Impaired Oxidation of Plasma-Derived Fatty Acids in Type 2 Diabetic Subjects During Moderate-Intensity Exercise

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The present study was intended to investigate the different components of fatty acid utilization during a 60-min period of moderate-intensity cycling exercise (50% of Vo_{2max}) in eight male type 2 diabetic subjects (aged 52.6 ± 3.1 years, body fat 35.8 ± 1.3%) and eight male obese control subjects (aged 45.1 ± 1.4 years, body fat 34.2 ± 1.3%) matched for age, body composition, and maximal aerobic capacity. To quantitate the different components of fatty acid metabolism, an isotope infusion of [U-^{13}C]-palmitate was used in combination with indirect calorimetry. In separate experiments, the ^{13}C label recovery in expired air was determined during infusion of [1,2-^{13}C]-acetate (acetate recovery factor). There were no differences in energy expenditure or carbohydrate and total fat oxidation between the groups. The rate of appearance (R_a) of free fatty acid (FFA) ($P < 0.05$) and the exercise-induced increase in R_a of FFA were significantly lower ($P < 0.05$) in type 2 diabetic subjects compared with control subjects (baseline vs. exercise [40–60 min]; type 2 diabetes 11.9 ± 0.9 vs. 19.6 ± 2.2 µmol · kg^{-1} fat-free mass [FFM] · min^{-1} and control 15.8 ± 1.8 vs. 28.6 ± 2.1 µmol · kg^{-1} FFM · min^{-1}). The oxidation of plasma-derived fatty acids was significantly lower in type 2 diabetic subjects during both conditions ($P < 0.05$, baseline vs. exercise [40–60 min]; type 2 diabetes 4.2 ± 0.5 vs. 14.1 ± 1.9 µmol · kg^{-1} FFM · min^{-1} and control 6.2 ± 0.6 vs. 20.4 ± 1.9 µmol · kg^{-1} FFM · min^{-1}), whereas the oxidation of triglyceride-derived fatty acids was higher ($P < 0.05$). It is hypothesized that these impairments in fatty acid utilization may play a role in the etiology of skeletal muscle and hepatic insulin resistance. *Diabetes* 49:2102–2107, 2000

Alterations in skeletal muscle lipid metabolism may be of importance in the etiology of obesity and type 2 diabetes. The uptake and oxidation of fatty acids is diminished in skeletal muscle of type 2 diabetic subjects during the postabsorptive state (1) and during β-adrenergic stimulation (1,2). A lowered capac-ity of skeletal muscle to take up or oxidize plasma fatty acids may divert fatty acids toward increased storage in skeletal muscle, which is strongly linked to insulin resistance (3), or may divert fatty acids toward other tissues like adipose tissue (promoting large adipose tissue stores) or liver (increasing VLDL and glucose output [4,5]). The above-mentioned observation of a lowered fatty acid utilization in type 2 diabetes during basal and β-adrenergically mediated conditions gives rise to the idea that this impairment also extends to physical exercise.

Although the pattern of fat utilization during exercise is well described in healthy volunteers (6), less is known about fat utilization in type 2 diabetes during exercise. Kelley and colleagues (7,8) have shown that obese type 2 diabetic subjects show similar increases in whole-body fat and carbohydrate utilization compared with body fat–matched control subjects during moderate-intensity exercise, whereas the use of plasma glucose was increased, and the oxidation of muscle glycogen was diminished. In contrast, a study in lean type 2 diabetic subjects showed an increased carbohydrate and a lowered exercise-induced fat oxidation (9). So far, no information is available on the components of fatty acid oxidation in the exercising muscle of type 2 diabetic subjects.

The present study intended to investigate the different components of fatty acid metabolism (rate of appearance of free fatty acid [FFA], plasma-derived fatty acid oxidation, triglyceride-derived fatty oxidation, and total fat oxidation) during a 60-min period of moderate-intensity exercise in obese type 2 diabetic and control subjects matched for age, body composition, and maximal aerobic capacity. On the basis of the existing knowledge, it was hypothesized that in type 2 diabetes, total fatty acid oxidation and plasma-derived fatty acid oxidation are lowered during moderate-intensity exercise.

RESEARCH DESIGN AND METHODS

Eight male subjects with type 2 diabetes and eight male obese control subjects were studied. The diabetic subjects were treated with diet alone ($n = 3$) or diet together with sulfonylureas ($n = 5$). Blood glucose–lowering medication was withheld for 2 days before the experiment. No other medication was used. None of the subjects had serious health problems apart from their diabetes. A normal resting electrocardiogram reading was a prerequisite for participation. Subjects were matched for age, body composition, and maximal aerobic capacity. Subject characteristics are indicated in Table 1. All subjects engaged in sports no more than 3 h a week, and none had a physically demanding job. The study protocol was approved by the Medical Ethical Review Committee of Maastricht University, and all subjects gave written informed consent.

Experimental design. After determination of body composition and maximal aerobic power on a separate occasion, all subjects participated in two stable isotope experiments in random order, with 2 weeks in between. The subjects arrived at the laboratory at 8:00 A.M. by car or bus after an overnight fast of at least 12 h. They were studied while resting supine on a comfortable bed

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TABLE 1
Physical characteristics

	Obese subjects	Type 2 diabetic subjects
n	8	8
Age (years)	45.1 ± 1.4	52.6 ± 3.1
Body weight (kg)	103.7 ± 4.3	108.9 ± 6.4
Body fat (%)	34.2 ± 1.3	35.8 ± 1.3
Waist-to-hip ratio	1.04 ± 0.04	1.06 ± 0.03
VO_{2max} (l/min)	2.82 ± 1.68	2.54 ± 1.88
W_{max} (W)	199 ± 13	182 ± 14
VO_{2max} (ml · kg^{-1} FFM · min^{-1})	41.3 ± 1.0	36.7 ± 2.6
Blood glucose (mmol/l)	5.3 ± 0.1	7.7 ± .5

Data are means ± SE.

in a room kept at 23–25°C. In one study, whole-body fatty acid metabolism was studied during baseline conditions and during 1 h of cycling exercise at 50% of the predetermined VO_{2max} during continuous intravenous infusion of the stable isotope tracer [U-^{13}C]-palmitate (protocol 1). The fraction of $^{13}CO_2$ recovered in breath during intravenous infusion of the stable isotope tracer [1,2-^{13}C]-acetate (acetate recovery factor [10–12]) was determined in a separate experiment (protocol 2).

Body composition. Body weight was determined on an electronic scale accurate to 0.1 kg. Waist and hip circumference measurements to the nearest 1 cm were made with the subjects standing upright. Body composition was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000; Mijnhardt, Bunnik, the Netherlands). Body composition was calculated according to Siri (13).

Maximal aerobic capacity. The maximal aerobic capacity of the subjects was determined during an incremental protocol on a cycle ergometer. After two steps lasting 2.5 min at 0.75 and 1.5 W/kg fat-free mass (FFM), the workload was increased by 0.5 W/kg FFM every 2.5 min until exhaustion. Criteria for stopping the exercise were respiratory exchange ratio >1.15 and no further increase in oxygen uptake.

Protocol 1: [U-^{13}C]-palmitate infusion. Cannulae were inserted in a forearm vein for the infusion of the stable isotope tracer and in a dorsal hand vein of the contralateral arm to obtain arterialized venous blood. After placement of the catheters, the hand was placed in a warm air box in which air was circulated at 60°C. After taking background blood and breath samples (30 min after placement of the hand in the warm air box), an intravenous priming dose of 0.085 mg/kg NaH^{13}CO$_3$ was given. Then, a constant rate continuous infusion of [U-^{13}C]-palmitate was started (0.08 μmol · kg^{-1} body wt · min^{-1}) via a calibrated infusion pump (IVAC 560 pump; IVAC, San Diego, CA). After a 2-h baseline period (0–120 min), the rate of palmitate infusion was doubled during the 1-h cycling exercise period (121–180 min) at 50% VO_{2max}. The concentration of palmitate in the infusate was measured for each experiment (see BIOCHEMICAL METHODS) so the exact infusion rate could be determined. The palmitate tracer (60 mg of the 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 5% warm human serum albumin (Central Blood Bank, the Netherlands) to make a 0.65 mmol/l solution (0.650 ± 0.025 mmol/l [mean ± SD]).

Breath, blood, and urine sampling. Five minutes before the start of infusion (background) and at time points 100, 110, and 120 min during baseline and 150, 160, 170, and 180 during exercise, breath samples were taken to determine the enrichment of CO$_2$ (^{13}C/^{12}C ratio) in expired air. Expired air samples were obtained by having the subjects breathe normally for 3 min into a mouthpiece connected to a 6.75-liter mixing chamber and then collecting a breath sample into a 20-ml vacutainer tube. Additionally, at the above-indicated time points, arterialized blood samples were taken to determine the concentrations of glucose, insulin, glycerol, O$_2$, FFA, palmitate, and the ^{13}C/^{12}C ratio of palmitate. During the resting period and during the last 40 min of exercise, O$_2$ and CO$_2$ exchange were determined by means of open-circuit spirometry (Oxycon Beta; Jaeger, Breda, the Netherlands). Urine was collected overnight in containers with 10 ml H$_2$SO$_4$ to determine nitrogen excretion for calculating the nonprotein respiratory exchange ratio.

Protocol 2: [1,2-^{13}C]-acetate. The acetate recovery in expired air was determined in a separate experiment during a 2-h baseline period and a 1-h period of exercise at 50% VO_{2max}. After collection of a background breath sample, subjects received an intravenous priming dose of 0.085 mg/kg NaH^{13}CO$_3$. The stable iso-

tope tracer [1,2-^{13}C]-acetate was continuously infused during the baseline period, and the rate of infusion was doubled at the start of the exercise period. The acetate tracer was dissolved into 0.9% saline to obtain a 3.46 ± 0.02 mol/l solution. Acetate was infused at a rate of 0.064 μmol · kg^{-1} body wt · min^{-1} to obtain the same ^{13}C infusion rate per time unit as that for the palmitate tracer. Breath samples were taken at similar time points as those during the palmitate infusion.

Biochemical methods. Blood samples were collected in EDTA-containing tubes on ice and were immediately centrifuged at 4°C, and the plasma was put in liquid nitrogen until storage at –80°C. Breath samples were analyzed for ^{13}C/^{12}C ratio by injecting 20 μl of the gaseous head space into a gas chromatography (GC) continuous flow isotope ratio mass spectrometer (Finnigan MAT 252; Finnigan, Bremen, Germany). For the determination of plasma palmitate and total FFA kinetics, FFAs were extracted from plasma, isolated by thin-layer chromatography, and derivatized to their methyl esters. Isotope enrichment of palmitate was determined by GC–isotope ratio mass spectrometry after online combustion of fatty acids to CO$_2$ (Finnigan MAT 252). Palmitate concentrations were determined on an analytical GC with ion-flame detection using heptadecanoic acid as an internal standard, and on average, palmitate concentration comprised 24 ± 1% of total FFA concentration. Total plasma FFA, glucose, glycerol, lactate, and infusate acetate concentrations were measured using standard enzymatic techniques automated on the Cobas Fara centrifugal analyzer at 340 nm (for FFA: FFA-C test kit, Wako Chemicals, Neuss, Germany; for glucose: Roche Unikit III, Hoffman-La Roche, Basel, Germany; for glycerol and acetate: Boehringer Mannheim, Mannheim, Germany). Plasma insulin was measured using a specific double-antibody radioimmunoassay for human insulin (Kabi Pharmacia, Uppsala, Sweden). Nitrogen concentrations in urine were measured using a Carlo-Erba analyzer (type CN-O-Rapid).

Calculations. The metabolic rate was calculated from VO_2 and VCO_2 according to the equation of Weir (14). Carbohydrate and fat oxidation rates were calculated from VO_2 and VCO_2 and urinary nitrogen excretion (15). Protein oxidation (as calculated from nitrogen excretion) was assumed to be similar during the overnight fasted state and during exercise.

Tracer calculations. Fatty acid oxidation was calculated by converting the rate of triglyceride oxidation (grams per minute) to its molar equivalent, with the assumption of the average molecular weight of triglyceride to be 860 g/mol and with a tripled molar rate of triglyceride oxidation because each molecule contains 3 mol of fatty acids.

During the last 20 min of the baseline period (110 and 120 min), a physiological and isotopic state was present, and therefore Steele's equation for steady state was used to calculate palmitate flux (rate of appearance [R_a] or rate of disappearance [R_d], as reported previously [2]). During the exercise period, non–steady-state equations for R_a of FFA (R_aFFA) and R_d of FFA (R_dFFA) were used (16).

Fractional recovery of the acetate label in breath CO$_2$ was calculated as follows:

$$\text{Acetate recovery} = \frac{(\text{E}_{CO_2} - \text{E}_{bkg}) \times \text{V}_{CO_2}}{2 \times F}$$

where F equals the infusion rate of acetate in micromoles per minute, $\text{E}_{CO_2} - \text{E}_{bkg}$ equals the increase in the ^{13}C/^{12}C ratio in expired air during infusion (compared with background), V_{CO_2} is the expired CO$_2$ in micromoles per minute, and the number 2 in the denominator is to correct for the number of ^{13}C molecules in acetate.

The percent of infused oxidized [U-^{13}C]-palmitate was calculated as follows:

$$\% \text{ Infused tracer oxidized} = \frac{(\text{E}_{CO_2} - \text{E}_{bkg}) \times \text{V}_{CO_2}}{16 \times F \times \text{acetate recovery}} \times 100\%$$

where F equals the infusion rate of palmitate in micromoles per minute, and the number 16 in the denominator is to correct for the number of ^{13}C molecules in palmitate.

Plasma-derived fatty acid oxidation (micromoles per minute) was calculated as follows:

$$\text{Oxidation} = R_d\text{FFA} \times \% \text{ infused palmitate tracer oxidized}$$

The fatty acid oxidation from plasma triglycerides and intramuscular triglycerides was calculated as follows:

Sum of triglyceride-derived fatty acid oxidation =
    total fatty acid oxidation – plasma-derived fatty acid oxidation.

Statistical analysis. Data are represented as means ± SE. Differences between obese subjects and obese type 2 diabetic subjects were analyzed by a two-way repeated-measures analysis of variance. Statistical significance was set at $P < 0.05$.

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TABLE 2
Circulating concentrations of metabolites during baseline conditions and during a 60-min period of moderate-intensity exercise in obese subjects and obese type 2 diabetic subjects

	Baseline	Exercise (min)				Analysis of variance		
		150	160	170	180	Group	Exercise	Interaction
Glucose (mmol/l)								
Obese	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.2	5.3 ± 0.2	5.4 ± 0.2	0.01	0.05	0.02
Type 2 diabetic	7.6 ± 0.4	6.9 ± 0.4	6.8 ± 0.4	6.7 ± 0.5	7.3 ± 0.7			
FFA (µmol/l)								
Obese	724 ± 66	603 ± 50	751 ± 56	864 ± 61	902 ± 83	—	0.001	—
Type 2 diabetic	710 ± 87	609 ± 75	774 ± 107	854 ± 119	910 ± 119			
Glycerol (µmol/l)								
Obese	106 ± 9	268 ± 16	322 ± 32	371 ± 44	378 ± 43	—	0.001	—
Type 2 diabetic	117 ± 13	249 ± 44	315 ± 57	356 ± 61	351 ± 76			
Lactate (mmol/l)								
Obese	0.76 ± 0.10	2.30 ± 0.28	2.28 ± 0.26	2.14 ± 0.23	2.01 ± 0.24	—	0.001	—
Type 2 diabetic	1.09 ± 0.07	2.60 ± 0.54	2.56 ± 0.55	2.52 ± 0.55	2.69 ± 0.50			
Insulin (mU/ml)								
Obese	10.4 ± 0.6	8.8 ± 0.7	7.9 ± 0.6	8.8 ± 0.7	8.0 ± 0.4	0.03	0.0001	0.082
Type 2 diabetic	16.6 ± 2.6	12.2 ± 1.7	12.1 ± 1.9	12.0 ± 1.9	12.0 ± 1.9	—	—	—

Data are means ± SE. Levels of statistical significance are indicated in the right-hand columns.

RESULTS

Exercise intensity. Maximal aerobic capacity and maximal workload were not different between the obese subjects and obese type 2 diabetic subjects. All subjects cycled at 50% of Vo_{2max}. Whole-body oxygen consumption reached a plateau after 40 min of exercise. Average oxygen consumption during exercise (40–60 min) was not significantly different in obese subjects and obese type 2 diabetic subjects (obese, $22.9 ± 0.5$ ml · kg^{-1} FFM · min^{-1}; obese type 2 diabetic, $21.2 ± 0.7$ ml · kg^{-1} FFM · min^{-1}). Also, the absolute workload during exercise was not significantly different in obese subjects and obese type 2 diabetic subjects (obese, $77 ± 4$ W/min; type 2 diabetic, $72 ± 6$ W/min). The respiratory exchange ratio during exercise was comparable in both groups (obese, $0.864 ± 0.01$; type 2 diabetic, $0.874 ± 0.02$).

Arterialized concentrations of circulating metabolites. Glucose concentrations were higher throughout the experiment in type 2 diabetic subjects compared with control subjects (Table 2, $P < 0.05$). Glucose concentrations decreased in type 2 diabetic subjects during exercise, whereas no change was observed in the obese control subjects (interaction group × exercise: $P < 0.05$). Circulating FFA concentrations were comparable in both groups throughout the experiment and increased during exercise. Additionally, baseline and exercise-induced increases in plasma glycerol and in plasma lactate were similar in both groups. Insulin concentrations were higher in type 2 diabetic subjects compared with control subjects throughout the experiment, whereas the decrease in insulin concentrations during exercise tended to be more pronounced in type 2 diabetic subjects ($P = 0.08$).

Whole-body indirect calorimetry. Whole-body energy expenditure and carbohydrate and total fat oxidation reached a plateau after 40 min of exercise (data not shown). Mean values of the last 20 min of the baseline and exercise period (expressed as kilogram FFM) are indicated in Fig. 1. Energy expenditure increased four- to fivefold as a result of exercise. There were no significant differences in energy expenditure and fat and carbohydrate oxidation between groups throughout the experiment.

Stable isotope infusion. Acetate recovery increased gradually during baseline conditions and flattened during exercise (Fig. 2A). Throughout the experiment, the recovery factor was lower in type 2 diabetes compared with control subjects (group effect, $P < 0.05$). Palmitate tracer/tracee ratios tended to be higher in type 2 diabetic subjects, but differences did not reach statistical significance ($P = 0.08$, Fig. 2B).

R_aFFA and R_dFFA ($P < 0.05$) were significantly lower in type 2 diabetic subjects compared with control subjects during both baseline conditions and exercise (Fig. 3). Figure 4 shows the different components of fatty acid oxidation. Baseline and exercise-stimulated total fatty acid oxidation were not significantly different between the groups (Fig. 4A). The oxidation of plasma fatty acids was significantly lower in type 2 diabetes compared with control subjects during both conditions (group effect, $P < 0.05$, Fig. 4B), whereas the oxidation of triglyceride-derived fatty acids tended to be higher in type 2 diabetic subjects (group effect, $P < 0.05$, Fig. 4C).

The percentage of the R_dFFA that was oxidized was not significantly different during baseline conditions and exercise (baseline vs. exercise [average 40–60 min]: type 2 diabetes, $35.0 ± 2.4$ vs. $70.9 ± 3.6\%$; control, $42.7 ± 2.9$ vs. $74.7 ± 3.2\%$).

DISCUSSION

This study investigated the different components of fatty acid metabolism during exercise in type 2 diabetic subjects. Alterations in skeletal muscle fat metabolism during the postabsorptive state and during exercise have been suggested to be of importance in the etiology and pathophysiology of obesity and insulin resistance (1,2,17). A disturbance in skeletal muscle fatty acid uptake and oxidation has previously been reported in type 2 diabetic subjects during the postabsorptive state (1,2). The present study extends these findings toward physical exercise and indicates for the first time that during both baseline conditions and exercise, there is an impairment in the oxidation of, in particular, plasma-derived fatty acids in type 2 diabetic subjects compared with weight-matched control subjects. Interestingly, the oxidation of triglyceride-derived fatty acids (derived from intra-
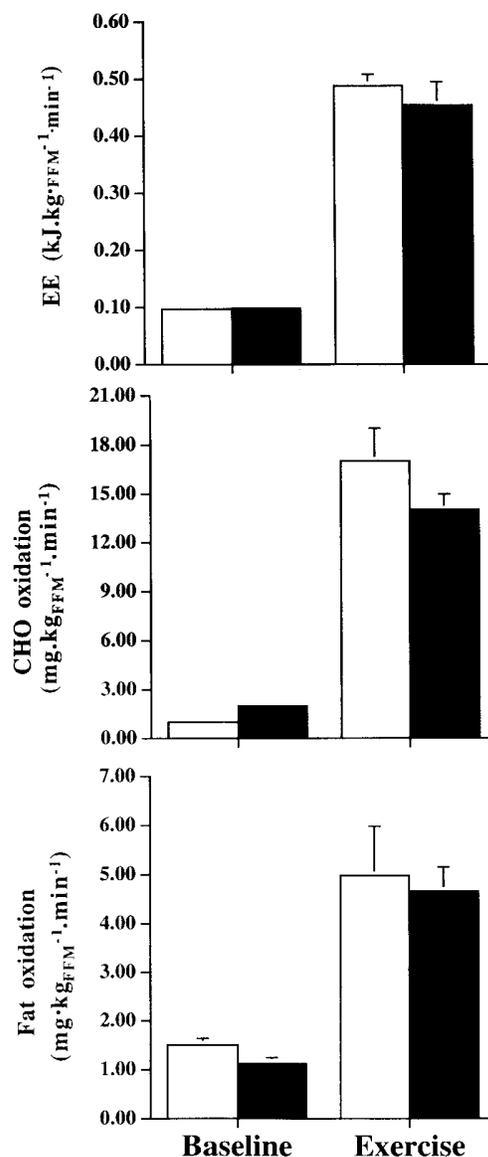
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FIG. 1. Whole-body energy expenditure (EE), carbohydrate (CHO) oxidation, and fat oxidation during baseline conditions and during moderate-intensity exercise in obese control subjects (□) and obese type 2 diabetic subjects (■).

FIG. 2. Fractional ^{13}C label recovery of [1,2-^{13}C]-acetate (*A*) and plasma palmitate tracer/tracee ratio (*B*) during baseline conditions (90–120) and during moderate-intensity exercise (121–160 min) in obese control subjects (□) and obese type 2 diabetic subjects (◇).

muscular triglyceride stores or VLDL triglycerides) was increased, resulting in equal rates of total fat oxidation. As indicated above, there are strong indications from previous studies (1,2) that skeletal muscle is the site responsible for this defective oxidation of plasma-derived FFA.

Acetate recovery factor. The acetate recovery factor has been proposed to correct for label fixation of the [U-^{13}C]-palmitate tracer into products of the tricarboxylic cycle and the bicarbonate pool (10–12). Previous studies in our laboratory have shown that the interindividual variance in recovery is high and amounts to 12% at rest and 16.1% during exercise (12), indicating that this recovery factor needs to be determined in every subject. In the present study, the acetate recovery factor was lower in type 2 diabetic subjects throughout the experiment. This result may possibly be related to an increased rate of gluconeogenesis in the diabetic
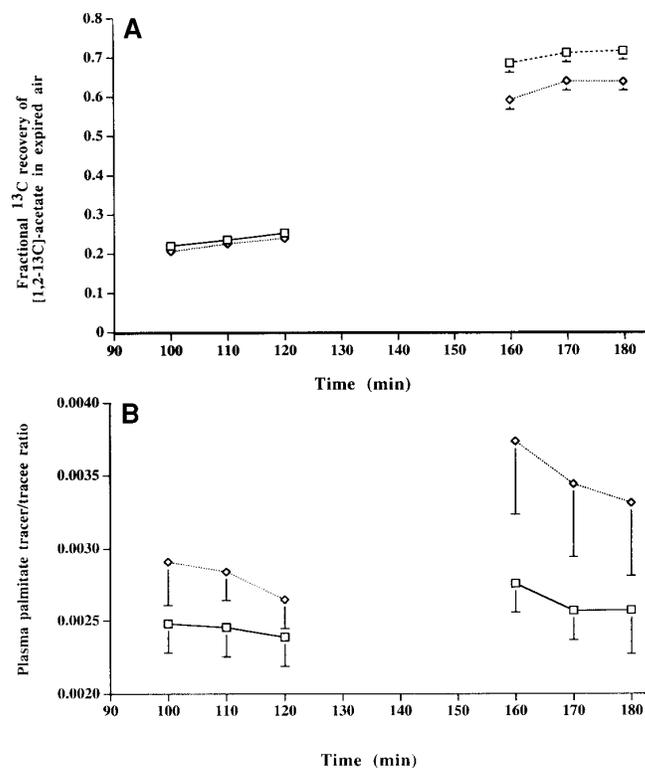
state leading to an increased loss of label from the tricarboxylic cycle, i.e., through oxaloacetate conversion to glucose. Further studies are necessary to elucidate the underlying mechanisms.

Underlying mechanisms. Several mechanisms may be responsible for this impaired plasma-derived fatty acid oxidation. First, R_aFFA (and R_d per kilogram FFM) was lower in type 2 diabetic subjects compared with control subjects at baseline conditions as well as during physical exercise, indicating that a diminished availability of FFAs may contribute to the diminished plasma-derived fatty acid oxidation. The lowered R_a may possibly be explained by an increased fatty acid re-esterification within adipose tissue because exercise-induced glycerol concentrations were comparable in both groups. Second, recent studies indicate that muscle lipolysis in type 2 diabetic subjects may be increased during both baseline and β-adrenergic stimulation (2). An increased muscle lipolysis may flood the muscle with FFA, thereby decreasing the blood tissue concentration gradient, which is one of the primary determinants of plasma fatty acid uptake and oxidation (18).

Third, a lowered skeletal muscle cytoplasmatic fatty acid transport may contribute to the disturbance in plasma-derived fatty acid oxidation. In a previous study (2), we found a diminished concentration of cytosolic fatty acid binding protein (h-FABP) in skeletal muscle of type 2 diabetic subjects, creating the possibility that, due to an impaired cytosolic fatty acid transport capacity, the oxidation of plasma-derived fatty acid is impaired. It can then be speculated that the increased triglyceride-derived fatty acid oxi-
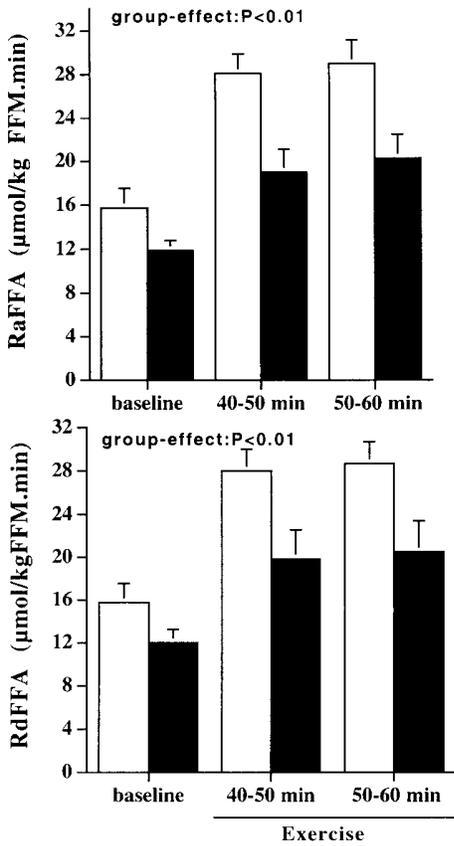
FIG. 3. R_aFFA and R_dFFA during baseline conditions and during moderate-intensity exercise (after 40–60 min) in obese control subjects (□) and obese type 2 diabetic subjects (■).

FIG. 4. Total fatty acid (FA) oxidation (A), plasma-derived fatty acid oxidation (B), and triglyceride-derived fatty acid oxidation (C) during baseline conditions and during moderate-intensity exercise (after 40–60 min) in obese control subjects (□) and obese type 2 diabetic subjects (■).

dation in the present study originated from an increased content of lipid droplets within the myocytes in the diabetic muscle, which are located near the mitochondria and are therefore not dependent on cytosolic transport capacity (19).

Lastly, hyperglycemia per se may stimulate glucose uptake and oxidation. Therefore, hyperglycemia has been proposed to be an important factor that may suppress muscle FFA utilization in type 2 diabetic subjects (1). However, from the present data, there are no indications that type 2 diabetic subjects relied to a greater extent on carbohydrate use compared with obese control subjects.

The impairments in fatty acid metabolism found in the present study may have several metabolic implications. A diminished use of plasma fatty acids by skeletal muscle may increase FFA uptake by the liver, resulting in a decreased hepatic insulin binding (20), a diminished hepatic insulin clearance (21), an increased hepatic glucose output (4), and an increased VLDL triglyceride output (5). Furthermore, it can be speculated that the strong tendency toward an increased triglyceride-derived fatty acid oxidation (possibly related to a mass action effect of increased muscle triglyceride stores [3,22]) is one of the mechanisms linking an increased triglyceride storage to insulin resistance by inhibiting glucose oxidation and uptake (23). Further studies are necessary to obtain more information on whether the impaired oxidation of plasma-derived FFAs is a primary factor or an adaptational response to the diabetic state.
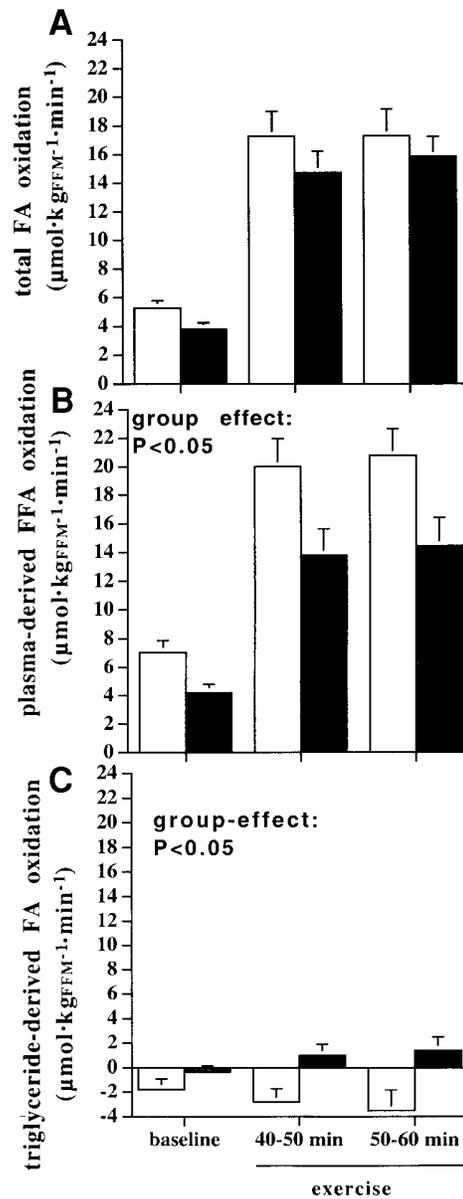
In conclusion, the oxidation of plasma-derived fatty acid is diminished in type 2 diabetic subjects during rest and exercise, whereas triglyceride-derived fatty acid oxidation tends to be increased. Mechanisms involved in the impaired plasma-derived fatty acid oxidation may be a diminished R_aFFA, increased intramuscular FFA concentrations, or a diminished cytosolic FFA transport capacity. We hypothesize that this impairment may play a role in the etiology of skeletal muscle and hepatic insulin resistance.

REFERENCES

1. Kelley DE, Simoneau J: Impaired free fatty acid utilization by skeletal muscle in non-insulin dependent diabetes mellitus. *J Clin Invest* 94:2349–2356, 1994
2. Blaak EE, Wagenmakers AJM, Glatz JFC, Wolffenbuttel BHR, Kemerink GJ, Langenberg CJM, Heidendal GAK, Saris WHM: Plasma free fatty acid utilization and fatty acid binding protein content are diminished in type 2 diabetic muscle. *Am J Physiol* 279:E146–E154, 2000
3. Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L: Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans. *Diabetes* 48:1600–1606, 1999
4. Lewis GF, Vranic M, Harley P, Giacca A: Fatty acids mediate the acute extra-hepatic effects on hepatic glucose output. *Diabetes* 46:1111–1119, 1997
5. Frayn KN, Williams CM, Arner P: Are increased plasma non-esterified fatty acid concentrations a risk marker for coronary heart disease and other chronic diseases? *Clin Sci* 90:243–253, 1996
6. Spriet LL: Regulation of fat/carbohydrate interaction in human skeletal muscle during exercise. In *Skeletal Muscle Metabolism in Exercise and Diabetes*. Richter EA, Ed. New York, Plenum, 1998, p. 249–261
7. Kang J, Kelley DE, Robertson RJ, Goss FL, Suminski RR, Utter AC, DaSilva SG: Substrate utilization and glucose turnover during exercise of varying intensities in individuals with NIDDM. *Med Sci Sports Exerc* 31:82–89, 1999
8. Colberg SR, Hagberg JM, McCole SD, Zmuda JM, Thompson PD, Kelley DE: Utilization of glycogen but not plasma glucose is reduced in individuals with NIDDM during mild-intensity exercise. *J Appl Physiol* 81:2027–2033, 1996
9. Martin IK, Katz A, Wahren J: Splanchnic and muscle metabolism during exercises in NIDDM patients. *Am J Physiol* 269:E583–E590, 1995
10. Sidossis LS, Coggan AR, Castaldelli A, Wolfe RR: A new correction factor for the use in tracer estimations of plasma fatty acid oxidation. *Am J Physiol* 269:E649–E656, 1995
11. Sidossis LS, Coggan AR, Gastaldelli A, Wolfe RR: Pathway of free fatty acid oxidation in human subjects. *J Clin Invest* 95:278–284, 1995
12. Schrauwen P, Blaak EE, Van Aggel-Leijssen DPC, Borghouts LB, Wagenmakers AJM: Determination of the acetate recovery factor: implications for estimations of [^{13}C]substrate oxidation. *Clin Sci* 98:587–592, 2000
13. Siri WE: The gross composition of the body. *Adv Biol Med Physiol* 4:239–280, 1956
14. Weir JB: New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* 109:1–9, 1949
15. Frayn KN: Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 55:628–634, 1983
16. Steele R: Influence of glucose loading and of injected insulin on hepatic glucose output. *Ann N Y Acad Sci* 82:420–430, 1959
17. Wade AJ, Marbutt MM, Round JM: Muscle fibre type and aetiology of obesity. *Lancet* 335:805–808, 1990
18. Van der Vusse GJ, Reneman RS: Lipid metabolism in muscle. In *Handbook of Physiology. Section 12. Exercise: Regulation and Integration of Multiple Systems*. Bethesda, MD, American Physiological Society, 1966, p.952–994
19. Hoppeler H: Skeletal muscle substrate metabolism. *Int J Obes* 23 (Suppl. 3):S7–S10, 1999
20. Svedberg J, Björntorp P, Smith U, Lönnroth P: Free-fatty acid inhibition of insulin binding, degradation, and action in isolated rat hepatocytes. *Diabetes* 39:570–574, 1990
21. Hennes MMI, Dua A, Kissebah AH: Effects of free fatty acids and glucose on splanchnic insulin dynamics. *Diabetes* 46:57–62, 1997
22. Rudermann NB, Saha AK, Vavvas D, Witters LA: Malonyl-CoA, fuel sensing, and insulin resistance. *Am J Physiol* 276:E1–E18, 1999
23. Randle PJ, Garland PB, Hales CN: The glucose-fatty acid cycle: its role in insulin sensitivity and the metabolic disturbances in diabetes mellitus. *Lancet* 1:785–789, 1963

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