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Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle

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Blaak, Ellen E., Anton J. M. Wagenmakers, Jan F. C. Glatz, Bruce H. R. Wolffenbuttel, Gerrit J. Kemerink, Chris J. M. Langenberg, Guido A. K. Heidendal, and Wim H. M. Saris. Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. *Am J Physiol Endocrinol Metab* 279: E146–E154, 2000.—In this study, we investigated the hypothesis that impairments in forearm skeletal muscle free fatty acid (FFA) metabolism are present in patients with type 2 diabetes both in the overnight fasted state and during β -adrenergic stimulation. Eight obese subjects with type 2 diabetes and eight nonobese controls (Con) were studied using the forearm balance technique and indirect calorimetry during infusion of the stable isotope tracer [U-¹³C]palmitate after an overnight fast and during infusion of the nonselective β -agonist isoprenaline (Iso, 20 ng · kg lean body mass⁻¹ · min⁻¹). Additionally, activities of mitochondrial enzymes and of cytoplasmatic fatty acid-binding protein (FABP) were determined in biopsies from the vastus lateralis muscle. Both during fasting and Iso infusion, the tracer balance data showed that forearm muscle FFA uptake (Con vs. type 2: fast 449 ± 69 vs. 258 ± 42 and Iso 715 ± 129 vs. 398 ± 70 nmol · 100 ml tissue⁻¹ · min⁻¹, $P < 0.05$) and FFA release were lower in type 2 diabetes compared with Con. Also, the oxidation of plasma FFA by skeletal muscle was blunted during Iso infusion in type 2 diabetes (Con vs. type 2: Iso 446 ± 274 vs. 16 ± 70 nmol · 100 ml tissue⁻¹ · min⁻¹, $P < 0.05$). The net forearm glycerol release was increased in type 2 diabetic subjects ($P < 0.05$), which points to an increased forearm lipolysis. Additionally, skeletal muscle cytoplasmatic FABP content and the activity of muscle oxidative enzymes were lowered in type 2 diabetes. We conclude that the uptake and oxidation of plasma FFA are impaired in the forearm muscles of type 2 diabetic subjects in the overnight fasted state with and without Iso stimulation.

type 2 diabetes; skeletal muscle; free fatty acids; fatty acid-binding protein; stable isotopes

OBESITY AND TYPE 2 DIABETES frequently occur together, indicating that these two conditions may share common pathological mechanisms. Increased circulating plasma free fatty acid (FFA) concentrations have been

indicated as an important risk factor in the etiology of both conditions and in the predisposition toward other chronic diseases like cardiovascular disease (9). An increased delivery of FFA to the liver may underlie many metabolic disturbances in obesity and type 2 diabetes like a decreased insulin binding to hepatocytes (24), a diminished (hepatic) insulin clearance (12), an impaired insulin-mediated suppression of hepatic glucose output (15), and an increased very low density lipoprotein (VLDL)-triacylglycerol output (9). Additionally, it has been proposed that chronically elevated FFA concentrations may reduce insulin secretion in type 2 diabetes (17).

On one hand, the elevated FFA concentrations may result from an increased release from the expanded adipose tissue stores (13) and impaired insulin-mediated suppression of lipolysis (18). On the other hand, defects in muscle FFA uptake exist in obesity and type 2 diabetes (2, 3, 5, 14). Previously, we have shown a diminished skeletal muscle uptake of FFA in (abdominally) obese subjects during β -adrenergic stimulation (2, 3), suggesting that the sympathetic nervous system may be of importance in the disturbances in fat utilization. Arteriovenous difference measurements across leg muscle of Kelley and co-workers indicate that there may be a defect in uptake and oxidation of plasma FFA in the postabsorptive state in (visceral) obesity (5) and in obesity-associated type 2 diabetes (14). Because of its large total mass and increased energy expenditure during physical activity, skeletal muscle is one of the most important organs involved in FFA metabolism in healthy humans (6), indicating that a diminished muscle utilization of fatty acids may lead to an increase in the plasma FFA concentrations, especially under conditions with a high lipolytic rate (starvation, exercise, adrenergic stimulation).

The aim of the present study was, first, to investigate whether the observed impairment in muscle fat metabolism in type 2 diabetes (14) also extends to catecholamine-stimulated conditions. Second, we intended

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Table 1. *Characteristics of the subjects*

	Control	Type 2 Diabetes
Age, yr	50.6 ± 2.4	51.8 ± 2.4
Weight, kg	73.7 ± 2.6	103.6 ± 5.5
Height, cm	178 ± 2	177 ± 2
Body mass index, kg/m ²	23.6 ± 0.7	32.6 ± 1.4
Body fat, %	16.9 ± 1.8	33.8 ± 2.1
Waist-to-hip ratio	0.94 ± 0.02	1.06 ± 0.03
Fasting blood glucose, mM	5.31 ± 0.19	7.69 ± 0.92
Hb _{A1c} , %	5.1 ± 0.1	6.4 ± 0.3

Data are means ± SE; $n = 8$ subjects in each group.

to better define previous findings on an increased muscle respiratory quotient in type 2 diabetes (14) by measuring the oxidation of plasma-derived fatty acids at the forearm muscle and whole body level. Third, muscle biopsies of the vastus lateralis were taken to determine indicators of fatty acid transport, β -oxidation, and general oxidative capacity.

We clearly want to indicate that it is not the aim of the present study to investigate the relative importance of obesity and type 2 diabetes in the development of the observed impairment in fat metabolism. Follow-up research will be needed to answer this aspect.

SUBJECTS AND METHODS

Eight healthy lean males and eight obese male subjects with type 2 diabetes (diabetes duration 2 yr, range 0.5–8 yr) were studied. The diabetic subjects were treated with diet alone ($n = 2$) or with diet in combination with oral blood glucose-lowering agents ($n = 6$ were treated with low doses of sulfonylureas, which were withheld for 2 days before the experiments). Beside this, no other medication was used. None of the subjects had serious health problems apart from their diabetes. A normal resting electrocardiogram and blood pressure was a prerequisite for participation. Subject characteristics are indicated in Table 1. All subjects engaged no more than 3 h/wk in sports, 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.

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 subject standing upright. Body composition was determined by hydrostatic weighing with simultaneous lung volume measurement (Volugraph 2000, Mijndhardt, The Netherlands). Body composition was calculated according to Siri (23).

Experimental Design

All subjects participated in two experiments in random order, with 2 wk in between. *Protocol 1* was performed to determine FFA kinetics during baseline and during intravenous infusion of the nonselective β -agonist isoprenaline (Iso). *Protocol 2* was performed to determine the acetate recovery factor, which was used to correct the tracer estimations of plasma palmitate oxidation in *protocol 1* for label fixation into products of the tricarboxylic acid cycle and in the bicarbonate pool (21). For *protocols 1* and *2*, the subjects arrived at the laboratory after an overnight fast (of at least 12 h) at 8:00

AM by car or by bus. They were studied while resting supine on a comfortable bed in a room kept at 23–25°C.

On a separate occasion, needle muscle biopsies were taken percutaneously from the vastus lateralis muscle after an overnight fast, using the Bergström method with suction (8). The biopsies were immediately frozen in liquid nitrogen and stored at -80°C until the determination of the activities of mitochondrial enzymes, lactate dehydrogenase (LDH), and cytoplasmatic fatty acid-binding protein (FABP; see *Biochemical Methods*).

Protocol 1. Whole body and forearm skeletal muscle metabolism were studied by means of the forearm muscle balance technique during continuous intravenous infusion of the stable isotope tracer [$\text{U-}^{13}\text{C}$]palmitate.

Before the start of the experiment, three canulas were inserted. One canula was inserted under local anesthesia in the radial artery of the forearm for sampling of arterial blood. In the same arm, a second canula was inserted in a forearm vein for the infusion of Iso and the stable isotope tracer. In the contralateral arm, a third catheter was inserted in retrograde direction in an antecubital vein for the sampling of deep venous blood, draining forearm muscle. Measurements were done during the last 30 min of a 90-min baseline period (0–90 min) and a subsequent 60 min period of intravenous infusion of the nonselective β -agonist Iso [90–150 min, in a dose of $20 \text{ ng} \cdot \text{kg lean body mass (LBM)}^{-1} \cdot \text{min}^{-1}$].

ISO TOPE INFUSION. After taking background blood and breath samples (see below), an intravenous priming dose of 0.085 mg/kg $\text{NaH}^{13}\text{CO}_3$ was given. Next, a constant-rate continuous infusion of [$\text{U-}^{13}\text{C}$]palmitate was started ($0.011 \mu\text{mol} \cdot \text{kg body wt}^{-1} \cdot \text{min}^{-1}$) and continued during the entire period via a calibrated infusion pump (IVAC 560 pump, San Diego, CA). The concentration of palmitate in the infusate was measured for each experiment (see *Biochemical Methods*) so that the exact infusion rate could be determined. The palmitate tracer (60 mg of the potassium salt of [$\text{U-}^{13}\text{C}$]palmitate, 99% enriched; Cambridge Isotope Laboratories, Andover, MA) was dissolved in heated sterile water and was passed through a 0.2- μm filter to 5% warm human serum albumin (Central Blood Bank) to make a 0.670 mM solution [0.668 ± 0.016 (SD) mM].

BREATH, BLOOD, AND URINE SAMPLING. Arterial and deep venous blood samples and a breath sample were obtained before the start of the experiment to determine background isotopic enrichment. At *time points* 10, 20, 30, 40, 50, 60, 75, and 90 min during the baseline period and at *time* 100, 110, 120, 135, and 150 min during Iso infusion, breath samples were taken to determine the enrichment of CO_2 (^{13}C -to- ^{12}C ratio) in expired air. Expired air samples were obtained by having the subjects breath normally for 3 min into a mouthpiece connected to a 6.75-liter mixing chamber, and the sample was collected in a 20-ml vacutainer tube. During the entire experiment, O_2 and CO_2 exchange were determined with an open-circuit ventilated hood system (Oxycon Beta, Mijndhardt, The Netherlands). Urine was collected overnight and at the end of the Iso infusion to calculate the nonprotein respiratory exchange ratio.

After 60, 75, and 90 min (baseline period) and after 120, 135, and 150 min (Iso infusion), forearm blood flow and arterial and venous concentrations of glucose, lactate, glycerol, O_2 , FFA, palmitate, and CO_2 as well as the ^{13}C -to- ^{12}C ratio of palmitate and CO_2 were determined.

BLOOD FLOW. Total forearm blood flow (TBF) was measured by venous occlusion plethysmography with a mercury strain gauge (Periflow 0699; Janssen Scientific Instruments), as reported previously (4). To obtain an indication whether TBF was representative of forearm skeletal muscle blood flow

(SMBF), an estimation of "pure" SMBF was made by simultaneously determining forearm skin blood flow (SBF), forearm subcutaneous adipose tissue blood flow (ATBF), and forearm composition, as described in detail previously (4).

Protocol 2. The acetate recovery in expired air was determined in a separate experiment during a 90-min baseline period and a 60-min period of Iso infusion. After collection of a background breath sample, subjects received an intravenous priming dose of 0.085 mg/kg body wt $\text{NaH}^{13}\text{CO}_3$. Next, a continuous infusion of [1,2- ^{13}C]acetate was started and continued during the entire period. The acetate tracer (sodium salt of [1,2- ^{13}C]acetate, 99% enriched; Cambridge Isotope Laboratories) was dissolved in 0.9% saline to obtain a 3.11 ± 0.16 mM solution. Acetate was infused at a rate of $0.088 \mu\text{mol} \cdot \text{kg body wt}^{-1} \cdot \text{min}^{-1}$ to obtain the same ^{13}C infusion rate per time unit as for the palmitate tracer. Breath samples were taken at similar time points as during the palmitate infusion.

Biochemical Methods

Blood samples were taken simultaneously from the radial artery and deep forearm vein after the blood flow measurement while the hand circulation was still occluded. Duplicate 1-ml blood samples were immediately injected with a needle through the rubber stopper of preweighed vacutainer tubes, without disturbing the vacuum, for the determination of $^{13}\text{CO}_2/^{12}\text{CO}_2$. After being weighed again, 1 vol of 1 M sulfuric acid (H_2SO_4) was injected through the rubber stopper in the tubes to direct all blood CO_2 in the gaseous head space. The tubes were weighed again to determine the dilution factor. The gaseous head space was finally brought to atmospheric pressure with helium. The same procedure was applied to bicarbonate standards of known concentration. The coefficient of variation of this method is 0.4% for CO_2 concentrations in the range of 15–30 mM. The coefficient of variation between duplicate measurements of CO_2 concentrations is 0.09%.

All other 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 and blood samples were analyzed for the ^{13}C -to- ^{12}C ratio and CO_2 content by injecting 20 μl of the gaseous head space in a gas chromatography (GC) continuous-flow isotope ratio-mass spectrometer (Finnigan MAT 252).

For the determination of plasma palmitate and total FFA kinetics, FFA were extracted from plasma, isolated by TLC, and derivatized to their methyl esters. Isotope enrichment of palmitate was determined by GC-isotope ratio mass spectrometry after on-line combustion of the fatty acids to CO_2 .

Palmitate concentrations were determined on an analytical GC with ion flame detection using heptadecanoic acid as internal standard, and, on average, it comprised $24.5 \pm 0.6\%$ of total FFA concentration. Total plasma FFA, glucose, glycerol, lactate, and infusate acetate 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, Switzerland; for glycerol and lactate: Boehringer, Mannheim, Germany). Plasma insulin was measured using a specific double-antibody RIA for human insulin (Kabi Pharmacia, Uppsala, Sweden). Also, the hematocrit was determined in heparinized blood using a microcapillary system.

The muscle biopsies were homogenized in ice-cold Tris-EDTA buffer at pH 7.4. The homogenates were subsequently

sonicated for 4×15 s and were centrifuged at 10,000 g for 2 min at 4°C to remove cell debris. Tissue content of heart-type or muscle-type cytoplasmic FABP in skeletal muscle was measured by means of a newly developed ELISA, using recombinant human FABP as standard (27). Citrate synthase (CS) was determined by the method of Shepherd and Garland (20), whereas 3-hydroxyacyl-CoA dehydrogenase (HAD) and LDH activities were assayed according to Bergmeyer (1).

Calculations

Blood flow. SMBF was calculated according to the following equation

$$\text{TBF} = \text{amount of muscle} \times \text{SMBF} + \text{amount of skin} \\ \times \text{SBF} + \text{amount of fat} \times \text{ATBF}$$

where units for the amount of tissue are percent of total forearm area/100; units for blood flow are milliliters per 100 ml tissue per minute.

This equation and the assumptions underlying the estimation of SMBF have been discussed in detail elsewhere (2, 4).

Gas exchange monitoring. The metabolic rate was calculated from the rates of O_2 uptake ($\dot{V}\text{O}_2$) and CO_2 production ($\dot{V}\text{CO}_2$) according to the equation of Weir (26). Carbohydrate and fat oxidation rates were calculated from $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ and urinary nitrogen excretion (9).

Tracer calculations. The palmitate flux (rate of appearance or rate of disappearance) was calculated as

$$\text{palmitate flux } (\mu\text{mol}/\text{min}) = \frac{F}{E_{\text{plasma palmitate}} - E_{\text{bkg}}} - F$$

where F is the infusion rate of palmitate in micromoles per minute, and $E_{\text{plasma palmitate}} - E_{\text{bkg}}$ is the increase in the ^{13}C -to- ^{12}C ratio of plasma palmitate during infusion (compared with background).

The FFA flux was calculated by dividing palmitate flux by the fractional contribution of palmitate to total FFA concentration. Fractional recovery of the acetate label in breath CO_2 was calculated as follows

$$\text{acetate recovery} = \frac{(E_{\text{CO}_2} - E_{\text{bkg}}) \times \dot{V}\text{CO}_2}{2 \times F}$$

where F is the infusion rate of acetate in micromoles per minute; $E_{\text{CO}_2} - E_{\text{bkg}}$ is the increase in the ^{13}C -to- ^{12}C ratio in expired air during infusion (compared with background); $\dot{V}\text{CO}_2$ 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 [^{13}C]palmitate oxidized was calculated as

$$\% \text{infused tracer oxidized} = \frac{(E_{\text{CO}_2} - E_{\text{bkg}}) \times \dot{V}\text{CO}_2}{16 \times F \times \text{acetate recovery}} \times 100\%$$

where F is 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 FFA oxidation ($\mu\text{mol}/\text{min}$) was calculated as

$$\text{oxidation} = \text{FFA flux} \times \% \text{infused palmitate tracer oxidized}$$

Forearm muscle. The exchange of metabolites across forearm muscle was calculated by multiplying the arteriovenous

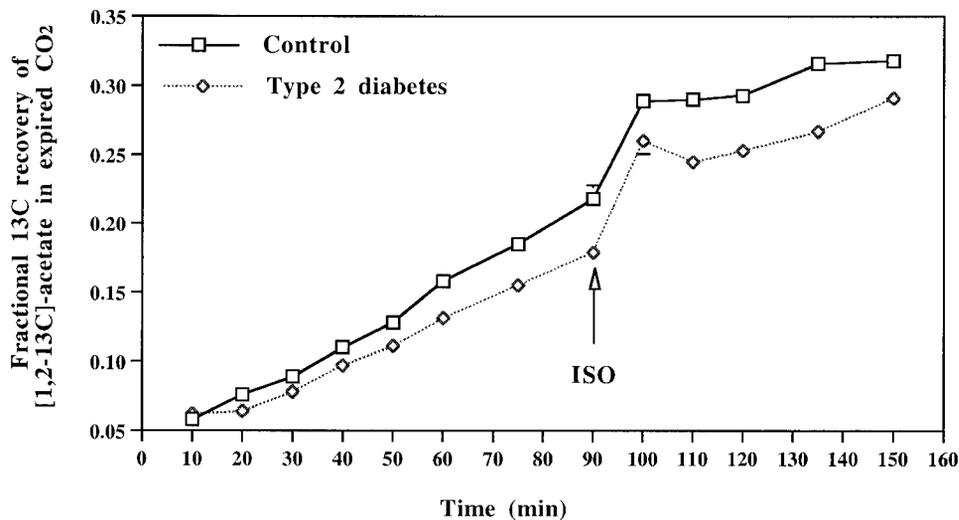


Fig. 1. Fractional ^{13}C label recovery of $[1,2-^{13}\text{C}]$ acetate during baseline (0–90 min) and isoprenaline (Iso)-stimulated conditions (91–150 min) in control and type 2 diabetic subjects. For the Iso effect, $P < 0.001$; for group effect, $P < 0.001$; and for interaction of group \times Iso effect, $P < 0.001$.

concentration difference of metabolites ($\mu\text{mol/l}$) by forearm plasma flow ($\text{ml} \cdot 100 \text{ ml forearm muscle}^{-1} \cdot \text{min}^{-1}$) or by TBF (for CO_2 exchange). Forearm plasma flow was calculated by multiplying forearm blood flow by $(1 - \text{hematocrit})/100$. A positive exchange indicates uptake.

The fractional extraction (Fract) of palmitate across the forearm was calculated by dividing the arteriovenous concentration differences of $[\text{U-}^{13}\text{C}]$ palmitate by the arterial $[\text{U-}^{13}\text{C}]$ palmitate concentration. Forearm FFA uptake was calculated as follows

$$\text{forearm muscle FFA uptake} = \text{fract} \times [\text{FFA}_{\text{art}}] \times \text{PBF}$$

where the units are nanomoles per 100 ml forearm tissue per minute; $[\text{FFA}_{\text{art}}]$ is arterial FFA concentration; and PBF is forearm plasma blood flow.

Forearm FFA release was calculated from the formula

forearm muscle FFA uptake

$$= \text{forearm muscle FFA net balance}$$

$$- \text{forearm muscle FFA uptake}$$

The oxidation of plasma FFA by skeletal muscle was calculated as

forearm muscle plasma FFA oxidation

$$= \frac{\text{TBF} \times (\text{ven } ^{13}\text{CO}_2 \text{ concn} - \text{art } ^{13}\text{CO}_2 \text{ concn})}{16 \times [^{13}\text{C}] \text{palmitate uptake} \times \text{acetate recovery}} \times \text{muscle FFA uptake}$$

where the units for oxidation and palmitate flux are nanomoles per 100 ml per minute; ven $^{13}\text{CO}_2$ concn is venous $\text{E}_{\text{CO}_2} \times \text{venous } \text{CO}_2$ concentration; and art $^{13}\text{CO}_2$ concn is arterial $^{13}\text{CO}_2$ concentration.

In this equation, the acetate recovery across forearm muscle is assumed to be similar to the whole body acetate recovery factor (16).

Statistical Analysis

Data are expressed as means \pm SE. To compare baseline and Iso-induced responses between groups, a two-factor repeated-measures ANOVA was performed. A P value < 0.05 was regarded as statistically significant.

RESULTS

Tracer Enrichment in Blood and Expired Air

The plasma palmitate concentration and the ^{13}C -to- ^{12}C ratio of plasma palmitate did not change significantly during the sampling times from 60 to 90 min during the baseline period and from 30 to 60 min of Iso infusion in both groups, allowing the applications of steady-state equations for the tracer data (data not shown). The acetate recovery (Fig. 1) was significantly lower in type 2 diabetes compared with controls, and the increase in this variable throughout the experiment was significantly blunted in type 2 diabetes. Palmitate recovery followed the same pattern (at 90 min: controls 8.0 ± 0.3 vs. type 2 diabetes $6.0 \pm 0.3\%$ and at 150 min: 12.0 ± 0.4 vs. $9.3 \pm 0.7\%$, $P < 0.001$) and was on average threefold lower than acetate recovery.

Arterial Hormone and Substrate Concentrations

Arterial glucose, lactate, insulin, and glycerol concentrations were significantly higher in type 2 diabetes both during basal conditions and during Iso infusion, whereas FFA concentrations were not significantly different between groups (Table 2). Basal insulin concentrations and the Iso-induced increase in insulin concentrations were higher in type 2 diabetes compared with control ($P < 0.01$).

Whole Body Energy Expenditure and Substrate Utilization

After 20 min of Iso infusion, a steady state was reached for O_2 exchange, CO_2 exchange, and energy expenditure. Resting energy expenditure and the Iso-induced thermogenesis (%increase above baseline: control 13.7 ± 2.2 vs. type 2 diabetes $14.4 \pm 1.3\%$) were comparable in both groups (Table 3). Resting fat oxidation tended to be higher ($P = 0.11$), whereas resting carbohydrate oxidation was lower ($P < 0.05$) in type 2 diabetic subjects compared with controls. The Iso-in-

Table 2. Arterial concentrations of metabolites during baseline conditions and during intravenous infusion of the nonselective β -agonist Iso

	Baseline	Isoprenaline			ANOVA		
		30 min	45 min	60 min	Group	Iso	Interaction
Glucose, mM							
Control	5.31 \pm 0.19	5.33 \pm 0.16	5.29 \pm 0.14	5.27 \pm 0.14	0.04	0.001	0.002
Type 2 diabetes	7.69 \pm 0.92	7.29 \pm 0.85	7.20 \pm 0.84	7.15 \pm 0.84			
Lactate, μ M							
Control	467 \pm 34	540 \pm 30		548 \pm 43	0.002	0.02	
Type 2 diabetes	779 \pm 66	807 \pm 60		822 \pm 72			
Glycerol, μ M							
Control	59 \pm 5	83 \pm 7	81 \pm 8	82 \pm 8	0.02	0.001	
Type 2 diabetes	99 \pm 14	132 \pm 16	127 \pm 18	117 \pm 10			
FFA, μ M							
Control	636 \pm 41	1,210 \pm 76	1,157 \pm 93	1,138 \pm 91	0.001		
Type 2 diabetes	688 \pm 38	1,170 \pm 89	1,195 \pm 61	1,181 \pm 58			
Palmitate, μ M							
Control	160 \pm 5	283 \pm 15	273 \pm 20	267 \pm 21	0.001		
Type 2 diabetes	159 \pm 17	270 \pm 41	271 \pm 35	272 \pm 35			
Insulin, mU/ml							
Control	4.6 \pm 0.5	8.5 \pm 0.7	8.7 \pm 0.9	8.8 \pm 1.8	0.002	0.001	0.02
Type 2 diabetes	12.9 \pm 2.4	22.4 \pm 3.5	23.0 \pm 3.6	21.8 \pm 3.2			

Data are means \pm SE. Iso, isoprenaline; FFA, free fatty acid. Levels of statistical significance are indicated on *right*.

duced increase in fat oxidation was less pronounced in type 2 diabetes compared with controls ($P = 0.05$). Furthermore, Iso infusion led to a decrease in carbohydrate oxidation in controls, whereas there was a slight increase in type 2 diabetes ($P < 0.05$).

The mean FFA flux per kilogram LBM was not significantly different between groups both during baseline conditions and Iso infusion (Table 3).

Plasma FFA oxidation was not significantly different between both groups during baseline, whereas the Iso-induced increase in plasma FFA oxidation was significantly blunted in type 2 diabetes ($P <$

0.001, Table 3). The proportion of the FFA flux that was oxidized was similar during baseline conditions in both groups (controls 39 ± 2 and type 2 diabetes $40 \pm 3\%$), whereas the Iso-induced decrease in this variable was significantly more pronounced in type 2 diabetes compared with controls (controls 38 ± 1 and type 2 diabetes $34 \pm 2\%$, $P < 0.05$).

Forearm Muscle Metabolism

Forearm composition. The total forearm area was significantly higher in type 2 diabetes (control vs. type

Table 3. Whole body energy expenditure and substrate utilization and plasma FFA flux and oxidation during baseline conditions and during intravenous infusion of the nonselective β -agonist Iso

	Baseline	Isoprenaline			ANOVA		
		30 min	45 min	60 min*	Group	Iso	Interaction
Energy expenditure, $\text{kJ} \cdot \text{kg LBM}^{-1} \cdot \text{min}^{-1}$							
Control	0.087 \pm 0.002			0.099 \pm 0.003	0.001		
Type 2 diabetes	0.084 \pm 0.001			0.096 \pm 0.001			
CHO oxidation, $\mu\text{mol} \cdot \text{kg LBM}^{-1} \cdot \text{min}^{-1}$							
Control	10.2 \pm 0.6			5.5 \pm 1.3	0.05		
Type 2 diabetes	5.5 \pm 1.3			7.8 \pm 0.8			
Fat oxidation, $\mu\text{mol} \cdot \text{kg LBM}^{-1} \cdot \text{min}^{-1}$							
Control	1.5 \pm 0.1			2.3 \pm 0.1	0.001	0.05	
Type 2 diabetes	1.7 \pm 0.2			2.1 \pm 0.1			
Plasma FFA flux, $\mu\text{mol} \cdot \text{kg LBM}^{-1} \cdot \text{min}^{-1}$							
Control	13.1 \pm 0.8	24.7 \pm 1.3	23.2 \pm 1.3	22.8 \pm 1.5	0.001	0.09	
Type 2 diabetes	13.4 \pm 1.0	22.4 \pm 1.2	22.7 \pm 0.9	22.6 \pm 0.9			
Plasma FFA oxidation, $\mu\text{mol} \cdot \text{kg LBM}^{-1} \cdot \text{min}^{-1}$							
Control	5.14 \pm 0.34	9.19 \pm 0.54	8.67 \pm 0.50	8.56 \pm 0.53	0.001	0.001	
Type 2 diabetes	5.22 \pm 0.34	7.87 \pm 0.52	7.86 \pm 0.62	7.28 \pm 0.71			

Data are means \pm SE. CHO, carbohydrate; LBM, lean body mass. *For energy expenditure, CHO and fat oxidation data in the time range of 41–60 min were averaged. Level of statistical significance is indicated on *right*.

Table 4. *TBF, SMBF, and skeletal muscle substrate exchange during baseline conditions and during intravenous infusion of the nonselective β -agonist Iso*

	Baseline	Isoprenaline			ANOVA		
		30 min	45 min	60 min	Group	Iso	Interaction
TBF, ml · 100 ml tissue ⁻¹ · min ⁻¹							
Control	2.47 ± 0.32	3.46 ± 0.60	3.64 ± 0.69	3.63 ± 0.70			0.001
Type 2 diabetes	1.89 ± 0.13	3.19 ± 0.31	3.25 ± 0.30	3.25 ± 0.3			
SMBF, ml · 100 ml tissue ⁻¹ · min ⁻¹							
Control	2.99 ± 0.50	3.92 ± 0.90	4.20 ± 1.10	4.18 ± 1.1			0.001
Type 2 diabetes	2.11 ± 0.17	2.92 ± 0.46	2.99 ± 0.54	3.09 ± 0.50			
Glucose, nmol · 100 ml tissue ⁻¹ · min ⁻¹							
Control	470 ± 107	469 ± 166	237 ± 152	288 ± 177	0.02	0.01	
Type 2 diabetes	136 ± 37	66 ± 92	10 ± 66	-78 ± 62			
Lactate, nmol · 100 ml tissue ⁻¹ · min ⁻¹							
Control	-217 ± 39	-441 ± 95		-409 ± 91			0.001
Type 2 diabetes	-243 ± 48	-455 ± 86		-529 ± 91			
Glycerol, nmol · 100 ml tissue ⁻¹ · min ⁻¹							
Control	-10 ± 4	-11 ± 19	-18 ± 6	-5 ± 10	0.05	0.001	
Type 2 diabetes	-28 ± 14	-44 ± 10	-43 ± 18	-57 ± 22			
FFA, nmol · 100 ml tissue ⁻¹ · min ⁻¹							
Control	69 ± 38	246 ± 173	172 ± 112	196 ± 126			0.02
Type 2 diabetes	19 ± 31	82 ± 60	64 ± 55	34 ± 43			

Data are means ± SE. TBF, total forearm blood flow; SMBF, skeletal muscle blood flow. Levels of statistical significance are indicated on right.

2 diabetes $5,840 \pm 132$ vs. $7,039 \pm 430$ mm², $P < 0.05$). When expressed as the percentage of total forearm area, the percentages of skin (5.9 ± 0.1 vs. $5.4 \pm 0.2\%$, $P < 0.05$) and muscle (66.8 ± 1.0 vs. $61.3 \pm 1.0\%$, $P < 0.001$) were significantly lower in type 2 diabetes, whereas the amount of subcutaneous adipose tissue was significantly higher in type 2 diabetes (13.5 ± 1.1 vs. $20.9 \pm 1.2\%$, $P < 0.001$). Baseline TBF and the Iso-induced increase in TBF ($P < 0.001$) tended to be lower in type 2 diabetes (Table 4), but differences between groups did not reach statistical significance. Forearm subcutaneous ATBF also significantly increased during Iso infusion in both groups ($P < 0.001$), and there were no differences between both groups (baseline vs. Iso: control 2.40 ± 0.49 vs. 4.22 ± 1.04 and type 2 diabetes: 1.82 ± 1.04 vs. 5.29 ± 1.23 ml · 100 g tissue⁻¹ · min⁻¹). Relative values for SBF were comparable in both groups and were similar during baseline and Iso-stimulated conditions (baseline vs. Iso: control 5.2 ± 0.6 vs. 5.1 ± 0.3 and type 2 diabetes: 5.0 ± 0.5 vs. 4.8 ± 0.5 ml · 100 g tissue⁻¹ · min⁻¹). Estimated SMBF and TBF were not significantly different in controls and type 2 diabetes (Table 4). It can be concluded that changes in TBF are similar to changes in SMBF. For this reason, TBF (or PBF) is used in calculating skeletal muscle substrate exchange.

Forearm muscle substrate exchange. Deep venous O₂ saturation of Hb was $40.0 \pm 2.7\%$ in controls and $43.9 \pm 2.6\%$ in type 2 diabetes, indicating that the forearm substrate exchange mainly reflects muscle metabolism. Net muscle glucose uptake was significantly lower in type 2 diabetes compared with controls both under basal conditions and during Iso infusion (Table 4). In both groups, there was an increase in muscle lactate release during Iso infusion. Both during baseline and during Iso infusion, muscle glycerol release

was higher in type 2 diabetes, whereas net muscle FFA uptake tended to be lower in type 2 diabetes compared with controls. The net plasma FFA uptake by the forearm is two- and sixfold higher than FFA delivery derived from forearm lipolysis (3 times glycerol release) in controls during overnight-fasted conditions and Iso stimulation, respectively, whereas in type 2 diabetic subjects net FFA uptake is more than twofold lower than FFA delivery derived from forearm lipolysis in both conditions.

Components of forearm muscle FFA metabolism. Both during basal conditions and Iso infusion, skeletal muscle FFA uptake and FFA release were significantly lowered in type 2 diabetes (Fig. 2). Expressing the contribution of muscle FFA uptake to whole body rate of disappearance of FFA yields significantly lower values in type 2 diabetes compared with controls (on average: type 2 diabetes 10% vs. control 15%; Fig. 3).

During baseline conditions, no significant muscle ¹³C₂ production and thus no significant oxidation of plasma FFA by muscle could be detected in both groups. During Iso infusion, plasma FFA oxidation by the forearm muscle was significantly increased in controls and still was not significantly different from a zero exchange in type 2 diabetes. A correction for the ¹³C label fixation by means of the whole body acetate recovery factor yielded an oxidation of plasma FFA accounting for 50% of muscle FFA uptake in controls and no significant oxidation of plasma FFA in type 2 diabetes (Fig. 2).

Skeletal muscle biopsies. Cytoplasmatic FABP content of the vastus lateralis muscle was significantly higher in controls than in type 2 diabetic subjects (Table 5). Also, muscle oxidative capacity, reflected by the activity of HAD and CS, was lowered in type 2 diabetes, whereas LDH activity was comparable in both groups.

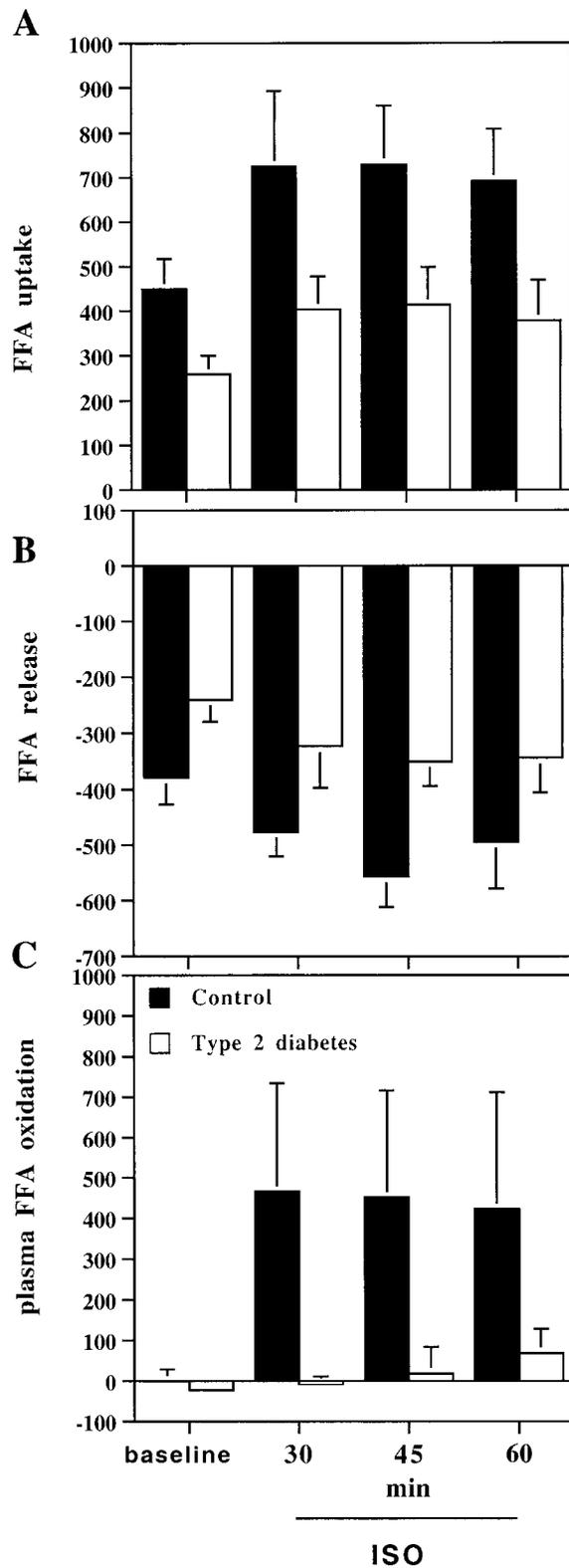


Fig. 2. Forearm skeletal muscle free fatty acid (FFA) uptake, release, and oxidation (in $\text{nmol} \cdot 100 \text{ ml forearm tissue}^{-1} \cdot \text{min}^{-1}$) during baseline and Iso-stimulated conditions in control and type 2 diabetic subjects. In A and B, $P < 0.05$ for Iso effect and group effect. In C, $P < 0.05$ for Iso effect and interaction of group \times Iso effect.

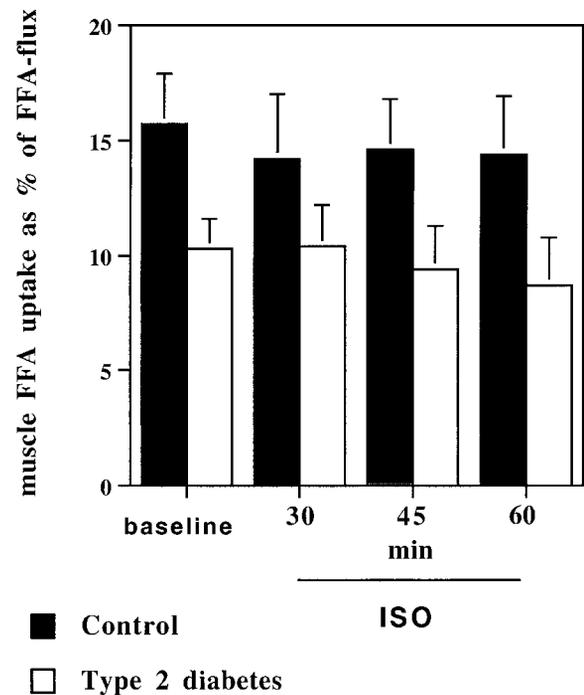


Fig. 3. Forearm skeletal muscle FFA uptake expressed as percentage of total FFA flux during baseline and Iso-stimulated conditions in control and type 2 diabetic subjects. For group effect: $P < 0.05$.

DISCUSSION

We have used an isotopic tracer approach in combination with the forearm balance technique to quantify the uptake, release, and oxidation of plasma FFA by skeletal muscle in obesity-associated type 2 diabetes during postabsorptive and catecholamine-stimulated conditions. The present data show a reduced uptake of plasma FFA by skeletal muscle in obese subjects with mild type 2 diabetes under baseline conditions and during intravenous infusion of the nonselective β -agonist Iso. Additionally, during Iso infusion, the oxidation of plasma FFA by skeletal muscle was significantly increased in controls (accounting for 50% of muscle FFA uptake), whereas in type 2 diabetic subjects muscle $^{13}\text{CO}_2$ production was not significantly different from zero. The impairments in skeletal muscle plasma fatty acid uptake and oxidation are accompanied by a diminished skeletal muscle content of cytoplasmic FABP and a diminished muscle oxidative capacity.

Table 5. Skeletal muscle cytoplasmic FABP content and CS, HAD, and LDH activity

	Control	Type 2 Diabetes	P Value
FABP, mg/g total protein	1.190 ± 0.197	0.498 ± 0.118	0.01
HAD, U/g total protein	58.3 ± 9.2	29.2 ± 7.2	0.02
CS, U/g total protein	105.1 ± 19.4	62.5 ± 10.9	0.08
LDH, U/g total protein	571 ± 136	562 ± 133	

Data are means \pm SE. FABP, fatty acid-binding protein; HAD, 3-hydroxyacyl-CoA dehydrogenase; CS, citrate synthase; LDH, lactate dehydrogenase.

Methodological Considerations

Forearm muscle blood flow. The results of the present study show that changes in TBF are similar to changes in SMBF in both control and type 2 diabetic subjects, indicating that it is valid to use TBF (or forearm plasma flow) for calculating forearm skeletal muscle substrate fluxes, as reported previously in obese subjects (2).

Acetate recovery factor. To correct the rate of plasma FFA oxidation for the incomplete label recovery in breath, an acetate recovery factor was used; the recovery factor has been proposed to correct for label fixation of a fatty acid tracer in pathways going from the entrance of labeled acetyl-CoA to the tricarboxylic acid cycle until the recovery of label in expired CO₂. This factor has been extensively validated in experiments of Sidossis et al. (21, 22) and more recently by Schrauwen et al. (19) for experiments lasting <2 h in lean and obese subjects. Additionally, the acetate recovery factor across (leg) muscle has been measured and was reported to be about equal to the whole body recovery factor (16).

Baseline Fatty Acid Utilization

During baseline conditions, there was an impaired uptake of FFA by muscle in type 2 diabetic subjects, resulting in a significant decrease in the contribution of skeletal muscle to whole body FFA disposal (10% in type 2 diabetes vs. 15% in control; Fig. 3). These data seem to correspond to data of Kelley and co-workers (5, 14), showing an impaired muscle FFA uptake during basal conditions in visceral obesity and type 2 diabetes.

In the present study, there was no significant forearm muscle ¹³CO₂ production under overnight-fasted conditions both in controls and in type 2 diabetic patients, suggesting no oxidation of plasma FFA by skeletal muscle. However, we cannot exclude the possibility that the ¹³CO₂ production cannot be measured during the 90-min baseline infusion period with the present tracer infusion rate, as the recovery of ¹³CO₂ from a palmitate tracer may be low (after 90 min infusion: 7% recovery in expired air) and may increase linearly in time for periods of 5–6 h. Thus, to draw definite conclusions on the quantitative differences in the oxidation of plasma fatty acids by skeletal muscle between patients and controls during overnight-fasted conditions, a higher infusion rate and/or a more prolonged infusion period may be required.

Iso-Mediated Fatty Acid Utilization

As during baseline conditions, we found a diminished forearm muscle FFA uptake in the type 2 diabetic subjects during Iso-stimulated conditions, resulting in an impaired systemic FFA clearance by skeletal muscle. Additionally, in type 2 diabetic subjects, muscle ¹³CO₂ production was not significantly different from zero during Iso infusion, whereas the oxidation of plasma FFA by skeletal muscle was significantly increased in controls (accounting for 50% of muscle FFA

uptake). In accordance with the forearm muscle data, the increases in the whole body oxidation of plasma FFA and total fat oxidation were significantly blunted in type 2 diabetes compared with controls. Thus these data strongly point to an impairment of Iso-induced fat oxidation in type 2 diabetes.

Underlying Mechanisms

Forearm muscle glycerol release was three and five times as high in type 2 diabetic subjects compared with controls during basal and Iso-stimulated conditions, respectively (Table 4). On one hand, this may be explained by a higher intramuscular or plasma VLDL triglyceride lipolysis and/or a higher lipolysis in adipose cells in the perimysium covering muscle, which indicates, in view of the lowered FFA release, an increased utilization (oxidation and/or reesterification) of FFA released by forearm lipolysis in type 2 diabetic subjects. Increased forearm lipolysis in fact may flood the muscle with FFA and reduce the FFA concentration gradient between blood and muscle, which is one of the primary determinants of plasma FFA uptake and oxidation (25). Therefore, an increased forearm lipolysis may be an important mechanism for the reduced uptake and oxidation of plasma FFA. However, on the other hand, care has to be taken in interpreting muscle glycerol metabolism from net release data as glycerol tracer balance studies have shown that glycerol disappears into muscle and may be metabolized (7). Therefore, the increased forearm glycerol release in type 2 diabetic subjects also may be a consequence of a decreased rate of intramuscular glycerol metabolism. Further studies are necessary to elucidate this issue.

To examine other potential mechanisms behind the lowered plasma FFA utilization by skeletal muscle in type 2 diabetes, FABP and activities of mitochondrial enzymes were examined. Muscle biopsy analyses showed a lower cytoplasmatic FABP content and lower HAD and CS activities in type 2 diabetes compared with controls. As far as we are aware, this is the first study that demonstrates a diminished cytoplasmatic FABP content in skeletal muscle of type 2 diabetic subjects. Because FABP functions in the intracellular transport of long-chain fatty acids (11), these data suggest that a lowered cytoplasmatic fatty acid transport may also contribute to the disturbances in muscle fat utilization in type 2 diabetes.

In summary, this study demonstrates that the previously reported impairment in skeletal muscle fat oxidation in type 2 diabetic subjects also extends to β-adrenergically mediated conditions. Additionally, it was demonstrated that both the uptake and oxidation of plasma fatty acids are impaired in skeletal muscle of type 2 diabetic subjects. We suggest that the mechanisms behind the reduced plasma FFA uptake and oxidation in skeletal muscle of type 2 diabetic patients may involve 1) the flooding of muscle with FFA generated by muscle lipolysis, leading to a decrease of the blood-muscle FFA gradient and 2) a decrease of the

fatty acid transport capacity as a consequence of a reduced FABP content.

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