\text{total carbohydrate oxidation (g/min)} = 4.585\ V_{CO_2} - 3.226\ V_{O_2}$$

with V_{O_2} and V_{CO_2} in liters per minute.

Total fatty acid oxidation was determined by converting the rate of total fat oxidation to its molar equivalent—with the assumption that the average molecular weight of triglyceride is 860 g/mol—and multiplying the molar rate of triglyceride oxidation by 3 because each molecule contains 3 mol of fatty acids.

Fractional recovery of label in breath CO_2, derived from the infusion of labeled acetate, was calculated as follows:

$$\text{fractional recovery of label (ar, \%)} = (TTR_{CO_2} \cdot V_{CO_2})/(F) \cdot 100\%$$

where TTR_{CO_2} is the TTR in breath CO_2, V_{CO_2} is carbon dioxide production (mmol/min), and F is infusion rate (mmol/min).

The rate of [U-^{13}C]palmitate oxidation was calculated as follows:

$$\text{palmitate oxidation (µmol/min)} = (TTR_{CO_2} \cdot V_{CO_2})/(TTR_p \cdot ar) \cdot 1{,}000$$

where TTR_p is the TTR of fatty acid carbon in plasma and ar is the fractional acetate recovery.

Total plasma fatty acid oxidation was then calculated by dividing palmitate oxidation rate by the fractional contribution of palmitate to the total FFA concentration.

Rate of appearance (R_a, µmol/min) of palmitate in plasma, which under steady-state conditions is equal to the rate of disappearance (R_d) minus tracer infusion rate, was calculated as $R_a = F \cdot (TTR_i/TTR_p)$, where TTR_i is the TTR of fatty acid carbon in infusion. Percentage of plasma FFA cleared from the circulation that was oxidized (%R_a oxidized) was calculated as

$$\%R_a \text{ oxidized} = \text{plasma FFA oxidation}/R_a \text{ FFA}$$

Statistical analysis. All data are presented as means ± SE, and $P < 0.05$ is considered to be significant. Equality of respiratory quotient (RQ) and food quotient (FQ) was tested using paired t tests. Repeated measures one-way analysis of variance was used to detect differences in any variables between treatments. When significant differences were found, a Scheffé post hoc test was used to determine the exact location of the difference. For testing differences in blood parameters between treatments, areas under the curve were calculated for $t = 0$ to $t = 120$ at rest and for $t = 150$ to $t = 180$ during exercise.

TABLE 2
Twenty-four–hour energy intake, energy expenditure, energy balance, and RQ as measured in the respiration chamber for four different treatments

Treatment	Energy intake	Energy expenditure	Energy balance	RQ
LF	10.86 ± 0.35	10.42 ± 0.54	0.44 ± 0.34	0.91 ± 0.01
HF1	11.05 ± 0.45	10.24 ± 0.23	0.81 ± 0.26	0.85 ± 0.01*
HF7	11.06 ± 0.45	10.07 ± 0.25	0.98 ± 0.26	0.83 ± 0.01*
HF1+EX	10.88 ± 0.52	10.45 ± 0.47	0.43 ± 0.22	0.82 ± 0.01*

Data are means ± SE. *$P < 0.01$ vs. LF.

RESULTS

Twenty-four–hour substrate oxidation determined in respiration chamber. While in the respiration chamber, subjects consumed diets based on their energy requirements. On average, subjects were in a positive energy balance (0.44 ± 0.34, 0.81 ± 0.26, 0.98 ± 0.26, and 0.43 ± 0.22 MJ/day for LF, HF1, HF7, and HF1+EX, respectively). There were no significant differences in energy balance between treatments, allowing comparison of the treatments (Table 2).

Twenty-four–hour RQ was significantly higher in the LF treatment than in the HF1, HF7, and HF1+EX treatments ($P < 0.0001$, Fig. 2). Furthermore, RQ was significantly different from FQ in the HF1, HF7, and HF1+EX treatments ($P < 0.05$), but not in the LF treatment.

Twenty-four–hour fat oxidation, as measured in the respiration chamber, was significantly different between treatments ($P < 0.002$). Fat oxidation was lower in the LF treatment compared with the HF7 and HF1+EX treatments, but the difference with HF1 did not reach statistical significance. Twenty-four–hour carbohydrate oxidation was also signifi-

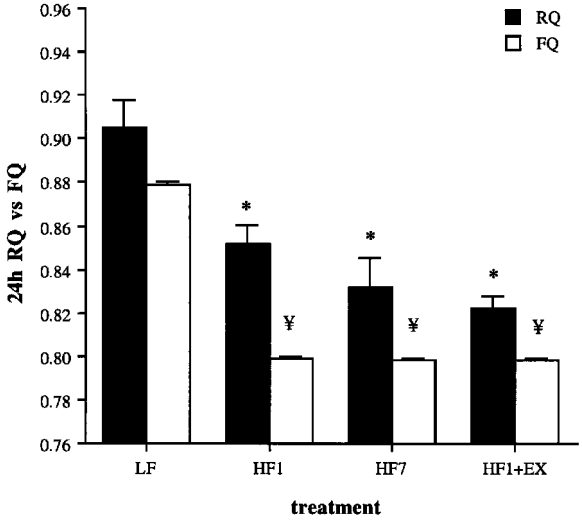

FIG. 2. Twenty-four–hour RQs and FQs as measured in the respiration chamber (means ± SE). *$P < 0.01$ vs. LF; ¥$P < 0.05$ vs. FQ.

TABLE 3
Plasma (tracers) and total (indirect calorimetry) fatty acid kinetics at rest and during exercise in different treatments

Treatment	Total FA oxidation (μmol/min)	Rate of FA appearance (μmol/min)	Plasma FA oxidation (μmol/min)	R_a oxidized (%)	Triglyceride-derived FA oxidation (μmol/min)
Rest					
LF	227 ± 27	661 ± 71	220 ± 26	33.0 ± 1.6	8 ± 18
HF1	240 ± 17	653 ± 47	217 ± 18	33.2 ± 1.7	24 ± 18
HF7	270 ± 24	623 ± 54	213 ± 26	34.1 ± 2.5	57 ± 13†
HF+EX	281 ± 15	758 ± 68	265 ± 16	35.8 ± 2.1	15 ± 15
Exercise					
LF	1,282 ± 130	1,215 ± 160	867 ± 103	72.2 ± 4.6	398 ± 160
HF1	1,415 ± 96	1,279 ± 139	940 ± 81	75.0 ± 3.5	475 ± 59
HF7	1,780 ± 202	1,301 ± 104	1,007 ± 106	77.0 ± 3.4	773 ± 127*
FH+EX	1,955 ± 204*	1,676 ± 205†	1,245 ± 175†	74.0 ± 2.9	710 ± 108

Data are means ± SE for seven volunteers. *P < 0.05 vs. LF; †P < 0.1 vs. LF. FA, fatty acid.

cantly different between treatments (P < 0.0001). Post hoc testing revealed that carbohydrate oxidation was significantly higher in the LF treatment than in the HF1, HF7, and HF1+EX treatments. No differences in 24-h protein oxidation between treatments were observed.

Substrate oxidation in postabsorptive state at rest and during exercise. At rest, whole-body fat oxidation (indirect calorimetry) tended to be different between treatments (P < 0.1). The difference became significant during exercise (P < 0.05, Table 3). Post hoc testing revealed that fat oxidation was significantly lower in the LF treatment compared with the HF1+EX treatment.

Acetate recovery gradually increased at rest from 20 to 26% during all treatments and was not significantly different between treatments (data not shown). During exercise, a plateau in acetate recovery was present at ~80% in all treatments. Plasma palmitate enrichment reached a plateau both at the end of the resting period and at the end of the exercise period (Fig. 3) in all treatments.

R_a of FFAs was not significantly different between treatments at rest, but it tended to be higher in the HF1+EX treatment during exercise (P = 0.1, Table 3). In Fig. 4*A* and *B*, the relative contribution of plasma-derived FFA and triglyceride-derived FFA to total FFA oxidation is depicted, both at rest and during exercise. Plasma-derived fatty acid oxidation was not significantly different between treatments at rest or during exercise, but tended to be higher in the HF1+EX treatment during exercise (P < 0.07, Table 3). Triglyceride-derived fatty acid oxidation tended to be higher in the HF7 treatment than in the LF treatment at rest (P = 0.06, Table 3). This difference became significant during exercise (P < 0.05, Table 3). Percentage of palmitate uptake that was oxidized was not significantly different between treatments, neither at rest nor during exercise (Table 3).

Blood parameters. At baseline, triglyceride concentration was significantly lower in the HF7 and HF1+EX treatments compared with the LF treatment (P < 0.01, Table 4). There were no significant differences in baseline concentrations of any of the other blood parameters. Both at rest and during exercise, the area under the curve for triglycerides was significantly different between treatments (P < 0.01). The triglyceride concentration was significantly higher in the LF compared with the HF7 and HF1+EX treatments. Similar results were found for glycerol during exercise (P < 0.01) but not at rest. The area under the curve for insulin during exercise was significantly different between treatments (P < 0.05). Post hoc testing revealed that this area was higher in the LF treatment than in the HF7 treatment. No differences in areas under the curve for any other blood parameters were found.

DISCUSSION

There is a clear hierarchy in substrate oxidation, with priority for protein and carbohydrate balance to be maintained (3). Fat oxidation, on the other hand, is only marginally influenced by fat intake. On a high-fat diet, positive fat balances can easily occur. We have previously shown that it takes several days before fat oxidation is increased sufficiently to match fat intake on a high-fat diet (4). Here, we confirm these findings, showing that 24-h fat oxidation only gradually increases on a high-fat diet, but can be increased rapidly when glycogen stores are lowered. Furthermore, we here show that this increase in fat oxidation is primarily due to an increase in triglyceride-derived fatty acid oxidation.

The mechanism behind the increase in fat oxidation on a high-fat diet is unknown. Randle et al. (17) introduced the glucose-fatty acid cycle in 1963 to describe the interaction between fat and carbohydrate oxidation. It was suggested that

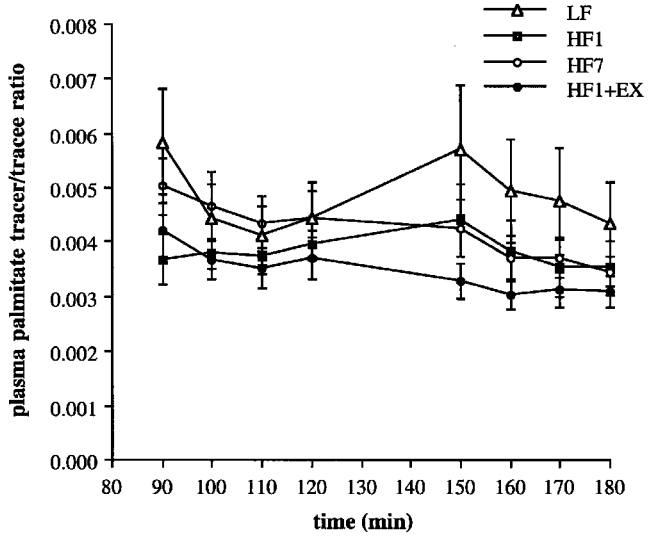

FIG. 3. Plasma palmitate enrichment during the different treatments.

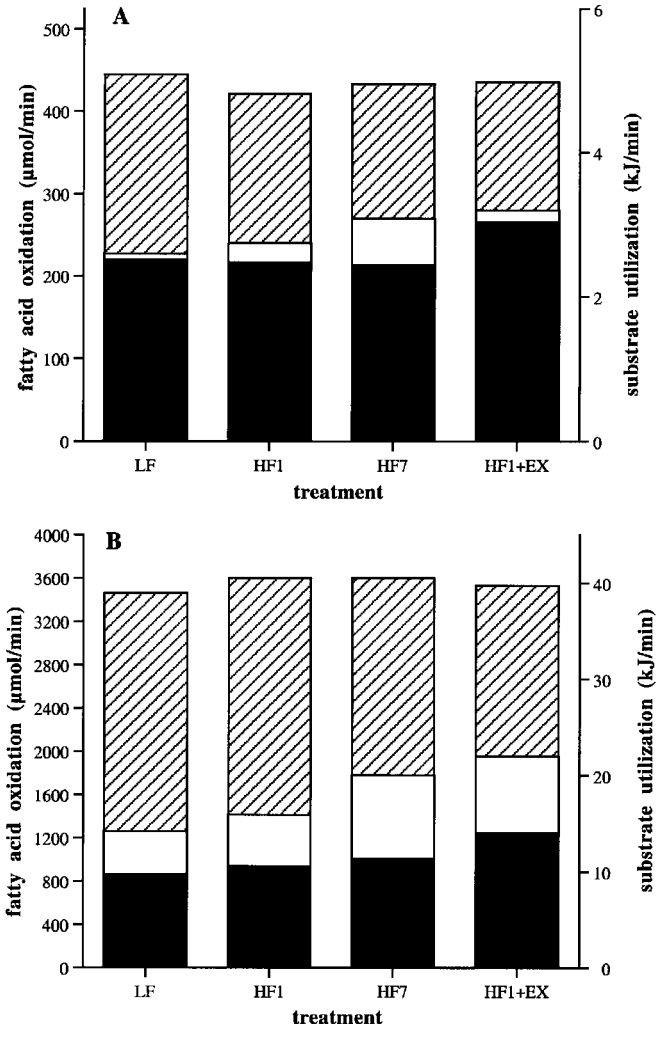

FIG. 4. *Left axis*: Relative contribution of plasma-derived and triglyceride-derived fatty acid oxidation to total fat oxidation at rest (*A*) and during exercise (*B*) in the different treatments (in µmol/min). *Right axis*: Relative contribution of plasma-derived, triglyceride-derived fatty acid oxidation and carbohydrate oxidation to total energy expenditure (in kJ/min) at rest (*A*) and during exercise (*B*) in the different treatments. ■, Plasma-derived fatty acid oxidation; □, triglyceride-derived fatty acid oxidation; ▨, carbohydrate oxidation (right axis, kJ/min).

the availability of FFA determines the rate of fat oxidation. On a high-fat diet, an increased availability of FFA would then lead to an increased fat oxidation. However, in our study, the increase in fat oxidation after 7 days on the high-fat diet was not accompanied by an increase in R_a of plasma FFA and/or higher plasma FFA levels.

Saha et al. (18) and Sidossis and colleagues (8,19) have suggested that the availability of carbohydrate rather than that of fat determines the rate of fat oxidation. An increased carbohydrate availability can increase acetyl-CoA and malonyl-CoA concentrations in muscle and liver. The latter is an important inhibitor of carnitine palmitoyltransferase-I (CPT-I). Inhibition of CPT-I on a high-carbohydrate diet will lead to reduced uptake of fatty acids into the mitochondria and thus decreased fatty acid oxidation. Switching to a high-fat diet would reverse this mechanism and increase fat oxidation. Interestingly, it has been shown in rats that a high-fat diet increases CPT-1 activity in muscle (20). Our results of the present study are in accordance with the hypothesis that carbohydrate availability controls the rate of fat oxidation. While on the high-fat diet in the respiration chamber, subjects were in negative carbohydrate balance; this must have resulted in a gradual decrease of the glycogen stores in liver and/or muscle. Thus, the gradually declining carbohydrate availability and oxidation was paralleled by a gradual increase in fat oxidation. In accordance with the findings of Turcotte et al. (9) in rats, this increase in fat oxidation was accounted for by an increase in triglyceride-derived fatty acid oxidation (intramuscular and/or VLDL triglycerides). Furthermore, even after acute lowering of the glycogen stores, followed by an acute increase in fat oxidation, the increase in plasma FFA availability could not completely account for the observed increase in fat oxidation, as would have been predicted from the glucose-fatty acid cycle hypothesis. Therefore, our data support the hypothesis that carbohydrate availability controls the rate of fatty acid oxidation. Future studies are needed to examine whether lower malonyl-CoA concentrations are responsible for this increased fat oxidation on a high-fat diet.

In humans, little data are available on the contribution of plasma-derived fatty acid oxidation to total fat oxidation, especially at rest. The main reason is that the use of ^{13}C- and ^{14}C-fatty acid tracers to estimate the oxidation of plasma fatty acids has been questioned, especially in the resting state (21). The appearance of $^{13}CO_2$ (and $^{14}CO_2$) in breath, coming from the oxidation fatty acid tracers, is very low in the first hours of infusion, especially under resting conditions. Recently, Sidossis et al. (22) showed that part of the ^{13}C (or ^{14}C) tracer accumulates in products of the tricarboxylic acid (TCA) cycle and that the fixation of the tracer in these products, together with fixation in the bicarbonate pool, was the main reason for the delayed and incomplete appearance of $^{13}CO_2$ ($^{14}CO_2$) in the breath. Furthermore, they showed that the amount of this label fixation can be determined by mea-

TABLE 4
Blood parameters at baseline during the different treatments

Treatment	Glucose (mmol/l)	Insulin (µU/ml)	Triglycerides (µmol/l)	Fatty acids (µmol/l)	Glycerol (µmol/l)
LF	5.17 ± 0.21	5.1 ± 1.0	687 ± 85	356 ± 63	53 ± 7
HF1	5.10 ± 0.23	5.5 ± 2.0	556 ± 66	401 ± 64	52 ± 3
HF7	4.79 ± 0.12	4.4 ± 0.8	435 ± 57*	380 ± 89	49 ± 5
HF1+EX	4.84 ± 0.13	3.5 ± 0.5	454 ± 55*	385 ± 50	60 ± 9

Data are means ± SE. *P < 0.01 vs. LF.

suring $^{13}CO_2$ ($^{14}CO_2$) in breath after the infusion of ^{13}C- (or ^{14}C-) acetate, because acetate, like palmitate, is immediately converted to acetyl-CoA and from that point on is treated the same as fatty-acid derived acetyl-CoA (22). Therefore, the fraction of acetate label retained in the body is equal to the fraction of the palmitate label retained in the body, and the acetate recovery can be used to correct the plasma-derived fatty acid oxidation rate for loss of label in the TCA cycle. We recently showed that this acetate recovery factor is reproducible but has a high interindividual variation (23). Furthermore, it increases linearly with isotope infusion duration (23,24). The acetate recovery factor is also much higher during exercise than at rest (12). Therefore, this acetate recovery factor has to be determined at similar time points and under similar conditions as the measurement of plasma-derived fatty acid oxidation and has to be repeated in every single individual. In the present study, we measured the acetate recovery factor in each individual both on the low-fat and high-fat diet and in the HF1+EX treatment. In this way, we were able to correct plasma-derived fatty acid oxidation rate for loss of label in the TCA cycle.

The finding that at least a part of the increase in fat oxidation on a high-fat diet was accounted for by an increase in triglyceride-derived fatty acid oxidation is interesting. Sidossis et al. (22) recently assumed that, at rest, total fat oxidation is completely covered by the oxidation of plasma-derived fatty acids (12). However, during exercise, the contribution of triglyceride-derived fatty acid oxidation to total fat oxidation has been estimated to account for 15–35% (25,26), although results are contradictory (27). In our study, at rest, the contribution of triglyceride-derived fatty acid oxidation to total fat oxidation was also negligible, but only on a low-fat diet! The prolonged consumption of a high-fat diet resulted in an increased contribution of triglyceride-derived fatty acid oxidation to ~20% after 7 days. Similarly, during exercise, the contribution of triglyceride-derived fatty acid oxidation to total fat oxidation increased from ±27% on the low-fat diet to ±42% after 7 days on the high-fat diet. These results indicate that the previous estimations of triglyceride-derived fatty acid oxidation might hold true when consuming "normal" diets, but can be influenced by the dietary fat content.

A role for triglyceride-derived fatty acids in the adaptation of fat oxidation to fat intake is not surprising in the context of other findings. It has previously been shown that after consumption of a high-fat diet, intramuscular triglyceride concentration was elevated in healthy subjects (11,28), and this may suggest that an increased intramuscular triglyceride concentration allows an increased intramuscular triglyceride oxidation. However, another source for the increased triglyceride-derived fatty acid oxidation could be the VLDL triglycerides. A major part of the fatty acids liberated by LPL are immediately extracted by the skeletal muscle without mixing with the plasma fatty acid pool and directly contribute, when oxidized, to triglyceride-derived fatty acid oxidation. The contribution of VLDL triglyceride oxidation to total fat oxidation during exercise is not clear, because studies have been published with conflicting data (11,29). Activity of LPL, the enzyme responsible for releasing fatty acids from the lipoproteins (VLDL), is increased after consumption of a high-fat diet (11). Furthermore, a relation between LPL activity and whole-body fat oxidation has been shown (30). These findings suggest that a higher LPL activity on a high-fat diet facilitates the release of fatty acids from VLDL triglycerides, which might be directly oxidized in the muscle, especially in the postabsorptive state and during exercise. However, it is also possible that the higher LPL activity on a high-fat diet facilitates the storage of fatty acid derived from VLDL triglycerides into intramuscular triglycerides, and that these intramuscular triglycerides are oxidized in the postabsorptive state and during exercise. With the tracer methodology, we cannot conclude what the relative contribution of intramuscular and VLDL triglyceride oxidation to total fat oxidation is.

In conclusion, the results from this study show that triglyceride-derived fatty acid oxidation (VLDL and/or intramuscular triglycerides) plays an important role in the increase in fat oxidation on a high-fat diet in lean subjects. Future studies should examine the relative contribution of triglyceride-derived fatty acid oxidation in obesity-susceptible humans both on normal and on high-fat diets.

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