

Substrate source utilisation in long-term diagnosed type 2 diabetes patients at rest, and during exercise and subsequent recovery

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Abstract

Aims/hypothesis Disturbances in substrate source metabolism and, more particularly, in fatty acid metabolism, play an important role in the aetiology and progression of type 2 diabetes. However, data on substrate source utilisation in type 2 diabetes are inconclusive.

Methods [$U\text{-}^{13}\text{C}$]palmitate and [$6,6\text{-}^2\text{H}_2$]glucose tracers were used to assess plasma NEFA and glucose oxidation rates and to estimate the use of muscle- and/or lipoprotein-derived triacylglycerol and muscle glycogen. Subjects were ten male patients who had a long-term (7 ± 1 years) diagnosis of type 2 diabetes and were overweight, and ten matched healthy, male control subjects. Muscle biopsy samples were collected before and after exercise to assess muscle fibre type-specific intramyocellular lipid and glycogen content.

Results At rest and during exercise, the diabetes patients had greater values than the controls for palmitate rate of appearance (Ra) (rest, 2.46 ± 0.18 and 1.85 ± 0.20 respec-

tively; exercise, 3.71 ± 0.36 and 2.84 ± 0.20 $\mu\text{mol kg}^{-1} \text{min}^{-1}$) and rate of disappearance (Rd) (rest, 2.45 ± 0.18 and 1.83 ± 0.20 ; exercise, 3.64 ± 0.35 and 2.80 ± 0.20 $\mu\text{mol kg}^{-1} \text{min}^{-1}$ respectively). This was accompanied by significantly higher fat oxidation rates at rest and during recovery in the diabetes patients (rest, 0.11 ± 0.01 in diabetes patients and 0.09 ± 0.01 in controls; recovery, 0.13 ± 0.01 and 0.11 ± 0.01 g/min respectively), despite significantly greater plasma glucose Ra, Rd and circulating plasma glucose concentrations. Furthermore, exercise significantly lowered plasma glucose concentrations in the diabetes patients, as a result of increased blood glucose disposal.

Conclusion This study demonstrates that substrate source utilisation in long-term-diagnosed type 2 diabetes patients, in whom compensatory hyperinsulinaemia is no longer present, shifts towards an increase in whole-body fat oxidation rate and is accompanied by disturbances in fat and carbohydrate handling.

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Abbreviations

AU	arbitrary unit
IMTG	intramyocellular triacylglycerol
$\text{Pr}^{13}\text{CO}_2$	production of $^{13}\text{CO}_2$
Ra	rate of appearance
Rd	rate of disappearance
Rox	rate of oxidation
TTR	plasma enrichment (tracer/tracee ratio)
$\dot{V}\text{O}_2$	oxygen uptake
$\dot{V}\text{CO}_2$	CO_2 production
$\dot{V}\text{O}_{2\text{max}}$	maximal oxygen uptake capacity
W_{max}	maximal workload capacity

Introduction

Disturbances in fatty acid metabolism, as found in sedentary, obese and/or type 2 diabetes patients, are an important factor in the development of skeletal muscle insulin resistance [1–3]. Elevated fatty acid delivery and/or impaired fatty acid oxidation result in net intramyocellular accumulation of triacylglycerol and fatty acid metabolites (such as fatty acyl-CoA, ceramides and diacylglycerol). The latter are likely to induce defects in the insulin signalling cascade, thus causing insulin resistance [4–10] in skeletal muscle.

On a whole-body level, conflicting data exist on the proposed disturbances in substrate metabolism in type 2 diabetes patients. In these patients, basal oxidation rates of whole-body total fat have been reported to be both increased [11–13] and similar [14–19] compared with lean [11–13] and overweight/obese [14–19] controls respectively. Whole-body plasma NEFA uptake and/or oxidation rates at rest have been reported to be either similar [17, 18] or decreased [16] in the type 2 diabetes patient. However, data on arteriovenous differences in plasma NEFA concentrations over the leg or arm have more consistently displayed reduced fasting plasma NEFA uptake and/or oxidation in type 2 diabetes patients compared with normoglycaemic controls [11, 14, 20], despite elevated systemic plasma NEFA and glucose levels [14, 20]. During exercise, in both the prediabetes and/or type 2 diabetes state, the uptake and oxidation of plasma NEFA and other lipid sources have been shown to be similar [17, 18] or decreased [16, 21]. A cross-sectional review of the literature on the use of lipoprotein and/or muscle-derived triacylglycerol suggests that type 2 diabetes patients have a reduced capacity to mobilise and/or oxidise the intramyocellular lipid stores [22]. However, a direct comparison of intramyocellular triacylglycerol (IMTG) use between diabetes patients and matched controls has not yet been made.

Exercise can lower plasma glucose concentrations in type 2 diabetes patients. This can be attributed to a blunted increase in hepatic glucose output [23] and/or an increase in whole-body glucose uptake rate [15, 17, 18, 24]. The latter has been shown to result in greater carbohydrate oxidation rates during exercise in type 2 diabetes patients in some [24] but not all studies [15–18]. Data on differences in substrate source utilisation during post-exercise recovery between diabetes patients and healthy, normoglycaemic controls are entirely lacking in the literature.

The apparently inconsistent findings on aberrations in whole-body substrate utilisation in the type 2 diabetes state can be explained by differences in the methods used and in the selected subpopulations of type 2 diabetes patients and controls. These equivocal data restrict conclusive insight into the exact nature and extent of the metabolic disturbances

that play a key role in the aetiology and progression of type 2 diabetes. In the present study, we investigated the disturbances in whole-body substrate source utilisation in long-standing type 2 diabetes at rest, during exercise and subsequent recovery by using contemporary stable isotope methods combined with skeletal muscle biopsy sampling. Patients with long-standing type 2 diabetes were selected to assess the metabolic disturbances when compensatory hyperinsulinaemia is no longer present. Careful matching of the type 2 diabetes patients with normoglycaemic controls was performed to be able to determine the effect of type 2 diabetes on substrate use independently of age, body composition and aerobic capacity. The present study is the first to provide a complete overview of substrate source utilisation rates at rest, during exercise and post-exercise recovery in overweight patients with long-standing type 2 diabetes, in whom compensatory hyperinsulinaemia is no longer present.

Subjects and methods

Subjects Ten male, sedentary, overweight type 2 diabetes patients and ten male, sedentary, weight-matched healthy controls (Table 1) participated in this study. All patients were using metformin with ($n=7$) or without ($n=3$) sulphonylurea derivatives (gliclazide, glibenclamide or tolbutamide). Exclusion criteria were impaired liver function, renal failure and/or a history of severe cardiovascular problems. Diabetic status was verified with an OGTT according to WHO

Table 1 Subjects' characteristics

	Control group ($n=10$)	Diabetes group ($n=10$)
Age (years)	60±2	60±2
Height (m)	1.76±0.01	1.79±0.02
Body mass (kg)	87±2	91±4
BMI (kg/m ²)	28±0	28±1
Body fat (%)	29±1	29±2
Fat-free mass (kg)	61±1	64±2
Basal plasma glucose (mmol/l)	5.56±0.14	9.44±0.59 ^a
Plasma glucose _{120min} (mmol/l) ^b	5.55±0.5	16.7±1.2 ^a
Basal plasma insulin (pmol/l)	47.16±9.48	52.20±6.06
Plasma insulin _{120min} (pmol/l) ^b	290.40±48.24	270.06±46.50
HbA _{1c} (%)	5.83±0.2	7.30±0.3 ^a
VO _{2max} (l/min)	3.2±0.2	2.9±0.2
W _{max} (W)	203±16	200±15
Maximal heart rate (beats/min)	164±7	161±4
Diagnosed with diabetes (years)	–	7±1 ^a

Data are mean±SEM

VO_{2max}, maximal oxygen uptake capacity; W_{max}, maximal workload capacity

^aSignificantly different from control group ($p<0.05$)

^bConcentrations at $t=120$ min during OGTT

criteria [25]. Medication was withheld for 24 h prior to the trials. Subjects were informed about the nature and risks of the experimental procedures before their written informed consent was obtained. The study was performed according to the principles of the Declaration of Helsinki and was approved by the local medical ethical committee.

Pretesting Maximal workload capacity (W_{\max}) and maximal oxygen uptake capacity ($\dot{V}O_{2\max}$) were determined with an electronically braked cycle ergometer (Excalibur, Lode, Groningen, the Netherlands) during an incremental exhaustive exercise test 2 weeks prior to the first trial [17]. Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured with an Oxycon β (Mijnhart, Breda, the Netherlands). Body composition was assessed using the hydrostatic weighing method. Body fat percentage was calculated using Siri's equation [26].

Diet and physical activity prior to testing All subjects were instructed to refrain from strenuous physical activity for 2 days prior to each trial. In addition, they recorded dietary intake during 2 days prior to the first trial and repeated this diet prior to the second trial. The evening before each trial, all subjects received the same standardised meal (42 kJ [10 kcal]/kg; consisting of 61% of energy as carbohydrate, 24% as fat and 15% as protein). There were no differences between groups in daily energy intake and macronutrient composition of the diet.

Experimental trials Each subject performed one main trial and an additional test to determine the acetate recovery factor. Both tests were separated by at least 1 week. Each trial consisted of 120 min of resting measurements followed by 60 min of cycling exercise at an exercise intensity set at 50% W_{\max} , and a subsequent 120 min recovery period. In the main trial, a [U- ^{13}C]palmitate and [6,6- 2H_2]glucose tracer was infused and breath, blood and muscle samples were collected at regular intervals. The acetate test was identical with the exception of the infusion of [1,2- ^{13}C]acetate and the collection of breath samples only [27].

Protocol After an overnight fast, subjects arrived at the laboratory at 08.00 hours by car or public transport. After 30 min of supine rest, a percutaneous muscle biopsy [28] was taken from the vastus lateralis muscle. A Teflon catheter (Baxter, Utrecht, the Netherlands) was inserted into an antecubital vein of one arm for blood sampling and another catheter was inserted in the contralateral arm for isotope infusion. Subsequently, subjects were administered a single intravenous dose of $NaH^{13}CO_3$ (0.06375 mg/kg), followed by a [6,6- 2H_2]glucose prime (13.5 $\mu\text{mol/kg}$). Thereafter, a continuous infusion of [6,6- 2H_2]glucose (0.3 $\mu\text{mol kg}^{-1} \text{min}^{-1}$) and [U- ^{13}C]palmitate (0.01 μmol

$\text{kg}^{-1} \text{min}^{-1}$) (or [1,2- ^{13}C]acetate) was started ($t=0$ min) via a calibrated IVAC pump (IVAC 560; Ivac, San Diego, CA, USA). At $t=120$ min subjects started to exercise on a cycle ergometer at 50% W_{\max} for a 60 min period. Whilst the subject was at rest, $\dot{V}O_2$ and $\dot{V}CO_2$ were measured from $t=60$ to 120 min (Oxycon- β ; Mijnhart); during exercise $\dot{V}O_2$ and $\dot{V}CO_2$ were measured for 5 min every 15 min prior to sampling of blood and expired breath. Immediately after cessation of exercise, a second muscle biopsy was taken, after which the subject rested supine for 2 h. $\dot{V}O_2$ and $\dot{V}CO_2$ were measured during recovery from $t=210$ to 270 min.

Blood and breath samples Blood samples (7 ml) were collected in EDTA-containing tubes and centrifuged at 1,000 g for 10 min at 4°C. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at -80°C. Plasma concentrations of glucose (Roche, Basel, Switzerland), lactate, NEFA (Wako Chemicals, Neuss, Germany), glycerol (Roche Diagnostics, Indianapolis, IN, USA) and triacylglycerol (Sigma Diagnostics, St Louis, MO, USA) were analysed with a COBAS semi-automatic analyser (Roche). Plasma insulin was measured by radioimmunoassay (Linco, St Charles, MO, USA). Blood HbA_{1c} content was analysed by high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany). Expired breath samples were analysed for $^{13}C/^{12}C$ ratio using a gas chromatograph–isotope ratio mass spectrometer system (GC-IRMS; Finnigan MAT 252, Thermo Electron Corp., Bremen, Germany). For determination of plasma palmitate and NEFA kinetics, NEFA were extracted, isolated by thin-layer chromatography and derivatised to their methyl esters. Palmitate concentration was determined on an analytical GC with flame ionisation detection using heptadecanoic acid as internal standard, and constituted 21.3±0.7% of total NEFA. The isotope tracer/tracee ratio (TTR) of [U- ^{13}C]palmitate was determined using GC combustion isotope ratio MS (Finnigan MAT 252). Following derivatisation, plasma [6,6- 2H_2]glucose enrichment was determined by electron ionisation GC-MS (Finnigan). Palmitate, glucose and acetate tracer concentrations in the infusates averaged 1.05±0.01, 34.4±0.9 and 4.92±0.03 mmol/l respectively. Therefore, the exact tracer infusion rates averaged 9.1±0.1, 277±2 and 75±1 nmol $\text{kg}^{-1} \text{min}^{-1}$ respectively.

Calculations From respiratory measurements, total fat and carbohydrate oxidation rates were calculated using the non-protein respiratory quotient [29]:

$$\text{fat oxidation rate} = 1.695 \dot{V}O_2 - 1.701 \dot{V}CO_2 \quad (1)$$

$$\begin{aligned} \text{carbohydrate oxidation rate} \\ = 4.585 \dot{V}CO_2 - 3.226 \dot{V}O_2 \quad (2) \end{aligned}$$

where $\dot{V}O_2$ and $\dot{V}CO_2$ are in l/min and oxidation rates in g/min. Rate of appearance (Ra) and rate of disappearance (Rd) of palmitate and glucose were calculated using the single-pool non-steady state Steele equations adapted for stable isotope methodology [30]:

$$Ra = \frac{F - V[(C_2 + C_1)/2][(E_2 - E_1)/(t_2 - t_1)]}{(E_2 + E_1)/2} \quad (3)$$

$$Rd = Ra - V \cdot \left(\frac{C_2 - C_1}{t_2 - t_1} \right) \quad (4)$$

where F is the infusion rate ($\mu\text{mol kg}^{-1} \text{min}^{-1}$), V is the distribution volume for palmitate or glucose (40 and 160 ml/kg, respectively), C_1 and C_2 are palmitate or glucose concentrations (mmol/l) at times 1 (t_1) and 2 (t_2) respectively, and E_2 and E_1 are the plasma palmitate or glucose enrichments (TTR) at times 1 and 2, respectively. Production of $^{13}\text{CO}_2$ ($\text{Pr}^{13}\text{CO}_2$; mol/min) from the infused palmitate tracer was calculated as:

$$\text{Pr}^{13}\text{CO}_2 = (\text{TTRCO}_2 \times \text{VCO}_2)/(k \times \text{Ar}) \quad (5)$$

where TTRCO_2 is the breath $^{13}\text{C}/^{12}\text{C}$ ratio at a given time point, VCO_2 is carbon dioxide production (l/min), k is the volume of 1 mole of CO_2 (22.4 l/mol), and Ar is the fractional ^{13}C label recovery in breath CO_2 observed after the infusion of labelled acetate [27, 31, 32], calculated as:

$$\text{Ar} = ([\text{TTRCO}_2 \times \text{VCO}_2]/[k \times 2F]) \quad (6)$$

where F is the infusion rate of [1,2- ^{13}C] acetate (mol/min). Plasma palmitate oxidation (Rox) (mol/min) can subsequently be calculated as:

$$\text{Rox palmitate} = \text{Rd palmitate} \times (\text{Pr}^{13}\text{CO}_2/F \times 16) \quad (7)$$

where Rd palmitate is the rate of disappearance of palmitate (mol/min), F is the palmitate infusion rate (mol/min) and 16 is the number of labelled carbon atoms in palmitate. Total plasma NEFA oxidation was calculated by dividing palmitate oxidation rates by the fractional contribution of plasma palmitate to total plasma NEFA concentration. The contribution of fat sources other than plasma NEFA was calculated by subtracting plasma NEFA oxidation from total fat oxidation.

In a previous study it has been shown that during exercise (50% W_{max}) plasma glucose Rd equals its Rox (96–100%) [33]. Therefore, plasma glucose oxidation rate during exercise was estimated as:

$$\text{Rox plasma glucose} = \text{Rd plasma glucose} \quad (8)$$

Whole-body muscle glycogen use was calculated by subtracting plasma glucose oxidation from total carbohydrate oxidation. As plasma glucose Rd does not match Rox

during resting conditions [34], plasma glucose oxidation rates cannot be calculated accurately at rest when using a [6,6- $^2\text{H}_2$]glucose tracer.

Muscle sample analysis Muscle samples were dissected, freed from any visible non-muscle material, frozen in nitrogen-cooled isopentane and embedded in Tissue-Tek (Sakura, Zoeterwoude, the Netherlands). Multiple serial sections (5 μm) were thaw-mounted together on uncoated, precleaned glass slides for each subject. To determine muscle fibre type-specific IMTG content, cross-sections were stained with Oil red O together with immunolabelled cellular constituents using a protocol described before [2]. For each muscle biopsy a total of 58 ± 7 and 49 ± 4 muscle fibres were analysed for lipid content for diabetes patients and control subjects respectively. To permit quantification of intramyocellular glycogen we used the modified PAS stain [35]. For each muscle biopsy, 148 ± 15 and 157 ± 13 muscle fibres were analysed for glycogen content in diabetes and control subjects respectively. Large microscopic overviews containing 167 ± 24 and 174 ± 20 fibres per subject (diabetes and control subjects respectively) were used to determine muscle fibre type composition.

Statistics All data are expressed as mean \pm SEM. To compare tracer kinetics, substrate utilisation rates, IMTG contents and/or plasma metabolite concentrations over time, repeated measures ANOVA was applied. A Scheffé post hoc test was applied in case of a significant F ratio, to locate specific differences. To determine differences between diabetes patients and control subjects, Student's t test for unpaired observations was used. Simple linear regression was used to investigate specific correlations. Significance was set at the 0.05 level of confidence.

Results

Tracer kinetics Plasma palmitate and glucose Ra, Rd and Rox are shown in Fig. 1 and Table 2. As plasma palmitate and glucose concentrations were subject to changes throughout rest, exercise and/or recovery (Fig. 2), non-steady-state Steele equations were applied. Table 3 shows breath $^{13}\text{CO}_2$ enrichment and plasma palmitate and glucose enrichment throughout the test. At rest, plasma palmitate Ra, Rd and Rox were stable, and significantly higher in the diabetes patients compared with the controls (Ra and Rd, $p < 0.05$; Rox, $p = 0.07$). During exercise, plasma palmitate Ra, Rd and Rox increased over time in both groups ($p < 0.01$) and were higher in the diabetes group than the control group (Ra and Rd, $p < 0.05$; Rox, $p = 0.08$). During post-exercise recovery, plasma palmitate Ra, Rd and Rox were similar between groups. Acetate recovery factors in

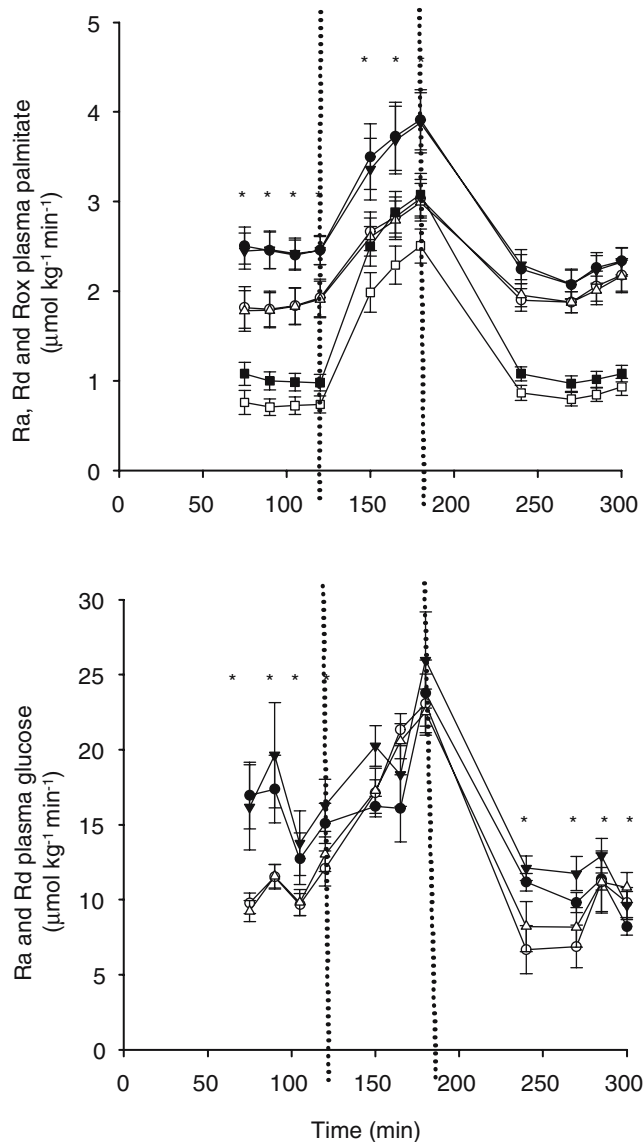


Fig. 1 **a** Plasma rates of appearance (Ra), disappearance (Rd) and oxidation (Rox) of palmitate at rest and during exercise and post-exercise recovery ($\mu\text{mol kg}^{-1} \text{min}^{-1}$) in the diabetes group (closed symbols) and the control group (open symbols). Circles, Ra; triangles, Rd; squares, Rox. **b** Plasma glucose rates of appearance (Ra), disappearance (Rd) and oxidation (Rox) at rest and during exercise and post-exercise recovery ($\mu\text{mol kg}^{-1} \text{min}^{-1}$). Data are mean \pm SEM ($n=20$). *Ra and Rd significantly different from control group ($p<0.05$). Vertical dotted lines mark the beginning and end of exercise

the diabetes and control groups respectively averaged 0.11 ± 0.00 and 0.13 ± 0.00 at rest, 0.87 ± 0.00 and 0.91 ± 0.00 during exercise, and 0.24 ± 0.00 and 0.26 ± 0.00 during subsequent recovery (no significant difference between groups).

At rest, plasma glucose Ra and Rd were stable, and significantly higher in the diabetes patients ($p<0.05$). During exercise, plasma glucose Ra and Rd increased over time in both groups ($p<0.01$) and were similar between groups. During post-exercise recovery, plasma glucose Ra

Table 2 Tracer kinetics

	Control group ($n=10$)	Diabetes group ($n=10$)
Rest		
Ra palmitate	1.85 ± 0.20	2.46 ± 0.18^a
Rd palmitate	1.83 ± 0.20	2.45 ± 0.18^a
Rox palmitate	0.73 ± 0.10	1.01 ± 0.10
%Rd ox palmitate	39.9 ± 2.0	40.9 ± 1.9
Ra glucose	10.77 ± 0.42	15.53 ± 0.84^a
Rd glucose	10.91 ± 0.33	16.46 ± 0.76^a
%Ra Rd glucose	101 ± 2	105 ± 3
Exercise		
Ra palmitate	2.84 ± 0.20^b	$3.71\pm 0.36^{a,b}$
Rd palmitate	2.80 ± 0.20^b	$3.64\pm 0.35^{a,b}$
Rox palmitate	2.26 ± 0.20^b	2.82 ± 0.22^b
%Rd ox palmitate	80.8 ± 3.70^b	79.2 ± 3.9^b
Ra glucose	20.51 ± 0.91^b	18.69 ± 0.98^b
Rd glucose	20.11 ± 1.06^b	21.52 ± 1.21^b
%Ra Rd glucose	99 ± 4	$118\pm 6^{a,b}$
Recovery		
Ra palmitate	2.01 ± 0.14^c	2.23 ± 0.15^c
Rd palmitate	2.00 ± 0.14^c	2.24 ± 0.15^c
Rox palmitate	0.86 ± 0.08^c	1.04 ± 0.08^c
%Rd ox palmitate	43.2 ± 2.6^c	$46.5\pm 1.6^{b,c}$
Ra glucose	$8.63\pm 0.48^{b,c}$	$10.14\pm 0.47^{a,c}$
Rd glucose	$9.57\pm 0.45^{b,c}$	$11.59\pm 0.76^{a,c}$
%Ra Rd glucose	106 ± 6	114 ± 4

Tracer kinetics ($\mu\text{mol kg}^{-1} \text{min}^{-1}$) were determined at rest and during exercise ($50\% W_{\text{max}}$) and subsequent recovery
%Rd ox percentage of Rd oxidised (%); %Ra Rd percentage of Ra that disappeared

Values are mean \pm SEM ($2n=20$)

^a Significantly different from control group ($p<0.05$)

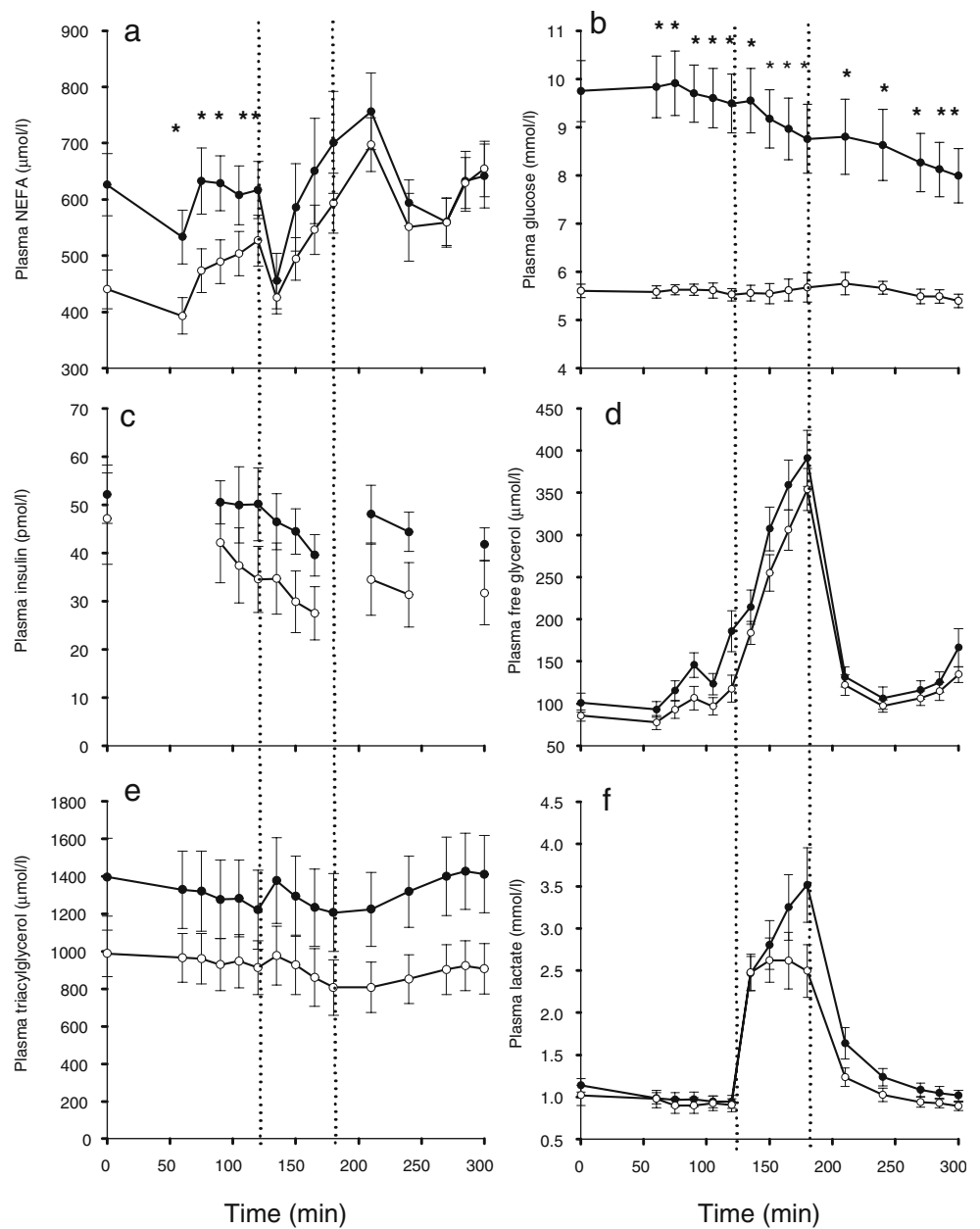
^b Significantly different from resting values ($p<0.01$)

^c Significantly different from exercise values ($p<0.01$)

and Rd were higher in the diabetes patients ($p<0.05$). No changes over time in plasma glucose Ra or Rd were observed. In the diabetes patients, the percentage of plasma glucose Ra that disappeared (%Ra/Rd) was significantly greater during exercise than with resting conditions. Furthermore, during exercise, %Ra/Rd was significantly greater in the diabetes group than in the control group.

Substrate utilisation Total energy expenditure and substrate source utilisation rates are illustrated in Fig. 3. Energy expenditure averaged 5.75 ± 0.22 and 5.28 ± 0.16 kJ/min in the diabetes and control groups respectively. Total fat oxidation rates (g/min) at rest were significantly higher in the diabetes group than in the control group (0.11 ± 0.01 and 0.09 ± 0.01 g/min respectively), contributing 77 ± 2 and $67\pm 3\%$ to total energy expenditure ($p<0.01$). The difference in fat oxidation rate was attributed to a significantly higher NEFA oxidation rate in the diabetes group ($p<0.05$), with no differences in muscle- and/or lipoprotein-derived triacylglycerol utilisation. The latter contributed only -2 ± 7

Fig. 2 Plasma concentrations of triacylglycerol (**a**), glucose (**b**), NEFA (**c**), non-esterified glycerol (**d**), lactate (**e**) and insulin (**f**) in the diabetes (closed circles) and control groups (open circles). Values are mean \pm SEM. *Significantly different from control group ($p < 0.05$). Vertical dotted lines mark the beginning and end of exercise



and $6 \pm 7\%$ to total energy expenditure in the diabetes and control groups respectively.

Exercise was performed at a 50% W_{\max} workload, which resulted in an absolute workload set at 100 ± 7 and 101 ± 8 W

(NS) or a relative workload of $63 \pm 2\%$ and $54 \pm 2\%$ of $VO_{2\max}$ ($p < 0.05$) in the diabetes and control groups, respectively.

Energy expenditure during exercise averaged 39 ± 2 and 36 ± 2 kJ/min, respectively. Fat oxidation contributed 42 ± 3

Table 3 ^{13}C -breath, ^{13}C -palmitate and $[6,6\text{-}^2\text{H}_2]$ glucose enrichments during rest, exercise and recovery conditions

	^{13}C -breath enrichment (TTR 10^{-5})		^{13}C -palmitate enrichment (TTR 10^{-3})		^2H -glucose enrichment (TTR 10^{-3})	
	Diabetes group	Control group	Diabetes group	Control group	Diabetes group	Control group
Rest	6.39 ± 0.74	6.88 ± 0.87	3.92 ± 0.0^a	5.59 ± 0.10	11.13 ± 0.76^a	18.44 ± 0.92
Exercise	12.07 ± 0.18	12.84 ± 0.38	2.78 ± 0.12^a	3.43 ± 0.16	14.87 ± 0.17^a	18.94 ± 1.18
Recovery	15.42 ± 1.03	15.68 ± 0.98	4.24 ± 0.18	4.69 ± 0.16	19.49 ± 0.10	22.41 ± 0.10

^a Significantly different from control group ($p < 0.05$)

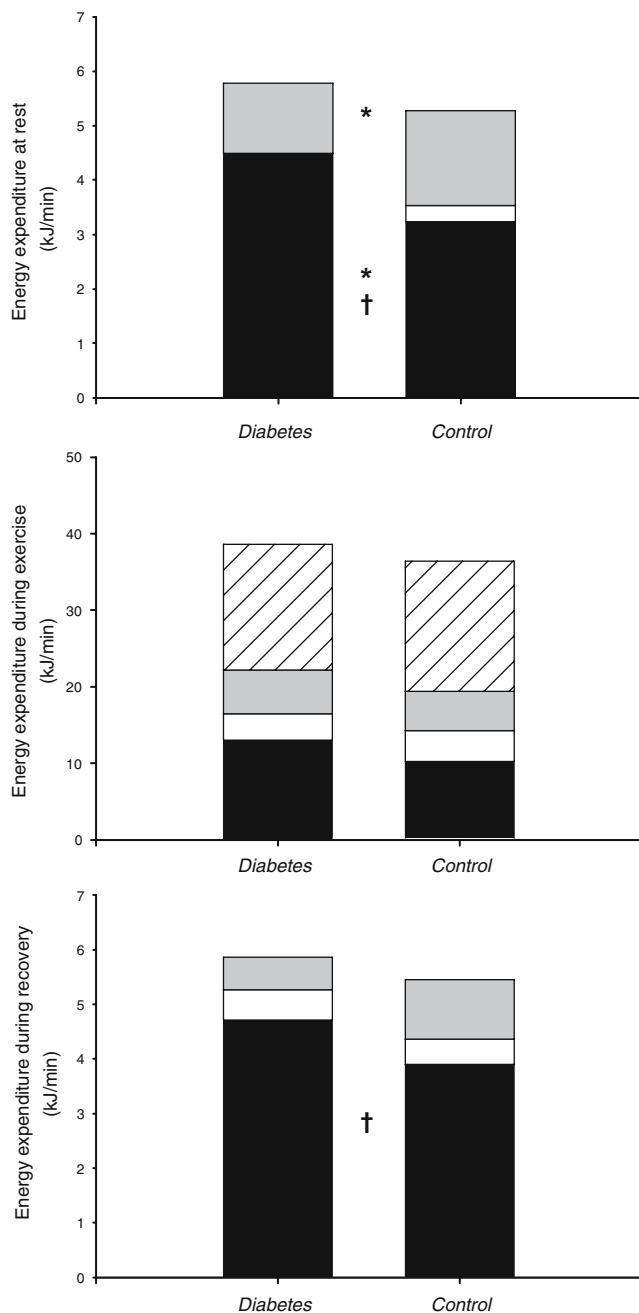


Fig. 3 Whole-body substrate source utilisation at rest (a) and during exercise (b) and post-exercise recovery (c). Black (a–c), plasma NEFA; white (a–c), muscle and lipoprotein-derived triacylglycerol; grey, carbohydrate in a and c, plasma glucose in b; hatching (b), muscle glycogen. *Substrate source oxidation significantly different from control group ($p<0.05$); †total fat oxidation significantly different from control group ($p<0.05$)

(0.40 ± 0.04 g/min) and $39\pm 3\%$ (0.35 ± 0.03 g/min) to total energy expenditure in the diabetes and control groups, respectively. Muscle- and/or lipoprotein-derived triacylglycerol use increased significantly during exercise conditions to 0.09 ± 0.03 and 0.10 ± 0.02 g/min in the diabetes and

control groups respectively, contributing 8 ± 3 and $11\pm 3\%$, respectively.

Total carbohydrate oxidation rates during exercise averaged 1.37 ± 0.09 and 1.37 ± 0.08 g/min in the diabetes and control groups respectively, contributing 58 ± 3 and $61\pm 3\%$ to total energy expenditure. In the diabetes and control groups, plasma glucose oxidation averaged 0.35 ± 0.03 and 0.32 ± 0.02 g/min respectively, contributing 15 ± 1 and $14\pm 1\%$ to total energy expenditure, with muscle glycogen contributing 43 ± 3 and $46\pm 3\%$ to energy expenditure. Aside from a tendency to greater plasma NEFA oxidation rates in the diabetes group ($p=0.08$), no significant differences in substrate source utilisation rates were observed between groups.

During post-exercise recovery, energy expenditure averaged 5.9 ± 0.2 in the diabetes group and 5.5 ± 0.2 kJ/min in the control group, fat oxidation contributing $90\pm 2\%$ (0.13 ± 0.01 g/min) and $80\pm 3\%$ (0.11 ± 0.01 g/min; $p<0.05$) to total energy expenditure, respectively. The greater fat oxidation rate in the diabetes group was attributed mainly to a greater plasma NEFA oxidation rate in the diabetes group ($p=0.10$), whereas no differences were observed in muscle- and/or lipoprotein-derived triacylglycerol. The greater fat oxidation rates were matched by lower total carbohydrate oxidation rates.

Plasma metabolite concentrations

Plasma metabolite concentrations are shown in Fig. 2. Plasma glucose concentrations were significantly higher in the diabetes group throughout the experiment. During exercise, there was a significant decline in plasma glucose concentration in the diabetes group only. Plasma NEFA concentrations at rest were higher in the diabetes group than the control group. No differences in plasma NEFA between groups were observed during exercise and recovery conditions. Glycerol concentrations increased significantly during rest and exercise and decreased during recovery. Free glycerol concentration tended to be higher in the diabetes group ($p=0.06$). Plasma triacylglycerol concentrations declined significantly at rest and during exercise in the diabetes patients, but were stable in control subjects. Plasma insulin levels declined during exercise in both groups ($p<0.01$), resulting in significantly lower insulin concentrations during recovery compared with resting levels. No significant differences in plasma insulin concentrations were observed between groups.

Muscle tissue analysis

Muscle fibre type analysis showed $46\pm 3\%$ type I muscle fibres and $54\pm 3\%$ type II fibres in the diabetes group, and

49±4% type I muscle fibres and 51±4% type II fibres in the control group. In both groups, type I fibres contained two to three times more IMTG than type 2 fibres ($p<0.005$). Type I and II muscle fibre lipid content averaged 24±4 and 11±2 arbitrary units (AU) respectively in the diabetes group and 34±5 and 11±2 AU respectively in the control group. No significant differences in type I or II muscle fibre lipid content were observed between groups. No significant net changes in type I or type II intramyocellular lipid content were observed following exercise in the diabetes or control group. In the diabetes and control groups, net changes were 8±4 and -6±6 AU respectively for type I fibres and 4±3 and 3±4 for type II fibres (all $p>0.05$), respectively. Type I and II muscle fibre glycogen content averaged 36±6 and 32±4 AU respectively in the diabetes group and 36±4 and 46±6 AU in the control group. No significant differences in type I or II muscle fibre glycogen content were observed between groups. After exercise, type II fibres contained significantly more glycogen than type I fibre type in both groups. However, no significant net changes in type I and II muscle fibre glycogen content (type I fibres, -13±6 AU in diabetes group and -6±3 AU in control group; type II fibres, 9±6 AU in diabetes group and -6±5 AU in control group; all $p>0.05$) were observed following exercise in both groups.

Discussion

The present study investigated the nature and extent of disturbances in different components of whole-body carbohydrate and fat metabolism in long-term-diagnosed type 2 diabetes patients. Using contemporary stable isotope methods, we found that basal whole-body fat oxidation rates were significantly greater in long-standing type 2 diabetes patients when compared with normoglycaemic controls matched for age, body composition and whole-body oxidative capacity (Fig. 3). This is attributed to significantly higher plasma NEFA appearance rates, resulting in greater NEFA uptake and oxidation rates (Fig. 1). In contrast, resting total carbohydrate oxidation rates were reduced in type 2 diabetes patients, despite substantially elevated plasma glucose appearance rates and the prevalence of hyperglycaemia. Exercise significantly reduces plasma glucose concentrations in type 2 diabetes patients, as glucose disposal exceeds its rate of appearance during exercise conditions.

Fasting whole-body fat oxidation rates were greater in the diabetes patients. This seems to be in contrast to some [14–19], but not all [12, 13, 36], previous studies, which describe normal and/or decreased basal whole-body fatty acid oxidation rates in the type 2 diabetic state [14–19]. These apparently contradictory findings are explained by

the fact that long-term-diagnosed diabetes patients were selected to participate in the present study. These patients differ significantly in metabolic profile from their recently diagnosed counterparts in that they no longer show any compensatory hyperinsulinaemia (Fig. 2, Table 1). As a consequence, plasma insulin levels do not compensate for the adipose tissue insulin resistance present in type 2 diabetes [37–40] and fail to inhibit adipose tissue lipolysis. This explains the greater plasma NEFA appearance rates and elevated plasma NEFA availability observed in the diabetes patients (Figs. 1 and 2). The elevated plasma NEFA availability drives a greater NEFA uptake rate and subsequently augments plasma NEFA oxidation in the diabetes patients. The higher basal plasma NEFA turnover and fat oxidation in the diabetes patients were accompanied by a lower carbohydrate oxidation rate (Fig. 3), despite substantially greater hepatic glucose output (Ra glucose) and the presence of marked hyperglycaemia. This clearly demonstrates that carbohydrate metabolism is significantly impaired in the long-term-diagnosed type 2 diabetes patient, and suggests that this might (in part) be attributed to the increased fat oxidation. Interestingly, despite previous studies suggesting substantial differences in the use of plasma glucose, glycogen, NEFA and/or IMTG as a substrate source during exercise conditions in type 2 diabetes patients and normoglycaemic controls [16–18, 24], substrate source utilisation rates were remarkably similar between type 2 diabetes patients and normoglycaemic controls (Fig. 3). However, it should be noted that in the type 2 diabetic state, such apparently normal substrate utilisation rates are observed in the presence of a significantly greater NEFA turnover.

Exercise significantly lowered blood glucose concentrations in type 2 diabetes patients. Though this has been observed before [15, 17, 18, 23, 41], the present study shows that this can be attributed to an improved plasma glucose disposal rate, with plasma glucose Rd representing 118±6% of its Ra during exercise (Table 2). The exercise-induced decline in blood glucose concentrations continued during subsequent recovery, blood glucose Rd remaining well above its Ra (114±1%). These data extend the observations made in previous studies [17, 23, 24, 42] and demonstrate that exercise forms a potent strategy to acutely improve glycaemic control in type 2 diabetes.

Our data do not support the hypothesis that the capacity to mobilise and/or oxidise the IMTG pool is substantially impaired in type 2 diabetes patients when compared with normoglycaemic controls, as suggested previously [2, 43, 44]. Tracer kinetic data (Fig. 1, Table 2) as well as histochemical analysis indicate that muscle (and/or lipoprotein)-derived triacylglycerol play only a relatively minor (quantitative) role as a substrate source at rest and/or during

exercise conditions in both sedentary type 2 diabetes patients and healthy sedentary men.

During post-exercise recovery, total fat oxidation rates were elevated compared with pre-exercise resting values in both the type 2 diabetes patients and the normoglycaemic controls (Fig. 3). Furthermore, total fat oxidation rates were greater in the type 2 diabetes patients compared with controls. In contrast, carbohydrate oxidation rates were reduced, which was accompanied by a decreased plasma glucose Ra and Rd.

The present data are not necessarily in contrast to previous findings, which generally show reduced NEFA uptake and/or oxidation rates over the leg or arm in recently diagnosed type 2 diabetes patients [11, 14, 16, 20, 45]. The greater whole-body NEFA turnover rate in long-term-diagnosed type 2 diabetes patients observed in the present study is most likely attributable to the presence of adipose tissue insulin resistance and the absence of compensating hyperinsulinaemia in the selected patient group. Disturbances in whole-body NEFA metabolism remain evident, since the higher total fat oxidation rates occurred under conditions of disproportionately elevated plasma NEFA appearance rates and concomitantly elevated plasma NEFA concentrations. Disturbances in glucose handling in these long-standing diabetes patients are more clearly visible, as total glucose oxidation rates are reduced, despite major upregulation of plasma glucose Ra and Rd and the concomitant hyperglycaemic state. Gaining more insight into the differences in disturbances of substrate utilisation between subpopulations of type 2 diabetes patients (i.e. recently or long-term-diagnosed) will be of great importance to our understanding of the aetiology and progression of the disease.

In conclusion, fasting whole-body fat oxidation rates are elevated in long-term-diagnosed type 2 diabetes patients, which is attributed to greater plasma NEFA appearance rates, elevated plasma NEFA availability, and increased plasma NEFA disappearance and oxidation rates. The greater plasma NEFA appearance rate is most likely attributable to disinhibition of adipose tissue lipolysis, secondary to adipose tissue insulin resistance and the absence of compensatory hyperinsulinaemia. Furthermore, hyperglycaemia in long-standing type 2 diabetes patients is associated with elevated plasma glucose appearance and disappearance rates, but is not accompanied by elevated carbohydrate oxidation rates. Moderate-intensity exercise improves plasma glucose disposal in long-standing type 2 diabetes patients, thereby effectively reducing blood glucose concentrations.

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Duality of interest None of the authors has a duality of interest.

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