

Disassociation of muscle triglyceride content and insulin sensitivity after exercise training in patients with Type 2 diabetes

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Abstract

Aim/hypothesis. We determined the effect of exercise training on insulin sensitivity and muscle lipids (triglyceride [TG_m] and long-chain fatty acyl CoA [LCACoA] concentration) in patients with Type 2 diabetes.

Methods. Seven patients with Type 2 diabetes and six healthy control subjects who were matched for age, BMI, % body fat and VO_{2peak} participated in a 3 days per week training program for 8 weeks. Insulin sensitivity was determined pre- and post-training during a 120 min euglycaemic-hyperinsulinaemic clamp and muscle biopsies were obtained before and after each clamp. Oxidative enzyme activities [citrate synthase (CS), β -hydroxy-acyl-CoA (β -HAD)] and TG_m were determined from basal muscle samples pre- and post training, while total LCACoA content was measured in samples obtained before and after insulin-stimulation, pre- and post training.

Results. The training-induced increase in VO_{2peak} (~20%, $p<0.01$) was similar in both groups. Compared with control subjects, insulin sensitivity was lower in

the diabetic patients before and after training (~60%; $p<0.05$), but was increased to the same extent in both groups with training (~30%; $p<0.01$). TG_m was increased in patients with Type 2 diabetes (170%; $p<0.05$) before, but was normalized to levels observed in control subjects after training. Basal LCACoA content was similar between groups and was unaltered by training. Insulin-stimulation had no detectable effect on LCACoA content. CS and β -HAD activity were increased to the same extent in both groups in response to training ($p<0.001$).

Conclusion/interpretation. We conclude that the enhanced insulin sensitivity observed after short-term exercise training was associated with a marked decrease in TG_m content in patients with Type 2 diabetes. However, despite the normalization of TG_m to levels observed in healthy individuals, insulin resistance was not completely reversed in the diabetic patients. [Diabetologia (2004) 47:23–30]

Keywords Muscle triacylglycerol · long-chain fatty acyl CoA · insulin resistance · oxidative capacity

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Abbreviations: β -HAD, β -hydroxy-acyl-CoA; CS, citrate synthase; DAG, diacylglycerol; DEXA, dual energy X-ray absorptiometry; LCACoA, long-chain fatty acyl CoA; PI3-kinase, phosphatidylinositol 3-kinase; TG_m, muscle triacylglycerol.

Type 2 diabetes is characterized by skeletal muscle insulin resistance and impaired glucose metabolism. In addition to disturbances in glucose homeostasis, individuals with Type 2 diabetes have an impaired lipid metabolism, reflected by increased circulating free fatty acids [1], reduced rates of whole body fat oxidation [2] and excessive deposition of lipids in various tissues including skeletal muscle [3]. With regard to muscle lipid status, there is evidence in humans that increased trigacylglycerol (TG_m) concentration [3, 4, 5, 6, 7] and increased long-chain fatty acyl CoA (LCACoA) content are negatively associated with whole body insulin action [8]. Considering the rela-

tionship between skeletal muscle lipid accretion and insulin resistance, it is of particular interest to determine whether interventions such as exercise training can reduce muscle lipid content and improve whole body insulin action.

In this regard, several recent studies have reported that short-term (4–10 weeks) aerobic exercise training programs were effective in improving whole body aerobic capacity and insulin sensitivity in both young, healthy subjects [9] and overweight and obese men [10], independent of any changes in TG_m content. Indeed, exercise training has been shown to increase TG_m content in muscle from healthy individuals [11], suggesting that the presence of triacylglycerol in the myocyte is not always associated with insulin insensitivity. In addition, it has been reported that the capacity to oxidize TG_m and/or VLDL-derived fatty acids is increased both at rest and during exercise following a 3-month low intensity (40% of VO_{2peak}) training program, and that increased rates of TG_m oxidation could be beneficial in the prevention/treatment of Type 2 diabetes [12]. However, to date the effects of exercise training on insulin sensitivity and muscle lipid status in Type 2 diabetic patients has not been determined. Accordingly, the purpose of this study was to investigate the effect of a short-term (8-week) program of aerobic exercise training on TG_m and LCACoA concentration in Type 2 diabetic individuals. We hypothesized that exercise training would result in a reduction in muscle lipid stores and that such changes would parallel improvements in insulin sensitivity.

Subjects and methods

Subjects. We studied seven male patients with Type 2 diabetes with a mean time since diagnosis of disease of 2.9 ± 0.5 years (range 1 to 5 years) and a control group consisting of six healthy male subjects matched for age, BMI, % body fat and VO_{2peak} (Table 1). Normal glucose tolerance was confirmed in control subjects by an OGTT (75 g glucose). The patients were being treated with diet ($n=1$) or oral hypoglycaemic agents ($n=1$ sulphonylureas, $n=2$ metformin, $n=3$ combination of sulphonylureas and metformin). Other than diabetes, none of these subjects had any medical problems, all were non-smokers and apart from diabetic patients, none were taking medications known to alter carbohydrate metabolism. All subjects had maintained a constant body mass (BM) during the 6 months preceeding the experiment and were not participating in any form of regular physical activity. As the principal aim of the investigation was to examine the effects of exercise training per se, subjects were studied under free-living conditions and were instructed not to make any dietary changes throughout the study. This has been common practice in a number of other investigations [9, 10]. The experimental protocol was approved by the Human Ethics Committee of RMIT University. The purpose, nature and potential risks of the study were explained to all subjects and written informed consent was obtained prior to participation.

Euglycaemic-hyperinsulinaemic clamp. Whole body insulin mediated glucose uptake was determined at 08:00 h following a

Table 1. Subject characteristics

	Type 2		Control	
	Pre	Post	Pre	Post
Age (yr)	48±2	–	46±3	–
Body mass (kg)	105.9±9.2	103.9±9.1	90.4±4.7	89.9±5.0
BMI (kg/m ²)	32.3±2.3	31.8±2.3	28.8±1.1	28.4±1.0
% body fat	26.8±3.5	25.5±3.2	29.6±2.3	29.1±2.5
VO_{2peak} (ml/min/kg) ^a	28.1±2.1	35.3±2.2	31.4±2.5	36.9±2.3

Data are means±SEM. ^aSignificant main effect of training, $p<0.001$

12–14 h overnight fast, 1 week before and 36–48 h after completion of the training program. Subjects were instructed to refrain from any form of vigorous physical activity for 72 h prior to the determination of pre-training insulin sensitivity. An antecubital vein was cannulated for infusion of glucose and insulin, and a hand vein was cannulated retrogradely and was heated for sampling of arterialized blood. After collection of baseline blood samples (5 ml), a percutaneous biopsy of the v. lateralis muscle was obtained. Briefly, local anaesthesia [1% lignocaine (lidocaine)] was administered to the skin, subcutaneous tissue and fascia of the v. lateralis and an incision was made. At this time a second site on the same leg (5 cm distal) was prepared for the post-clamp biopsy. Then, a resting muscle biopsy was obtained using a 6 mm Bergström needle modified to include suction. Muscle biopsy specimens (100–150 mg) were immediately frozen in liquid nitrogen and stored at -80°C until subsequent analysis. After resting for 5 min, a primed ($9 \text{ mU}\cdot\text{kg}^{-1}$) continuous infusion of insulin (Actrapid, Novo Nordisk, Sydney, Australia) was commenced at a rate of $40 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$, and blood glucose concentration was measured at 5-min intervals throughout the clamp. A variable rate infusion of 20% glucose was used to maintain euglycaemia (5 mmol/l) for the duration of the clamp (120 min). The blood glucose concentration in the diabetic subjects was allowed to decrease during the insulin infusion to 5 mmol/l and then maintained at this concentration for the remainder of the clamp. Samples (2 ml) for subsequent determination of plasma insulin concentration were obtained every 10 min, spun in a centrifuge and stored at -80°C . Whole body glucose uptake was calculated from the glucose infusion rate ($\text{mg}\cdot\text{kg FFM}^{-1}\cdot\text{min}^{-1}$) required to maintain a blood glucose concentration of 5 mmol/l throughout the last 30 min of the clamp. A second muscle biopsy was obtained 120 min after the initiation of the insulin infusion. To prevent a decrease in plasma potassium concentration during the clamp, 30 mmol KCl (Slow-K; Novartis, Sydney, Australia) was administered orally. A 120-min clamp was chosen as a similar protocol has previously been used to determine insulin sensitivity in patients with Type 2 diabetes [13]. However, there is the possibility that a clamp of this duration might not completely suppress hepatic glucose production in insulin-resistant patients. Accordingly, the glucose infusion rate for the Type 2 diabetic and control subjects might not have been at a steady state for the same length of time during the clamp.

Determination of peak oxygen uptake (VO_{2peak}). All subjects carried out a progressive, incremental cycling test to volitional fatigue on an electronically-braked ergometer (Lode, Groningen, The Netherlands) for determination of VO_{2peak} prior to, at the midpoint, and at the completion of the training program (described below).

Assessment of body composition. Whole body dual energy X-ray absorptiometry (DEXA; Lunar DPX, Lunar Radiation, Madison, Wis., USA), 1 week before and after the training program, was used to measure total and regional tissue composition (body fat, lean tissue mass). Prior to each scan, the DEXA was calibrated with known phantoms.

Exercise training program. The exercise-training program consisted of 8 weeks of ergometer cycling undertaken 3 days per week under supervision in the laboratory. Over the initial 4 weeks, subjects exercised a minimum of twice a week at 70% of pre-training $\text{VO}_{2\text{peak}}$, so that by the end of week 4 they were cycling continuously for 60 min. On the third day of training, and after a brief (10 min) warm-up, subjects carried out short exercise bouts (~3 min) of intense (~80 of $\text{VO}_{2\text{peak}}$) cycling separated by 2 min of active recovery (~50% of $\text{VO}_{2\text{peak}}$), for a total exercise time of about 50 min. The number of exercise bouts during interval training was progressively increased so that by the completion of week 4 subjects were able to do eight work bouts. After week 4 of exercise training, $\text{VO}_{2\text{peak}}$ was reassessed and the training program adjusted, so that for the remaining 4 weeks subjects cycled for about 60 min at 70% of their 'new' $\text{VO}_{2\text{peak}}$ twice a week, while the high intensity interval training session was undertaken at 85% of $\text{VO}_{2\text{peak}}$ with the same work-to-rest interval. At the completion of the 8-week training program, subjects carried out a third and final $\text{VO}_{2\text{peak}}$ test. Whole body rates of carbohydrate and fat oxidation were determined from indirect calorimetry during a 20-min ride at 65% of pre-training $\text{VO}_{2\text{peak}}$ during the first and last week of the program. During every training session, ratings of perceived exertion and heart rate were monitored (Polar Electro, Oy, Finland). During the training program, heart rate averaged 135 ± 2 beats·min⁻¹ for control subjects and 127 ± 14 beats·min⁻¹ for Type 2 diabetic patients, while the average ratings of perceived exertion averaged over all training sessions was 13 ± 1 for control subjects and 12 ± 1 for the patients with Type 2 diabetes. Subjects were allowed free access to water during the training sessions.

Blood biochemistry. Blood glucose concentration was measured using an automated analyzer (Yellow Springs Instruments 2300 Stat Plus Glucose and L-Lactate Analyzer, Yellow Springs, Ohio, USA). Plasma insulin concentration was determined by radioimmunoassay using a commercially available kit (LINCO Human Insulin Specific RIA Kit, St. Charles, Mo., USA). $\text{HbA}_{1\text{C}}$ was determined by specific ion-exchange chromatography (Sigma Diagnostics, NSW, Australia). Plasma non-esterified free fatty acid (NEFA) concentration was determined using an enzymatic colourimetric method (NEFA C test kit, Wako, Richmond, Va., USA). Plasma cholesterol and triglycerides were analyzed using an automated analyzer (Cholestech LDX, Cholestech, Hayward, Calif., USA).

Intramuscular triglyceride concentration. Approximately 30 mg of muscle was freeze-dried under vacuum for 24 h. The sample (~10 mg) was viewed under a microscope ($\times 6.3$) at room temperature for dissection and removal of all traces of adipose tissue, connective tissue and blood contaminants. This procedure yielded approximately 7–8 mg of dry weight dissected muscle from which a direct measure of TG_m content was determined, as previously described [5]. Total lipids were extracted according to a known method of [14] from the dried and dissected skeletal muscle in 4 ml of chloroform:methanol (2:1) and left to rotate at room temperature overnight. We added 0.6% sodium chloride and centrifugation (2000 rpm for 10 min) resulted in a separation of the aqueous and organic phases. The organic phase containing the TG was transferred to a glass vial and air

dried. The isolated lipids were re-suspended in 250 μl of ethanol and the triglyceride concentration determined spectrophotometrically at 490 nm using an enzymatic colourimetric test kit (Triglycerides GPO-PAP, Boehringer Mannheim, Sydney, Australia). The within-assay coefficient of variation for this assay in our laboratory is 8%.

Tissue LCACoA measurement. The total amount of LCACoA in muscle biopsy samples was measured using an assay adapted from another study [15]. Tissue was homogenized in 10% trichloroacetic acid. The acid insoluble pellet (which includes proteins, some lipids and LCACoAs) was washed with ether and water to remove unwanted lipids and water-soluble contaminants. The pellet was re-suspended in 10 mmol/l DTT and the pH raised to 11.5 with KOH. The sample was incubated at 55°C for 10 min to hydrolyse the ester bond between fatty acid and CoA. After hydrolysis the sample was neutralized with 1 mol/l HCl, centrifuged at 13 000 rpm and 250 μl of the supernatant used for the determination of CoA. The supernatant was added to a reaction buffer containing 50 mmol/l KH_2PO_4 , 100 $\mu\text{mol/l}$ α -ketoglutarate, 50 $\mu\text{mol/l}$ NAD^+ . After a basal reading 20 mU of α -ketoglutarate dehydrogenase was added and the reaction rate was followed to completion using a fluorimeter (340 nm excitation, 460 nm emission). The concentration of CoA in the sample was calculated with reference to a standard curve of known concentrations of CoA. The within-assay coefficient of variation for this assay in our laboratory is 10%.

Citrate synthase and β -hydroxy-acyl-CoA dehydrogenase (β -HAD) activity. Muscle (5–10 mg) was homogenized in 1:50 dilution (w/v) of a 175 mmol/l potassium buffer solution and citrate synthase activity was assayed spectrophotometrically at 25°C as previously described [16]. β -HAD activity was assayed spectrophotometrically at 25°C measuring the disappearance of NADH using the same homogenate as for citrate synthase [17].

Statistical analysis. Data are presented as means \pm SEM. Differences were determined using a two-way (group \times training) ANOVA for all measures except the effect of insulin-stimulation and training on LCACoA content. For this measure a three-way (group \times clamp \times training) ANOVA was used. Significant differences were located using a Newman Kuels post hoc test. A p value of less than 0.05 was considered statistically significant.

Results

Subject characteristics. BM, BMI and percentage body fat were similar between the two experimental groups (Table 1) and did not change after the training programme. The clinical characteristics of all subjects are shown in Table 2. There was a main effect for fasting blood glucose concentration, being higher in patients with Type 2 diabetes compared with control subjects ($p < 0.01$). Training resulted in a reduction in fasting plasma insulin ($p < 0.05$) and plasma NEFA levels ($p < 0.05$). $\text{HbA}_{1\text{C}}$ was increased in the diabetic patients ($p < 0.01$). Plasma triglycerides, cholesterol, HDL, LDL and VLDL were similar between the two experimental groups (Table 2) and did not change after the training program.

Table 2. Subject clinical characteristics

	Type 2		Control	
	Pre	Post	Pre	Post
Basal				
Blood glucose (mmol/l) ^a	8.3±1.0	7.9±1.0	4.7±0.1	4.9±0.2
Plasma insulin (pmol/l) ^b	178.2±62.1	127.8±41.3	78.7±13.0	70.6±13.0
Plasma NEFA (mmol/l) ^b	1.1±0.2	1.0±0.1	1.0±0.1	0.8±0.1
Triglycerides (mmol/l)	2.5±0.5	2.4±0.3	1.6±0.3	1.8±0.4
Cholesterol (mmol/l)	4.2±0.4	4.7±0.3	5.2±0.5	5.2±0.5
HDL cholesterol (mmol/l)	1.0±0.1	1.0±0.1	1.2±0.1	1.2±0.1
LDL cholesterol (mmol/l)	1.9±0.2	2.6±0.3	3.3±0.4	3.2±0.6
VLDL cholesterol (mmol/l)	0.9±0.2	1.1±0.1	0.8±0.1	0.8±0.2
HbA _{1c} ^a	7.9±0.6	7.0±0.7	5.3±0.2	5.4±0.2
Clamp				
Glucose (mmol/l)	5.1±0.1	4.9±0.1	4.9±0.1	4.8±0.1
Insulin (pmol/l)	755.7±72.7	765.0±81.0	792.0±45.0	808.5±51.5

Data are means±SEM. ^a Significant main effect of group, $p<0.01$. ^b Significant main effect of training, $p<0.01$

Effect of exercise training on aerobic capacity and rates of whole body substrate oxidation. The 8 weeks of endurance exercise training increased $\text{VO}_{2\text{peak}}$ by about 20% ($p<0.001$) and the magnitude of this increase was similar in both groups. During submaximal cycling at 65% of $\text{VO}_{2\text{peak}}$, whole body rates of fat oxidation were lower in Type 2 diabetic patients compared with control subjects (0.24 ± 0.02 vs 0.58 ± 0.10 g/min; $p<0.05$). In the patients with Type 2 diabetes, the exercise training program resulted in an increase in the rate of fat oxidation (0.24 ± 0.02 vs 0.32 ± 0.01 g/min; $p<0.05$) and a concomitant reduction in the rate of carbohydrate oxidation (2.32 ± 0.18 vs 1.77 ± 0.11 g/min; $p<0.05$) during a 20 min standardized ride at 65% of pre-training $\text{VO}_{2\text{peak}}$ (~55% of post-training $\text{VO}_{2\text{peak}}$). Unfortunately, due to technical difficulties we were only able to determine rates of substrate oxidation in two control subjects following training. In those two individuals the rates of carbohydrate oxidation were 2.36 ± 0.09 g/min, while whole body rates of fat oxidation were 0.46 ± 0.02 g/min.

Whole body insulin-stimulated glucose uptake. There were no significant differences in blood glucose (Fig. 1A) or plasma insulin (Fig. 1B) concentration during the final 30 min of the clamp procedure. Whole body insulin-stimulated glucose uptake was impaired in patients with Type 2 diabetes [60% reduction compared to control subjects ($p<0.05$)] before the exercise training intervention (Fig. 2). Despite a similar relative increase in insulin sensitivity in both groups after exercise training (~30%), whole body insulin-stimulated glucose uptake remained markedly impaired (60%) in the Type 2 diabetic subjects.

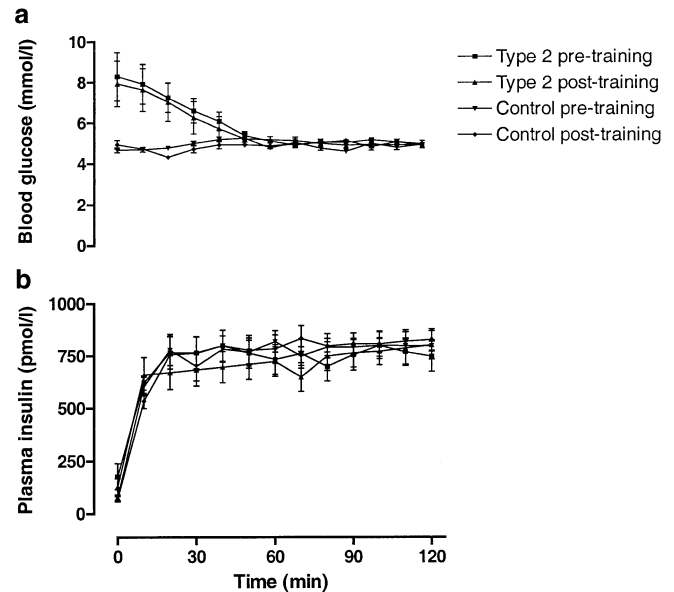


Fig. 1a, b. Blood glucose (a) and plasma insulin (b) concentrations during the euglycaemic hyperinsulinaemic clamp, pre- and post-training

Skeletal muscle lipids. TG_m content was about 170% higher in muscle from Type 2 diabetic individuals compared with control subjects [89.0 ± 23.2 vs 33.3 ± 4.3 mmol/kg dry weight (dw); $p<0.05$; Fig. 3]. Exercise training resulted in a reduction (47%) in TG_m content in Type 2 diabetic subjects (89.0 ± 23.2 vs 47.5 ± 11.4 mmol/kg d.w.; $p<0.05$), but not in control subjects. Basal skeletal muscle LCACoA content was similar between groups (Table 3). LCACoA content was not altered by the exercise training program. Insulin-stimulation had no detectable effect on total LCACoA content [~ 12 nmol/g wet weight (ww); Table 3], although there was a tendency for LCACoA content to be suppressed following insulin-stimulation

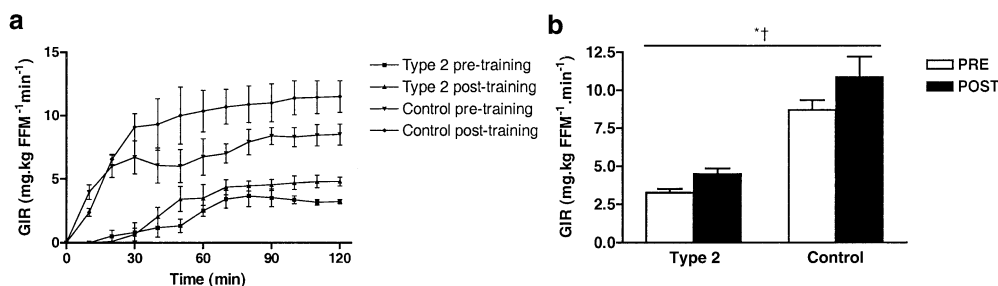


Fig. 2a, b. Whole body insulin-stimulated glucose infusion rate (GIR) averaged over 10 min intervals as measured throughout the euglycaemic-hyperinsulinaemic clamp before (pre) and after (post) an 8-week exercise training intervention (**a**) and steady state GIR determined during the final 30 min of the euglycaemic-hyperinsulinaemic clamp (**b**). Values are means \pm SEM. *Significant main effect of group, $p<0.001$. †Significant main effect of training, $p<0.01$

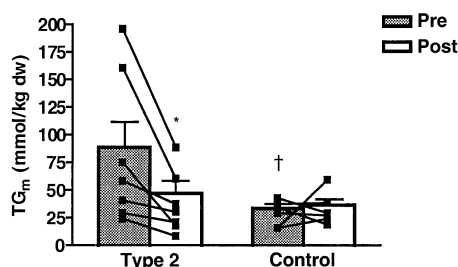


Fig. 3. Intramuscular triglyceride (TG_m) content in resting biopsy samples obtained from the v. lateralis before (pre) and after (post) an 8 week exercise training Intervention. Values are means \pm SEM. *-Significantly different to pretaining, $p<0.05$. †-Significantly different to subjects with Type 2 diabetes at same time point, $p<0.05$

Table 3. Basal and insulin-stimulated long-chain fatty acyl CoA content in muscle samples obtained before (pre) and after (post) an 8-week exercise training intervention

	Pre		Post	
	Basal	Insulin-stimulated	Basal	Insulin-stimulated
Type 2	11.9 \pm 1.9	12.3 \pm 0.5	10.6 \pm 1.5	11.4 \pm 1.0
Control	9.4 \pm 0.9	7.3 \pm 2.1	11.0 \pm 0.8	9.6 \pm 0.8

Data are means \pm SEM. Values are nmol/g w.w

in control subjects post-training (11.0 \pm 0.8 vs 9.6 \pm 0.8 nmol/g w.w.; $p=0.07$).

Skeletal muscle oxidative capacity. The activity of citrate synthase increased in response to training (main effect $p<0.001$) in skeletal muscle from both Type 2 diabetic (73%) and control subjects (85%; Table 4). Training resulted in an (main effect $p<0.001$) increase in β -HAD activity (Type 2 diabetic patients: 62%; control subjects: 48%; Table 4).

Table 4. Effects of an 8-week exercise training program on citrate synthase and β -hydroxyacyl CoA dehydrogenase activity

	Type 2		Control	
	Pre	Post	Pre	Post
Citrate synthase ^a	4.4 \pm 0.5	7.6 \pm 0.8	5.4 \pm 0.5	10.0 \pm 1.1
β -HAD ^a	5.3 \pm 0.7	8.6 \pm 0.9	4.2 \pm 0.6	6.2 \pm 0.7

β -HAD, β -hydroxyacyl CoA dehydrogenase. Data are means \pm SEM. ^a Significant main effect of training, $p<0.001$. Values are μ mol/g/min

Discussion

Evidence is accumulating to suggest that together with hyperglycaemia, Type 2 diabetes is associated with profound alterations in lipid metabolism. In particular, accretion of lipid in skeletal muscle seems to play a crucial role in the aetiology of insulin resistance: a number of independent research groups using a variety of different measurement techniques have reported negative correlations between TG_m and insulin action [3, 4, 5, 6, 7]. Here we report that exercise training in Type 2 diabetic individuals resulted in a “normalization” of TG_m content, but did not completely reverse insulin resistance in these patients.

It is well known that endurance training improves insulin action in healthy individuals [9], with an increase in insulin sensitivity being observed after only 7 days of training [18, 19, 20]. The mechanisms by which exercise training improves insulin action in healthy skeletal muscle have been attributed to increases in insulin-stimulated PI3-kinase activity [18], increased expression of GLUT4 [21], hexokinase [22] and glycogen synthase [22]. Exercise training has also been shown to increase insulin sensitivity in patients with Type 2 diabetes [23]. In this study, exercise training resulted in a similar increase in insulin sensitivity in both Type 2 diabetic and healthy control subjects matched for age, BMI, percentage of body fat and VO₂peak. However, despite the similar relative increase in insulin sensitivity, whole body insulin-stimulated glucose uptake remained markedly impaired in the Type 2 diabetic subjects.

The effects of exercise training on TG_m content in healthy subjects are equivocal, with some studies reporting an increase [11, 24, 25], decrease [26] or no

change [9, 10, 27] after short-term (6–12 weeks) endurance exercise. In our investigation, exercise training had little effect on TG_m concentrations in control subjects, but resulted in a substantial overall decrease in patients with Type 2 diabetes. Indeed, patients with the highest basal (pre-training) levels of TG_m seemed to have the largest decrease after the exercise training intervention. However, despite TG_m content being “normalized” to that of control subjects following the training program, insulin resistance (i.e., the glucose infusion rate during the clamp) was not fully reversed in the diabetic patients. Indeed, despite relatively large changes in lipid status following exercise training, the resting blood glucose concentration in patients with Type 2 diabetes was virtually unaltered. As glucose toxicity seems to reduce whole body insulin-stimulated glucose disposal in diabetic patients [28], this could contribute to the persistent insulin resistance observed in Type 2 diabetic subjects in this study following the exercise training intervention. Precisely how sustained hyperglycaemia produces insulin resistance is unknown. However, taken collectively, our findings strongly suggest that TG_m content is merely a reflection of the metabolic events that lead to a decreased insulin sensitivity rather than a direct causal factor of this state. Thus, muscle triacylglycerol content might merely act as a surrogate measure for other lipid-derived moieties that could have a more mechanistic and direct link with the pathophysiology of insulin resistance, such as the fatty acid composition of membrane phospholipids [9], diacylglycerol (DAG) [29] or the metabolically active LCACoA [30].

Investigations of the measurement of LCACoA in humans are scarce, but a recent study reported increased LCACoA content in skeletal muscle from overweight/obese and extremely obese individuals compared with normal weight subjects [31]. Furthermore, total LCACoA content has been reported to be a better correlate of insulin action than measurements of TG_m [8]. Hence, a second novel finding of the present investigation was that despite markedly different levels of insulin sensitivity and increased TG_m content in patients with Type 2 diabetes, total LCACoA at rest was similar between Type 2 diabetic and control subjects. Furthermore, 8 weeks of exercise training did not result in any detectable difference in resting muscle LCACoA content despite a reduction in TG_m and enhanced whole body insulin sensitivity. These findings are in agreement with those of another study [10] that reported that the improvement in insulin sensitivity following moderate intensity exercise was independent of changes in muscle LCACoA levels in overweight and obese men. Although speculative, it is possible that the endurance training intervention undertaken by subjects in the present investigation resulted in changes in the individual LCACoA species (i.e., a reduction in the saturated species). In support of such a contention,

exercise training has been shown to increase the degree of unsaturation of skeletal muscle membranes [9, 32, 33], suggesting that regular exercise modifies the fatty acid profile of skeletal muscle in favour of enhanced insulin sensitivity.

In the present study we were unable to detect any reduction in LCACoA content in muscle from Type 2 diabetic patients following acute insulin-stimulation either before or after training. This is in agreement with our previous findings that LCACoA content was not suppressed during a hyperinsulinaemic-euglycaemic clamp in a group of individuals with varying degrees of insulin sensitivity [34]. However, in healthy control subjects there was a tendency for insulin stimulation to suppress LCACoA content, with this decrease approaching statistical significance post-training ($p=0.07$). Although tentative, these data are suggestive that a suppression of LCACoA content could be associated with enhanced insulin sensitivity in muscle from healthy individuals. A common feature of both diet- and/or exercise-induced reversal of insulin resistance is a decrease in the muscle LCACoA levels during a glucose clamp [35]. Accordingly, it might have been predicted that insulin stimulation would have a greater potential to suppress LCACoA content in the Type 2 diabetic patients rather than the control subjects, as insulin sensitivity in these patients was markedly increased after the training intervention. We have no explanation why insulin stimulation would suppress LCACoA content in only healthy compared to insulin-resistant muscle. However, it should be remembered that although exercise training markedly improved insulin sensitivity in the Type 2 diabetic subjects, whole body insulin-stimulated glucose uptake was still markedly impaired compared to the control subjects.

It is well known that endurance training elicits an increase in the oxidative capacity of skeletal muscle from healthy, insulin-sensitive subjects [25, 36, 37]. Thus, regular exercise training might be therapeutic in overcoming derangements in lipid metabolism observed in Type 2 diabetes. Indeed, we observed a training-induced increase in muscle oxidative capacity that was of the same order of magnitude in both Type 2 diabetic and control subjects. This improved “metabolic fitness” was associated with an enhanced capacity for fatty acid utilization in the diabetic patients, reflected by an increase in whole body rates of fat oxidation during submaximal exercise conducted at the same absolute pre-training workload. Previous studies have also shown that a program of endurance training similar in intensity and duration to that used in the present investigation, results in an increase in whole body fat oxidation [27, 38]. Furthermore, in healthy subjects a short-term, low-intensity exercise training program has recently been shown to increase the capacity to oxidize TG_m and/or VLDL-derived fatty acids, both at rest and during submaximal exer-

cise [12]. It does not seem unreasonable, therefore, to suggest that part of the training-induced increase in rates of whole body fat oxidation during exercise in the Type 2 diabetic patients in the current study would have resulted in increased rates of TG_m oxidation (and turnover) during the actual exercise-training sessions, and that this, in part, could be responsible for the reduction in resting TG_m levels.

Finally, both Type 2 diabetic patients and the matched control subjects in this investigation were overweight/obese. However, the difference in the average body mass of participants in the two groups was not statistically significant. Notwithstanding this fact, the Type 2 diabetic subjects were about 15 kg heavier than the control subjects, and such a difference in body mass could have an influence on some of our findings. For example, it is likely that if the subjects were leaner, there could have been greater improvements in whole-body insulin-stimulated glucose utilization. While weight loss alone can reduce insulin resistance, physical activity (i.e., exercise training) and weight loss are generally recommended as a combined intervention as there is uncertainty regarding their respective effects on improvements in insulin resistance [10].

In conclusion, we observed that enhanced insulin action following 8 weeks of exercise training was associated with an increase in whole body rates of fat oxidation and a marked decrease in muscle TG_m content in patients with Type 2 diabetes. However, despite the "normalization" of TG_m concentration to values observed in healthy control subjects, insulin resistance was not completely reversed in the diabetic patients. These findings provide compelling evidence that TG_m per se does not confer insulin resistance, but possibly acts as a surrogate for other lipid-derived metabolites, such as diacylglycerol, that have a more mechanistic link with the development of insulin resistance.

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