

Substrate utilization in non-obese Type II diabetic patients at rest and during exercise

L. B. BORGHOUTS*, A. J. M. WAGENMAKERS†, P. L. L. GOYENS† and H. A. KEIZER*

*Department of Movement Sciences, Maastricht University, 6200 MD Maastricht, The Netherlands and †Human Biology, Maastricht University, 6200 MD Maastricht, The Netherlands

A B S T R A C T

Recently, we observed that impairments exist in skeletal muscle free fatty acid (FFA) utilization during exercise in obese subjects with Type II diabetes. The main objective of the present study was to investigate whether plasma FFA oxidation is impaired during exercise in non-obese Type II diabetic patients. Stable isotope tracers of palmitate and glucose were infused for 2 h at rest and 1 h of bicycle exercise at 40% peak oxygen consumption ($\dot{V}O_{2\max}$) in volunteers with Type II diabetes and a healthy control group. At rest, plasma FFA oxidation was not significantly different between subjects with Type II diabetes and control subjects (2.13 ± 0.51 versus $1.93 \pm 0.54 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ respectively). During exercise, Type II diabetic patients and control subjects had similar rates of total fat [Type II diabetes, $9.62 \pm 1.84 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; control, $12.08 \pm 4.59 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; not significant (NS)] and glucose oxidation (Type II diabetes, $44.24 \pm 10.36 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; control, $57.37 \pm 14.54 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; NS). No aberrations were present in plasma FFA uptake [rate of disappearance (R_d); Type II diabetes, 11.78 ± 4.82 ; control, 10.84 ± 3.39 ; NS] and oxidation rates (Type II diabetes 8.10 ± 1.44 ; control 8.00 ± 3.12 , NS) in Type II diabetic patients; triacylglycerol-derived fatty acid oxidation was 2.6-fold lower in Type II diabetic patients than in control subjects, but this difference was not statistically significant. Muscle glycogen oxidation was lower in diabetes patients than in control subjects (Type II diabetes, $25.16 \pm 13.82 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; control, $42.04 \pm 10.58 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; $P < 0.05$) and plasma glucose contributed more to energy expenditure in Type II diabetes ($26 \pm 3\%$ in diabetic versus $15 \pm 2\%$ in control, $P < 0.05$). We conclude that plasma FFA oxidation is not impaired during exercise in non-obese Type II diabetic patients. The data confirm that Type II diabetes is a heterogeneous disease, and that the adaptation at the substrate level differs between obese and non-obese patients and may contribute to differences in the final appearance of the various phenotypes.

INTRODUCTION

Under resting conditions, it has been demonstrated that skeletal muscle of Type II diabetic patients shows an impaired utilization of plasma free fatty acid (FFA), whereas whole-body lipid oxidation is normal [1,2].

Since skeletal muscle is responsible for the major part of plasma FFA oxidation during exercise, an impaired utilization of FFA by skeletal muscle could result in an altered pattern of substrate oxidation during exercise. In accordance, in a recently published study by our group [3], it was shown that both whole-body uptake and

Key words: free fatty acids, glucose turnover, muscle glycogen, muscle triacylglycerols, stable isotopes, Type II diabetes.

Abbreviations: ACF, acetate correction factor; BMI, body mass index; CHO, carbohydrate; FFA, free fatty acid; FFM, fat free mass; GC-IRMS, gas chromatograph–isotope ratio mass spectrometer system; NS, not significant; R_a , rate of appearance; R_d , rate of disappearance; TG-derived FA, triacylglycerol-derived fatty acid; TTR , tracer/tracee ratio; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}O_2$, oxygen consumption; $\dot{V}O_{2\max}$, peak oxygen consumption.

Correspondence: Dr H. A. Keizer (e-mail hans.keizer@bw.unimaas.nl).

oxidation of plasma FFA is diminished by approx. 30 % during exercise in obese Type II diabetic patients.

Although obesity is an important factor in the development of Type II diabetes in the majority of patients, between 20 to 40 % of Type II diabetic patients are non-obese [4]. Indeed, Type II diabetes has been subclassified according to association with obesity, and this subclassification may represent the existence of heterogeneous groups of patients [4]. It should be noted that studies reporting aberrations in skeletal muscle oxidation of plasma FFA [1,2] were conducted in obese diabetic patients. Thus, whether plasma FFA oxidation during exercise is equally diminished in non-obese Type II diabetic patients is not clear.

Therefore, the main objective of the present study was to investigate whether plasma FFA oxidation during moderate intensity exercise is decreased in Type II diabetic patients without overt obesity.

A characteristic finding in both obese and non-obese Type II diabetic patients is that plasma glucose often declines in the course of exercise [5,6]. This has been attributed to either an impaired splanchnic glucose output [7] or increased plasma glucose uptake [6,8] by exercising muscle. A secondary objective of this study was therefore to compare plasma glucose uptake during exercise between Type II diabetic patients and healthy control subjects.

We evaluated substrate utilization at rest and during 60 min of moderate intensity exercise in non-obese Type II diabetic patients, in comparison to a healthy control group matched for body composition. To this end, stable isotopes of palmitate and glucose were infused simultaneously to determine the rates of appearance (R_a) and disappearance (R_d) of these metabolites, and the rate of oxidation of plasma FFA.

METHODS

Subjects

The Medical Ethics Committee of Maastricht University approved the protocol of the study. Eight male Type II diabetic patients were recruited in collaboration with the Department of Internal Medicine of the St. Anna Hospital, Geldrop, The Netherlands. The subjects volunteered for the study after giving their written informed consent. Exclusion criteria were impaired renal or liver function, usage of corticosteroids, history of cardiac disease, hypertension, diabetic complications and insulin therapy. All Type II diabetic subjects were taking blood-glucose-lowering medication orally. Five were taking sulphonylureas only, and 3 were taking sulphonylureas in combination with metformin. All medication was discontinued 3 days before testing. Mean $\pm$ S.D. glycosylated haemoglobin was $7.7 \pm 2.0\%$. The subjects in the present study had a mean body mass index (BMI)

Table 1 Subject characteristics

Values are mean $\pm$ S.D. *Denotes significant difference from Type II diabetes with $P < 0.05$.

Characteristics	Type II diabetes	Control
Age (years)	58 $\pm$ 5	47 $\pm$ 5*
Body mass (kg)	86.4 $\pm$ 14.1	86.3 $\pm$ 9.5
BMI (kg $\cdot$ m ⁻²)	28.7 $\pm$ 4.2	27.8 $\pm$ 2.8
Body fat (%)	26.5 $\pm$ 8.4	29.2 $\pm$ 5.3
Lean body mass (kg)	59.9 $\pm$ 6.7	60.7 $\pm$ 3.9
$\dot{V}O_{2\max}$ (ml $\cdot$ kg ⁻¹ $\cdot$ min ⁻¹)	25.8 $\pm$ 3.3	36.2 $\pm$ 2.0*

similar to the average BMI for their age group [9], which according to international standards is overweight, but not obese. The healthy control group ($n = 8$) was matched for weight and body composition, and underwent a standard medical examination. Control subjects with a family history of diabetes were excluded. All volunteers were sedentary. Subject characteristics are presented in Table 1.

Body composition

Subjects were weighed and their body fat percentage and fat free mass (FFM) were determined using a skinfold calliper. Skinfolds measured were located subscapular, suprailiac, and over the biceps and triceps of the upper arm. From the sum of skinfolds, the body fat percentage was calculated according to the method of Durnin and Womersley [10].

Peak oxygen consumption ($\dot{V}O_{2\max}$) testing

A maximal bicycle test was performed in all volunteers. Subjects cycled on an electromagnetically braked bicycle ergometer (Lode, Groningen, The Netherlands) at a self-determined rate, but above 60 r.p.m., at a workload of $0.75 \text{ W} \cdot \text{kg of FFM}^{-1}$ for 3 min. After 3 min, the workload was adjusted to $1.5 \text{ W} \cdot \text{kg of FFM}^{-1}$ and increased every 3 min by $0.5 \text{ W} \cdot \text{kg of FFM}^{-1}$ until subjects were no longer able to maintain a pedalling frequency above 60 r.p.m. Open-circuit spirometry was performed with an Oxycon β (Mijnhardt Jaeger, Mannheim, Germany), oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured in the breath-by-breath mode and averaged over 15 s. $\dot{V}O_{2\max}$ was achieved in all tests as judged from the levelling off of the oxygen uptake at the end of the test.

Experimental protocol

Subjects were asked to consume a carbohydrate (CHO)-rich diet ($> 200 \text{ g of CHO/day}$) and to refrain from physical exercise 3 days prior to the test day. They were asked to abstain from food high in naturally enriched carbon 1 week before and during the test period, for which goal they were provided with a list of such products. In addition, Type II diabetic patients dis-

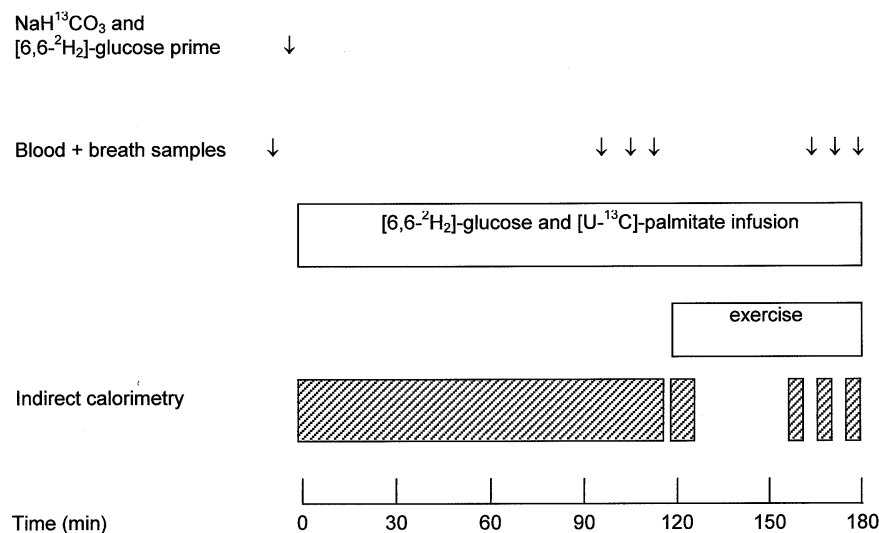


Figure 1 Experimental protocol

continued medication known to affect glucose uptake during this period.

On the test day, subjects reported to the laboratory at 08:30 hours after an overnight fasting period of 12 h. Subjects were recumbent with one hand in a heated air box (60 °C). This hand was cannulated in a dorsal hand vein in order to obtain arterialized blood samples. A second catheter was inserted in an antecubital vein of the contralateral arm for infusion of the tracers of glucose and palmitate. Figure 1 shows the experimental protocol. Basal blood samples (20 ml) were drawn into EDTA-containing tubes for determination of the concentrations of glucose, FFA, insulin and lactate, for measurement of plasma FFA profiles, and enrichment of glucose and palmitate. Blood was immediately centrifuged at 4 °C for 8 min and subsequently plasma was frozen in liquid N_2 and stored at -80 °C until analysis. Blood sampling was repeated at 90, 105, 120, 160, 170 and 180 min. Simultaneously, breath samples were taken from a mixing chamber into vacuum tubes for measurement of CO_2 enrichment. After basal sampling, a priming dose of sodium bicarbonate (0.085 mg/kg of $\text{NaH}^{13}\text{CO}_3$) was administered, followed by a priming dose of $[6,6-^2\text{H}_2]\text{-glucose}$ (equal to the accumulated dose of 1 h of continuous infusion). This marked the start of 2 h of rest, during which a continuous intravenous infusion of $[6,6-^2\text{H}_2]\text{-glucose}$ ($0.300 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and $[\text{U-}^{13}\text{C}]\text{-palmitate}$ ($0.0064 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was given. Isotopes were obtained from Cambridge Isotope Laboratories (Andover, MA, U.S.A.). Before infusion, palmitate tracer (60 mg of potassium salt of $[\text{U-}^{13}\text{C}]\text{-palmitate}$, enrichment 98.9%) was bound to albumin by dissolving it in heated (60 °C) sterile water and passing it through a $0.2 \mu\text{m}$ filter into a warm (60 °C) human serum albumin solution, giving a 0.670 mM infusion solution. Subjects were placed under a ventilated hood, with an airflow

of 35 l/min, for indirect calorimetry. At 120 min the subjects were placed on a magnetically braked cycle ergometer and commenced cycling at a load calculated to correspond to 40% $\dot{V}\text{O}_{2\text{max}}$. Isotope infusion rates were increased to $0.460 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and $0.0127 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for glucose and palmitate respectively. The true concentrations of palmitate, glucose and acetate in the infusate were determined for each experiment, so that the exact rate of infusion could be calculated. During the first 10 min of exercise, indirect calorimetry was performed and the load was adjusted such that the desired value of 40% $\dot{V}\text{O}_{2\text{max}}$ was approximated. Prior (5 min) to each blood and breath sampling, indirect calorimetry was repeated. Exercise was discontinued after 60 min.

Acetate recovery protocol

One week before the experimental trial, a similar test was performed in each subject in order to calculate the acetate correction factor (ACF). The ACF accounts for ^{13}C -label lost in exchange reactions in the tricarboxylic acid cycle [11–13]. In brief, the same protocol used during the experimental test was followed, with the omission of blood sampling. Instead of the palmitate and glucose infusions, a sodium bicarbonate-primed (0.085 mg/kg $\text{NaH}^{13}\text{CO}_3$) continuous infusion of $[1,2-^{13}\text{C}]\text{-acetate}$ (Cambridge Isotope Laboratories) was given, in which the amount of ^{13}C -label given equalled that of the palmitate infusion during the experimental test.

Sample analysis

Blood plasma was analysed for glucose using the hexokinase method (Roche, Basel, Switzerland), for lactate

[14] and for FFA (Wako Chemicals, Neuss, Germany) on a Cobas Fara[®] analyser (Roche). Insulin was measured with AutoDELFIA fluoroimmunoassay [15] (EG&G, Breda, The Netherlands; coefficient of variation 4.4%). Breath sample $^{13}\text{C}/^{12}\text{C}$ ratios were analysed using a gas chromatograph–isotope ratio mass spectrometer system (GC-IRMS; Finnigan MAT 252, Bremen, Germany). For determination of plasma palmitate, FFA were extracted from plasma, isolated by TLC, and derivatized to their methyl esters. Palmitate concentration was determined on a gas chromatograph with flame ionization detection using heptadecanoic acid as an internal standard. Palmitate concentration was on average $31 \pm 4\%$ 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 the derivative. For determination of glucose enrichment in plasma, aliquots of EDTA plasma were extracted first with methanol:chloroform and then with chloroform:water. The clear water layer was dried and a butylboronic acid-acetyl derivative was made. Subsequently, the enrichment of the glucose derivative was determined by electron ionization/GC-MS (Finnigan INCOS XL, San Jose, CA, U.S.A.).

Calculations

Breath and plasma enrichment is expressed as the TTR of the sample, corrected for background. TTR is defined as:

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

in which sa is the sample and bk is the background.

Total fat and CHO oxidation was calculated from indirect calorimetry using the following equations [16]:

$$\text{Total fat oxidation} = 1.695 \dot{V}\text{O}_2 - 1.701 \dot{V}\text{CO}_2$$

$$\text{Total CHO oxidation} = 4.585 \dot{V}\text{O}_2 - 3.226 \dot{V}\text{CO}_2$$

with $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ in litres per min, and total fat and CHO oxidation in g per min.

The rate of total fatty acid oxidation was calculated by converting total fat oxidation in g into its molar equivalent, assuming that 1 mole of triacylglycerols equals 860 g, and multiplying the molar rate of triacylglycerol oxidation by 3, since one molecule of triacylglycerols contains 3 fatty acids.

^{13}C recovery in breath from $[\text{U-}^{13}\text{C}]$ palmitate ($\mu\text{mol} \cdot \text{min}^{-1}$) was calculated as:

$$[(TTR_{\text{CO}_2} \times \dot{V}\text{CO}_2)/22.2966] \times 1000$$

where TTR_{CO_2} is the $^{13}\text{C}/^{12}\text{C}$ ratio in breath CO_2 , $\dot{V}\text{CO}_2$ the CO_2 production in $\text{ml} \cdot \text{min}^{-1}$ and 22.2966 the volume of 1 mole of CO_2 .

The ACF was calculated from the acetate infusion experiment as:

$$[(TTR_{\text{CO}_2} \times \dot{V}\text{CO}_2)/22.2966]/F \times 2$$

in which F is the rate of $[1,2\text{-}^{13}\text{C}]$ acetate infusion ($\mu\text{mol} \cdot \text{min}^{-1}$).

The rate of plasma palmitate oxidation ($\mu\text{mol} \cdot \text{min}^{-1}$) can subsequently be calculated as:

$$[(^{13}\text{C} \text{ recovery in breath from palmitate}/\text{ACF})/16]/TTR_p$$

in which 16 is the number of ^{13}C carbon atoms in $[\text{U-}^{13}\text{C}]$ palmitate and TTR_p is the TTR of palmitate in plasma. Total plasma fatty acid oxidation was subsequently calculated by dividing the palmitate oxidation rate by the proportion palmitate contributes to total plasma FFA. When indirect calorimetry-derived total fatty acid oxidation was higher than the total plasma fatty acid oxidation, the difference between these values was assumed to be accounted for by oxidation of fatty acids derived from inter- or intramuscular triacylglycerols and circulating triacylglycerols (mainly very-low-density lipoprotein) [17]. These sources will collectively be referred to as triacylglycerol-derived fatty acids (TG-derived FAs).

TG-derived FA oxidation

$$= \text{total fatty acid oxidation} - \text{plasma FFA oxidation}$$

Rate of appearance (Ra) of palmitate was calculated from steady-state equations, since at the time of sampling under both basal and exercise conditions, a tracer and tracee steady state was present (that is, both palmitate TTR_p and plasma palmitate concentration were stable). Therefore, Ra was assumed to be equal to the rate of disappearance (Rd) and calculated as:

$$Ra = F(TTR_i/TTR_p)$$

where TTR_i stands for the TTR of palmitate carbon in the infusate.

For glucose, non-steady-state equations were applied [18], since glucose has a slower turnover than FFA, and because in the diabetic subjects plasma glucose decreased during the course of the experiment, and thus Rd must have been higher than Ra .

These equations have been described elsewhere [19] as:

$$Ra = \frac{F - V[(C_2 + C_1)/2][(TTR_2 - TTR_1)/(t_2 - t_1)]}{(TTR_2 - TTR_1)/2}$$

in which F is the rate of $[6,6\text{-}^2\text{H}_2]$ glucose infusion ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$), and

$$Rd = Ra - V(C_2 - C_1)(t_2 - t_1)$$

in which V is the assumed volume of distribution for glucose, which was assumed to be $160 \text{ ml} \cdot \text{kg}^{-1}$, C_2 and C_1 are the concentrations at the time points t_2 and t_1 respectively.

When indirect calorimetry derived values of glucose oxidation were higher than glucose Rd , muscle glycogen oxidation was calculated as:

Muscle glycogen oxidation

$$= \text{total glucose oxidation} - \text{glucose } Rd$$

For this calculation to be valid, it has to be assumed that all glucose disappearing from the plasma is oxidized. A previous study [20] has shown that this condition is met during exercise. However, at rest this is not the case and, therefore, this calculation has only been made for the exercise period.

Statistics

Differences between groups were tested with two-tailed Student's *t* test for unpaired data, and within groups with two-tailed Student's *t* test for paired data.

RESULTS

Rest

The resting overnight fasted glucose concentration was higher in Type II diabetes than in control, and plasma insulin was similar (Table 2). Plasma glucose was stable during the resting period in control subjects, and declined at a rate of 0.6 ± 0.3 mM/h in Type II diabetes ($P < 0.05$).

Table 2 Blood plasma measurements, under post-absorptive resting conditions and during the 1 h exercise period

Values are mean $\pm$ S.D. from 90–120 min (rest) and 160–180 min (exercise). *Denotes significant difference from basal with $P < 0.05$; †denotes significant difference from Type II diabetes with $P < 0.05$.

	Rest		Exercise	
	Type II diabetes	Control	Type II diabetes	Control
Glucose (mmol/l)	7.7 ± 1.5	4.9 ± 0.3 †	6.3 ± 1.1 *	4.9 ± 0.5
Insulin (m-unit/l)	11 ± 3	9 ± 2	10 ± 2	8 ± 1
FFA (μ mol/l)	476 ± 92	447 ± 120	605 ± 130 *	639 ± 237 *
Lactate (mmol/l)	0.8 ± 0.1	0.8 ± 0.2	1.3 ± 0.5	1.2 ± 0.5

Insulin levels did not change in either group during the resting period. Plasma FFA levels were similar in Type II diabetes and control (Table 2).

Mean resting energy expenditure was 320 ± 67 KJ/h in Type II diabetes and 307 ± 95 KJ/h in control [not significant (NS)]. Total glucose oxidation and total fatty acid oxidation were not significantly different between Type II diabetes and control (Table 3). Plasma glucose *Ra* was similar in Type II diabetes and control (Table 3). Plasma glucose *Rd* was significantly greater than *Ra* ($P < 0.05$) in Type II diabetes, but not in control, as already indicated by the significant decline in plasma glucose in Type II diabetes only. There was no difference in plasma FFA *Ra* between groups. Plasma FFA oxidation, as measured from $^{13}\text{CO}_2$ production, was not significantly different between groups. The ACF was similar in Type II diabetes and control, 22.1 ± 0.02 and 22.4 ± 0.02 % respectively.

Exercise

Subjects cycled at a relative exercise intensity of 44 ± 3 % and 41 ± 6 % (Type II diabetes and control respectively, NS). Since $\dot{V}\text{O}_{2\text{max}}$ was somewhat higher in control than in Type II diabetes, the absolute power output during exercise was higher in the controls (69 ± 23 W versus 52 ± 11 W in Type II diabetes). During the 60 min of exercise, plasma glucose declined at a rate of 1.5 ± 1 mM/h in Type II diabetes only ($P < 0.05$). Insulin levels did not change significantly from basal values. Lactate levels rose slightly in the course of exercise, but this change was not significant compared with the resting condition. Plasma FFA levels increased significantly, with no difference between groups. Respiratory exchange ratios were similar between the two groups during exercise, 0.87 ± 0.04 in Type II diabetes and 0.86 ± 0.04 in control. Total glucose oxidation was increased approx. 9-fold compared with basal, and total fatty acid oxidation

Table 3 Glucose and lipid turnover, and oxidation

Values are in $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ and expressed as mean $\pm$ S.D. *Denotes significant difference from basal with $P < 0.01$; †significant difference from *Ra* with $P < 0.05$; ‡significant difference from Type II diabetes with $P < 0.05$.

	Rest ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)		Exercise ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	
	Type II diabetes	Control	Type II diabetes	Control
Glucose <i>Ra</i>	9.20 ± 1.71	9.79 ± 1.43	14.92 ± 4.76 *	16.14 ± 3.70 *
Glucose <i>Rd</i>	10.60 ± 0.98 †	10.06 ± 1.63	19.12 ± 4.92 *†	15.33 ± 4.34 *
Palmitate <i>Ra</i> (= <i>Rd</i>)	6.34 ± 1.83	5.12 ± 0.94	11.78 ± 4.82 *	10.84 ± 3.39 *
Plasma FFA oxidation	2.13 ± 0.51	1.93 ± 0.54	8.10 ± 1.44 *	8.00 ± 3.12 *
TG derived FA oxidation	1.97 ± 0.54	1.47 ± 0.81	1.52 ± 1.41	4.07 ± 4.47
Total fatty acid oxidation	4.10 ± 0.74	3.40 ± 0.90	9.62 ± 1.84 *	12.08 ± 4.59 *
Glycogen oxidation	—	—	25.16 ± 13.82	42.04 ± 10.58 ‡
Total CHO oxidation	5.37 ± 2.44	6.51 ± 3.12	44.28 ± 10.36 *	57.37 ± 14.54 *

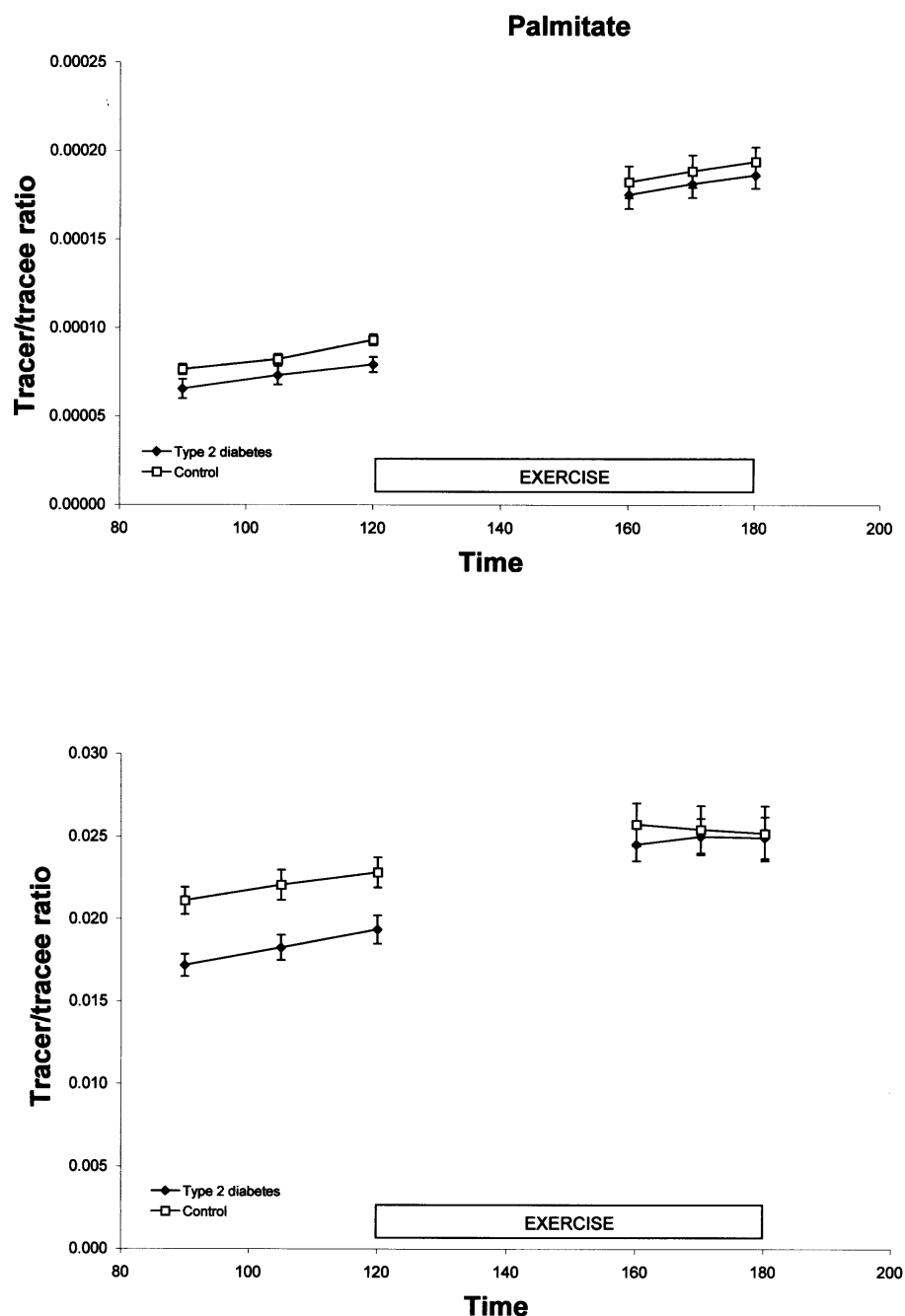


Figure 2 Plasma *TTRs* of glucose and palmitate at rest (90–120 min) and during exercise (160–180 min) in Type II diabetes (◆) and control (□) groups

increased approx. 3-fold; differences were not significant between groups (Table 3). Blood plasma *TTRs* for [6,6- $^2\text{H}_2$]glucose and [U- ^{13}C]palmitate during exercise are shown in Figure 2. With an inter-condition range in *TTR* of maximally 0.00001, palmitate enrichment could be regarded in steady state. Glucose *Ra* and *Rd* increased during exercise, with no significant difference between Type II diabetes and control. As under resting conditions, glucose *Rd* was significantly higher than *Ra* in Type II diabetes only ($P < 0.01$). Muscle glycogen oxidation was

17% lower ($P < 0.05$) in Type II diabetes than in control (Table 3). Plasma FFA levels were stable during the last 20 min of exercise, and elevated compared with basal. FFA *Rd* was similar in Type II diabetes and control. Plasma FFA oxidation was not significantly different between Type II diabetes and control (Table 3). Triacylglycerol-derived fatty acid oxidation was 2.6-fold lower in Type II diabetes than control, but this difference was not statistically significant ($P = 0.17$). The ACF was $54.5 \pm 0.06\%$ in Type II diabetes and $70.7 \pm 0.07\%$ in

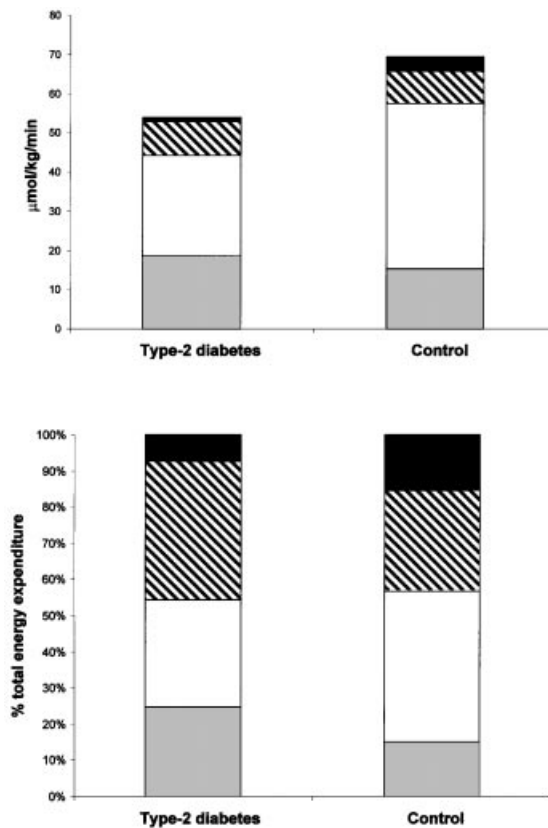


Figure 3 Substrate utilization during exercise in Type II diabetes and control groups

Upper panel: the absolute amounts oxidized. Lower panel: the relative contribution to energy expenditure. Grey-shaded bars, glucose *Rd*; open bars, glycogen oxidation; hatched bars, plasma FFA oxidation; closed bars, TG-derived FA oxidation.

control ($P < 0.05$). Figure 3 shows the contribution of the different substrates to energy provision during exercise.

Converting absolute amounts of substrate oxidized to their relative contribution to energy delivery (Figure 3) revealed that plasma glucose oxidation accounted for 26 ± 3 and $15 \pm 2\%$ ($P < 0.01$), and muscle glycogen oxidation for 29 ± 5 and $42 \pm 4\%$ ($P < 0.05$) of total energy expenditure in Type II diabetes and control, respectively. In Type II diabetes and control, 38 ± 5 and $30 \pm 12\%$ (NS) of total energy was derived from plasma FFA, and 7 ± 7 and $14 \pm 14\%$ (NS) from TG-derived FA, respectively.

DISCUSSION

This study investigated substrate metabolism during exercise in non-obese Type II diabetic patients. No alterations in fat metabolism were present in Type II diabetic patients; uptake and oxidation of FFAs from plasma were comparable with that in healthy control

subjects. The ratio of plasma FFA uptake to plasma FFA oxidation was comparable between groups (71 ± 15 and $75 \pm 13\%$ in Type II diabetic patients and control subjects respectively, NS). These findings are in contrast with those reported recently by our group in obese Type II diabetic patients using the same protocol [3]. Obese Type II diabetic patients had an impaired whole-body oxidation of plasma FFAs during exercise [3]. In accordance, in obese Type II diabetic patients, plasma FFA uptake by skeletal muscle is diminished under resting conditions [1,2]. This implies that there are differences between obese and non-obese Type II diabetic patients. If we assume that obese and non-obese Type II diabetic patients represent groups distinct in their aetiology of disease, then it may be that in patients with a normal body composition, aberrations in fat metabolism are not a primary factor in the development of insulin resistance. Thus, plasma FFA uptake and oxidation during exercise would be comparable with non-diabetics of similar body composition. In contrast, in obese populations prone to develop diabetes, obesity is most likely to be a major factor in the development of the disease, and is probably associated with the impairment in plasma FFA uptake found in skeletal muscle of these patients [2,3]. This is the first study to report a difference in whole-body fuel selection between obese and non-obese Type II diabetic patients. Unfortunately, to our knowledge, substrate utilization studies at skeletal muscle level discerning between obese and non-obese Type II diabetic patients are lacking.

The present study supports the notion that in Type II diabetes, plasma glucose uptake is elevated relative to splanchnic glucose production during exercise, causing plasma glucose levels to decline. In obese Type II diabetic patients, Minuk et al. [7] have attributed this decline in plasma glucose to impaired glucose production with glucose-uptake rates similar to control subjects. This conclusion opposes that of others [6,8] who studied non-obese patients and suggested that during exercise peripheral glucose utilization is enhanced in Type II diabetes, with normal splanchnic glucose output. The present study shows that both these mechanisms are present in non-obese Type II diabetic patients, their combined effect causing the decline seen in plasma glucose levels. Additionally, our present study shows that during moderate intensity exercise, muscle glycogen utilization is decreased in these patients, and that the contribution of plasma glucose oxidation to energy expenditure is increased. This finding illustrates that, although insulin-stimulated muscle glucose uptake is diminished, there is no impairment in contraction-stimulated plasma glucose uptake in (non-obese) Type II diabetes.

In the present study, all subjects were sedentary, and the Type II diabetes and control group had a similar lean body mass, but $\dot{V}O_{2\max}$ was significantly higher in control. This is in agreement with previously reported

reduced rates of maximal $\dot{V}O_2$ in Type II diabetic subjects [21]. Nonetheless, the difference in $\dot{V}O_{2\max}$ resulted in a somewhat higher absolute workload during exercise in control, despite the closely matched relative exercise intensity between groups. It should be noted that had the Type II diabetes group exercised at the same absolute intensity as the control group, then the contribution of CHO (both blood glucose and muscle glycogen) to energy expenditure would probably have increased at the expense of fat. This is because CHO utilization, both relatively and absolutely, increases with exercise intensity [22].

In conclusion, total CHO and fat oxidation at rest and during moderate intensity exercise are unaltered in non-obese Type II diabetic patients. During exercise, muscle glycogen oxidation is decreased in these patients, and the contribution of plasma glucose oxidation to energy expenditure is increased. No significant aberrations are present in plasma FFA uptake and oxidation rates. We have shown that the impairment in skeletal muscle fatty acid utilization recently reported [3] in obese Type II diabetes is not present in non-obese patients.

ACKNOWLEDGMENTS

This study was financially supported by the Diabetes Fund of The Netherlands (DFN).

REFERENCES

- Blaak, E. E., Wagenmakers, A. J., Glatz, J. F., Wolffenbuttel, B. H., Kemerink, G. J., Langenberg, C. J., Heidendal, G. A. and Saris, W. H. (2000) Plasma FFA utilization and fatty acid-binding protein content are diminished in type 2 diabetic muscle. *Am. J. Physiol. Endocrinol. Metab.* **279**, E146–E154
- Kelley, D. E. and Simoneau, J. A. (1994) Impaired free fatty acid utilization by skeletal muscle in non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* **94**, 2349–2356
- Blaak, E. E., van Aggel-Leijssen, D. P., Wagenmakers, A. J., Saris, W. H. and van Baak, M. A. (2000) Impaired oxidation of plasma-derived fatty acids in type 2 diabetic subjects during moderate-intensity exercise. *Diabetes* **49**, 2102–2107
- Porte, D. and Sherwin, R. S. (1998) *Ellenberg and Rifkin's Diabetes Mellitus: Theory and Practice*, McGraw-Hill, Seattle
- Schneider, S. H., Khachadurian, A. K., Amorosa, L. F., Gavras, H., Fineberg, S. E. and Ruderman, N. B. (1987) Abnormal glucoregulation during exercise in type II (non-insulin-dependent) diabetes. *Metabolism* **36**, 1161–1166
- Martin, I. K., Katz, A. and Wahren, J. (1995) Splanchnic and muscle metabolism during exercise in NIDDM patients. *Am. J. Physiol.* **269**, E583–E590
- Minuk, H. L., Vranic, M., Marliss, E. B., Hanna, A. K., Albisser, A. M. and Zinman, B. (1981) Glucoregulatory and metabolic response to exercise in obese non-insulin-dependent diabetes. *Am. J. Physiol.* **240**, E458–E464
- Colberg, S. R., Hagberg, J. M., McCole, S. D., Zmuda, J. M., Thompson, P. D. and Kelley, D. E. (1996) Utilization of glycogen but not plasma glucose is reduced in individuals with NIDDM during mild-intensity exercise. *J. Appl. Physiol.* **81**, 2027–2033
- Seidell, J. C., Verschuren, W. M. and Kromhout, D. (1995) Prevalence and trends of obesity in The Netherlands 1987–1991. *Int. J. Obes. Relat. Metab. Disord.* **19**, 924–927
- Durnin, J. V. and Womersley, J. (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br. J. Nutr.* **32**, 77–97
- Sidossis, L. S., Coggan, A. R., Gastaldelli, A. and Wolfe, R. R. (1995) A new correction factor for use in tracer estimations of plasma fatty acid oxidation. *Am. J. Physiol.* **269**, E649–E656
- Sidossis, L. S., Coggan, A. R., Gastaldelli, A. and Wolfe, R. R. (1995) Pathway of free fatty acid oxidation in human subjects. Implications for tracer studies. *J. Clin. Invest.* **95**, 278–284
- Schrauwen, P., van Aggel-Leijssen, D. P., van Marken Lichtenbelt, W. D., van Baak, M. A., Gijzen, A. P. and Wagenmakers, A. J. (1998) Validation of the [1,2- ^{13}C]acetate recovery factor for correction of [U- ^{13}C]palmitate oxidation rates in humans. *J. Physiol. (London)* **513**, 215–223
- Gutmann, I. and Wahlefeld, A. W. (1974) L-(+)-Lactate, determination with lactate dehydrogenase and NAD. In *Methods in Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 1464–1468, Academic Press, New York
- Andersen, L., Dinesen, B., Jorgensen, P. N., Poulsen, F. and Roder, M. E. (1993) Enzyme immunoassay for intact human insulin in serum or plasma. *Clin. Chem.* **39**, 578–582
- Peronnet, F. and Massicotte, D. (1991) Table of nonprotein respiratory quotient: an update. *Can. J. Sport. Sci.* **16**, 23–29
- Van der Vusse, G. J. and Reneman, R. S. (1996) Lipid metabolism in muscle. In *Exercise: Regulation and Integration of Multiple Systems*, vol. 12, (Rowell, L. B. and Shepherd, J. T., eds), pp. 953–994, Oxford University Press, Oxford
- Steele, R. (1959) Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann. NY. Acad. Sci.* **82**, 420–430
- Wolfe, R. R. (1992) Radioactive and stable isotope tracers. In *Biomedicine: Principles and Practice of Kinetic Analysis*, Wiley-Liss, New York
- Jeukendrup, A. E., Raben, A., Gijzen, A., Stegen, J. H., Brouns, F., Saris, W. H. and Wagenmakers, A. J. (1999) Glucose kinetics during prolonged exercise in highly trained human subjects: effect of glucose ingestion. *J. Physiol. (London)* **515**, 579–589
- Regensteiner, J. G., Sippel, J., McFarling, E. T., Wolfel, E. E. and Hiatt, W. R. (1995) Effects of non-insulin-dependent diabetes on oxygen consumption during treadmill exercise. *Med. Sci. Sports Exerc.* **27**, 875–881
- Romijn, J. A., Coyle, E. F., Sidossis, L. S., Gastaldelli, A., Horowitz, J. F., Endert, E. and Wolfe, R. R. (1993) Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. J. Physiol.* **265**, E380–E391

Received 18 June 2002; accepted 4 September 2002