Training-induced improvement in lipid oxidation in type 2 diabetes mellitus is related to alterations in muscle mitochondrial activity.

Effect of endurance training in type 2 diabetes

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Abstract

Aim. – We investigated whether or not, in type 2 diabetic (T2D) patients, an individualized training effect on whole-body lipid oxidation would be associated with changes in muscle oxidative capacity.

Methods. – Eleven T2D patients participated in the study. Whole-body lipid oxidation during exercise was assessed by indirect calorimetry during graded exercise. Blood samples for measuring blood glucose and free fatty acids during exercise, and muscle oxidative capacity measured from skeletal muscle biopsy (mitochondrial respiration and citrate synthase activity), were investigated in the patients before and after a 10-week individualized training program targeted at LIPOX max, corresponding to the power at which the highest rate of lipids is oxidized (lipid oxidation at LIPOX max).

Results. – Training induced both a shift to a higher-power intensity of LIPOX max (+9.1 ± 4.2 W; P < 0.05) and an improvement of lipid oxidation at LIPOX max (+51.27 ± 17.93 mg min⁻¹; P < 0.05). The improvement in lipid oxidation was correlated with training-induced improvement in mitochondrial respiration (r = 0.78; P < 0.01) and citrate synthase activity (r = 0.63; P < 0.05).

Conclusion. – This study shows that a moderate training protocol targeted at the LIPOX max in T2D patients improves their ability to oxidize lipids during exercise, and that this improvement is associated with enhanced muscle oxidative capacity.

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Résumé

L’amélioration de l’oxydation des lipides induite par l’entraînement est liée aux modifications de l’activité mitochondriale musculaire chez des diabétiques de type 2.

Objectif. – Le but de ce travail était d’étudier chez des diabétiques de type 2 (DT2) si les effets d’un entraînement individualisé sur l’oxydation des lipides étaient liés à l’amélioration des capacités oxydatives musculaires.

Méthodes. – Onze diabétiques de type 2 (DT2) ont participé à l’étude. L’oxydation des lipides à l’effort a été évaluée par une calorimétrie indirecte d’effort. Des prélèvements sanguins pour les dosages de la glycémie et des acides gras libres au cours de l’exercice ainsi qu’une biopsie musculaire destinée à étudier les capacités oxydatives musculaires (respiration mitochondriale et activité citrate synthase) étaient réalisés chez les patients avant et après dix semaines d’entraînement individualisé ciblé sur le LIPOX max, qui correspond à la puissance à laquelle l’oxydation des lipides est maximale (oxydation des lipides au LIPOX max).
**1. Introduction**

The prevalence of type 2 diabetes mellitus (T2D) continues to rise, and a sedentary lifestyle and obesity are recognized as key risk factors. T2D patients are characterized by impaired substrate uptake [1,2]. Using a specific protocol of exercise calorimetry [3–5], we recently reported [6] that the defect in lipid oxidation during exercise in such patients can be described as the balance of substrates used for oxidation during exercise being shifted towards a lower maximum peak of lipid oxidation (LIPOXmax) and lower exercise-intensity levels.

In addition, in T2D patients, skeletal muscle mitochondrial function appears to be disturbed, leading to, in particular, reduced activity of the electron transport chain [7–9]. However, this mitochondrial dysfunction remains controversial [10]. Nevertheless, it is well established that mitochondria are adaptable organelles directly involved in substrate oxidation, and skeletal muscle can manifest considerable plasticity of mitochondrial activity in response to training in insulin-resistant states, in obese individuals [11] and in T2D patients, as has been recently shown [12]. Interestingly, the defect in lipid oxidation found in both obese and insulin-resistant patients is also highly sensitive to training and, thus, is rapidly corrected by endurance training targeted at the LIPOXmax [4,13]. Therefore, it appeared to be logical to extend this approach to people with T2D.

Nevertheless, the mechanism of such training-induced improvement in lipid oxidation remains poorly understood as, to our knowledge, there has been no research into the effect of training on changes in whole-body lipid oxidation and skeletal muscle oxidative capacity in T2D patients.

Thus, the aim of our study was to investigate the effects of a 10-week individualized training program in T2D patients, carried out at the level of the LIPOXmax (power intensity at which lipid oxidation is maximum), on whole-body lipid oxidation and skeletal muscle oxidative capacity.

**2. Methods**

Eleven overweight T2D male patients were enrolled into the study: age (years): 55.4 ± 2.2; height (cm): 177 ± 1.3; weight (kg): 90.9 ± 3.1; body mass index (BMI, kg m⁻²): 29.0 ± 1.0; fasting blood glucose (mmol L⁻¹): 8.8 ± 1.0; fasting blood insulin (µIU mL⁻¹): 10.24 ± 2.18; and HbA₁c (%): 7.4 ± 0.4. These patients were all sedentary, with a score less than nine (5.70 ± 1.02) on a questionnaire commonly used for patients with chronic disease [14], and not engaged in any other training programs. All were treated with oral antidiabetic drugs only, and none received insulin or had clinical signs of long-term diabetic complications. All medications were withheld 24 hours before the experiment. Informed consent was obtained from all subjects after explanation of the nature of the study and the risks related to their participation. The study was approved by the local ethics committee (# 03/10/GESE).

**3. Experimental design**

Each patient visited the laboratory three times at 8 a.m. after an overnight fast. The first visit was for enrollment, and included a clinical examination, a physical-activity questionnaire, anthropometric measurements and obtaining the informed consent. Two days later, the patients returned to the laboratory for an exercise test (see below) and, after a further two days, for a skeletal muscle biopsy of the vastus lateralis. Then, two or three days later, the patients started the training program. At the end of the training, they all underwent a second exercise test and another skeletal muscle biopsy.

**4. Exercise testing**

The exercise test was performed at 8 a.m. after an overnight fast on an electromagnetically-braked cycle ergometer (550 ERG, Bosch, Germany) that was connected to a breath device (Zan 600, Zan, Germany) to measure gas exchanges (VO₂ and VCO₂). The test consisted of five six-minute submaximum steady-state workloads corresponding to 20, 30, 40, 50 and 60% of the maximum theoretical workload (Wmax th). This latter figure was calculated according to Wasserman’s equation [15]: \((0.79 \times H – 60.7) \times (50.72 – 0.372 \times A) – 350)/10.3 \) if the patient was obese, or \( W \times (50.72 – 0.372 \times A) – 350)/10.3 \) if the patient was not obese, where \( H \) = height in cm, \( A \) = age in years and \( W \) = weight in kg.

Blood samples were drawn at rest and during the last minute of each steady-state workload, using a 32-mm catheter placed into a superficial forearm vein, to measure blood glucose and free fatty acids.

Indirect calorimetric measurements were performed to determine whole-body lipid oxidation. VO₂ and VCO₂ were determined as the mean of the measurements taken during the fifth and sixth minute of each six-minute steady-state work-
load, according to Perez-Martin et al. [3]. Lipid oxidation was calculated on the basis of the gas-exchange measurements, according to the non-protein respiratory quotient (R) technique [16], as protein breakdown contributes little to energy metabolism during exercise [17]. The values of lipid oxidation were obtained according to the following equation [16]: Li p ox (mg min\(^{-1}\)) = 1.6946 \(V_{O_2}\) – 1.7012\(V_{CO_2}\) (with \(V_{O_2}\) and \(V_{CO_2}\) expressed in mL/min).

As has been previously described [3,13,18], we calculated a parameter representative of the whole-body lipid oxidation during exercise, which is the maximum lipid-oxidation point (LIPOX\textsubscript{max}) [3,13,18], expressed in watts (W), which corresponds to the exercise intensity at which the highest rate of lipid oxidation is achieved (lipid oxidation at LIPOX\textsubscript{max}, expressed in mg min\(^{-1}\)). The difference before and after training was expressed as \(\Delta V_\text{max}\).

5. Skeletal muscle biopsy

Vastus lateralis muscle biopsies were taken, according to the percutaneous Bergström technique, after local anaesthesia (xylocaine). The muscle samples (200–300 mg) were divided into two portions. One part was immediately frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\) for enzyme determinations (citrate synthase [CS] activity). The other portion was used for the mitochondrial respiration studies in situ and was immediately placed in an ice-cold relaxing solution containing the following (mM): EGTA–calcium buffer: 10 (free Ca\textsuperscript{2+} concentration 100 nmol L\(^{-1}\)); imidazole: 20; KH\textsubscript{2}PO\textsubscript{4}: 3; MgCl\textsubscript{2}: 1; taurine: 20; DTT: 0.5; MgATP: 5; and PCr: 15.

6. Mitochondrial respiration

Respiratory parameters of the total mitochondrial population were analyzed in situ on fresh skeletal muscle fibres, as previously described [19]. Bundles of muscle fibres were manually isolated and saponin-skinned (50 mg/mL saponin for 10 minutes at \(4^\circ\text{C}\)). To remove the saponin and free ATP, the fibres were washed with continuous stirring in relaxing solution for 10 minutes (\(4^\circ\text{C}\)), followed by two 5-minute washes in respiration solution at \(4^\circ\text{C}\) (same as the relaxing solution, except for substitution of MgATP and phosphocreatine by 3 mM of phosphate and 2 mM of fatty acid–free bovine serum albumin). After washing, the fibres were stored on ice until needed. Respiration rates were determined at 27 \(^\circ\text{C}\), using a Clark electrode (Strathkelvin Instruments, Scotland) and an oxygraphic cell, with continuous stirring in respiration solution. Respiration rates were recorded in the presence of pyruvate (10 mM)/malate (2 mM). For each sample, basal oxygen consumption without ADP was recorded first, followed by the ADP-stimulated maximum respiration rate (\(V_\text{max}\)), determined in the presence of a saturation concentration of ADP (2 mM). At the end of each measurement, cytochrome c was added to investigate the outer mitochondrial membrane integrity [20]. After the respiratory measurements (see below), the fibre bundles were dried overnight and weighed the next day. Respiration rates were expressed in \(\mu\text{mol O}_2/\text{min/g dry weight}\). The difference in \(V_\text{max}\) before and after training was expressed as \(\Delta V_\text{max}\).

7. Citrate synthase (CS) activity analysis

Frozen muscle biopsies were homogenized in 40 volumes of a solution (pH 8.2) containing (mM) Tris–HCl: 50, EDTA: 1 and MgSO\textsubscript{4}: 5 in a glass homogenizer. The crude homogenates were then centrifuged for 10 minutes at 500 g and assayed for supernatants. CS activity was then measured with oxaloacetate 0.38 mM and acetyl-CoA at 1.235 mM. Enzyme activity was monitored by recording the changes in absorbance at 412 nm for 2.5 minutes at 30 \(^\circ\text{C}\). Results were expressed in \(\mu\text{mol/min/g dry weight}\). The difference of CS activity before and after training was expressed as \(\Delta CS\).

8. Blood analysis

Glycated haemoglobin (HbA\textsubscript{1c}) was measured at rest. Blood glucose and free fatty acids (FFA) were measured at rest and at each step of the exercise test.

Fasting insulin was assessed by radioimmunoassay (Bi-Insulin IRMA Kit, Schering CIS bio international, France) and plasma glucose concentrations measured using an automated device (AU2700, Olympus, France). HbA\textsubscript{1c} was determined by high-performance liquid chromatography (HPLC; Ménarini, France), and FFA quantification was by the Wako NEFA C test kit (Wako Chemical GmbH, Dyasis France, France).

9. Individualized exercise training

The exercise training program entailed cycling on a cycle-ergometer for 45 minutes, twice a week, for 10 weeks. The patient’s heart rate, corresponding to LIPOX\textsubscript{max}, was continuously monitored during each training session (Polar Cardiometer, France). All training sessions took place at the laboratory under the supervision of a professional instructor.

10. Data analysis

All statistical analyses were performed using a commercial software package (StatView for Windows, version 5.0). Data are presented as means \(\pm\) S.E.M. Effects of training were tested with Student’s paired \(t\) test. The significance of differences between the exercise steps in blood glucose and FFA changes, and the exercise intensity, was determined by Anova for repeated measures. Post-hoc comparisons were made using Bonferroni–Dunn’s test for significant differences. Correlations were determined by Spearman analysis (\(P < 0.05\) was considered significant).

11. Results

Ten weeks of exercise training targeted at the LIPOX\textsubscript{max} did not significantly alter BMI (28.9 \(\pm\) 1 kg m\(^{-2}\)), fasting blood glucose (8.7 \(\pm\) 0.5 mmol L\(^{-1}\)), fasting blood insulin (9.13 \(\pm\) 1.59 \(\mu\)U mL\(^{-1}\)) or HbA\textsubscript{1c} (7.1 \(\pm\) 0.3%). During the
Table 1
Values of blood glucose and FFA at each step of exercise testing before and after training

<table>
<thead>
<tr>
<th></th>
<th>Rest (20%) W_{max th}</th>
<th>(20%) W_{max th}</th>
<th>(30%) W_{max th}</th>
<th>(40%) W_{max th}</th>
<th>(50%) W_{max th}</th>
<th>(60%) W_{max th}</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose (mmol L^{-1})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>8.81 ± 0.96</td>
<td>8.54 ± 0.87</td>
<td>8.35 ± 0.89</td>
<td>7.22 ± 0.89</td>
<td>7.71 ± 0.87</td>
<td>7.89 ± 1.03</td>
</tr>
<tr>
<td>After</td>
<td>8.73 ± 0.45</td>
<td>8.13 ± 0.65</td>
<td>8.01 ± 0.57</td>
<td>7.93 ± 0.60</td>
<td>8.16 ± 0.58</td>
<td>8.12 ± 0.52</td>
</tr>
<tr>
<td><strong>FFA (mmol L^{-1})</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.62 ± 0.1</td>
<td>0.46 ± 0.07</td>
<td>0.43 ± 0.06</td>
<td>0.40 ± 0.05</td>
<td>0.34 ± 0.05</td>
<td>0.36 ± 0.04</td>
</tr>
<tr>
<td>After</td>
<td>0.61 ± 0.1</td>
<td>0.48 ± 0.08#</td>
<td>0.44 ± 0.07#</td>
<td>0.43 ± 0.08#</td>
<td>0.42 ± 0.09#</td>
<td>0.41 ± 0.09#</td>
</tr>
</tbody>
</table>

Before: pretraining values; #P < 0.01 versus values at rest; After: post-training values.

exercise test, blood glucose did not change significantly, whereas FFA decreased significantly at each step of the exercise compared with the rest before and after training (Table 1). However, 10 weeks of exercise training did not significantly alter blood glucose and FFA values at rest and during exercise (Table 1).

The exercise training had, however, marked effects on whole-body lipid oxidation and muscle oxidative capacity. Indeed, after training, the LIPOX_{max} was shifted to a higher power of intensity (+9.14 ± 4.18 W; P < 0.05; Fig. 1A), and the lipid oxidation at LIPOX_{max} was significantly increased compared with pretraining values (+51.3 ± 17.9 mg min^{-1}; P < 0.05; Fig. 1B). Moreover, both V_{max} (Fig. 1C) and CS activity (Fig. 1D) also increased with the training program. Finally, changes in whole-body lipid oxidation were associated with changes in parameters of muscle oxidative capacity. Indeed, correlations were found, on the one hand, between Δ lipid oxidation at LIPOX_{max} and ΔV_{max} (r = 0.78; P < 0.01, Fig. 2) and, on the other hand, between Δ lipid oxidation at LIPOX_{max} and Δ CS (r = 0.63; P < 0.05, Fig. 3).

12. Discussion

This study shows that, in T2D patients, only moderate training over 10 weeks targeted at the level of LIPOX_{max} improves their ability to oxidize lipids during exercise, and that this improvement is correlated with an enhancement in both mitochondrial respiration and citrate synthase activity.

Fig. 1. Characteristics of lipid oxidation during exercise, and muscle oxidative capacity before and after training. (■) Pretraining; (□) Post-training; $P < 0.05$ versus pretraining values.
Based on our previous studies using calorimetry during steady-state workloads [21], we have developed an exercise test [3] that consists of five six-minute submaximum steps, during which we calculate lipid oxidation from gas exchanges during the fifth and sixth minute of each step [22]. This protocol has been used as a basis for targeting endurance training in adolescent and adult obese patients [4,13], with the aim of specifically improving lipid oxidation at exercise. It was logical to extend this approach to people with type 2 diabetes, in whom the ability to oxidize lipids during exercise has often been reported as impaired [1,2,6]. Indeed, low-intensity endurance training targeted at the level of the LIPOX max in subjects in whom lipid oxidation is impaired [4,13] has been shown to markedly improve the impairment, as observed in the present study.

In T2D, the exercise intensity that should be used for training remains controversial. In 27 studies reviewed in a recent meta-analysis by Snowling and Hopkins [23], intensities ranging from 40 to 85% of \( V_O^2 \) have been applied. On the whole, all protocols appear to be beneficial, but the meta-analysis was unable to demonstrate an influence of exercise intensity on the effectiveness of training. A likely explanation of this apparently paradoxical lack of intensity effect may be that the effect of exercise intensity on lipid oxidation is not linear, but a bell-shaped curve, reaching a peak at the LIPOX max, followed by a decrease at higher intensities. Since the LIPOX max occurs at very low intensities in T2D patients (38% of \( V_O^2 \) [6], 37% of \( V_O^2 \) in the present study), it is likely that most training protocols targeted at above 50% of \( V_O^2 \) will produce different effects. Exercise
sessions set at such levels, where carbohydrates are virtually the only substrate used for oxidation, may mostly enhance the ability to oxidize carbohydrates rather than lipids, as already demonstrated by our team in a longitudinal study of training in cyclists [24]. Presumably, procedures that increase lipid oxidation and those that increase carbohydrate oxidation result in different metabolic effects in the muscle cell. Therefore, training that is closely targeted at lipids and training that is targeted at carbohydrates may be different, complementary procedures.

In the present study, the goal was to test a protocol targeted at lipid oxidation, as this parameter is impaired in T2D, but has been shown to be markedly improved in non-diabetic obese adults and obese children [4,13], using a similar training protocol. The present study shows a similar improvement in lipid oxidation in a sample of T2D male patients, consistent with a recent study using endurance training at 60–70% of the maximum heart rate [12], a markedly higher intensity corresponding to approximately 50% of the $V_{O_2}$. What our study shows for the first time is that, in T2D, the improvement in whole-body lipid oxidation induced by training, and particularly the lipid oxidation rate at LIPOX$_{max}$ during exercise, are closely associated with an enhanced muscle oxidative capacity. This capacity was investigated by two complementary approaches. The first was to measure mitochondrial respiration on permeabilized skeletal muscle fibres to allow determination of all mitochondrial activity in situ. This method is now widely validated [10,25,26]. However, in our study, we used pyruvate/malate as the substrate instead of glutamate/malate, because pyruvate allows investigation of the whole of mitochondrial activity (Krebs cycle and all complexes of the respiratory chain). The second approach was to measure CS activity, a well-recognized marker of mitochondrial density in skeletal muscle [27]. Both techniques provided evidence of improvement in muscle oxidative capacity, proportional to the increase in the ability to oxidize lipids.

Training-induced improvements in skeletal muscle mitochondrial function have already been studied in healthy subjects [28] and in insulin-resistant patients, including T2D [12] and obesity, where training induced an increase in the size and content of the mitochondria [9], and enhanced mitochondrial fatty acid oxidation [11]. However, to our knowledge, the present study is the first to demonstrate a relationship between the improvement in whole-body lipid oxidation and enhanced mitochondrial respiration in T2D patients, who may have a disturbed skeletal muscle mitochondrial function by exercise training [7–9]. The present finding of a close relationship between mitochondrial adaptation to training and lipid oxidation during exercise in T2D is consistent with a recent report in non-diabetic subjects of a relationship between lipid oxidation and mitochondrial respiration [29]. However, Nordby et al. [30] reported no relationship between muscle oxidative capacity and whole-body peak lipid oxidation in either trained or untrained non-diabetic subjects. Presumably, the Nordby paper, which involved healthy young controls, was unable to detect such a relationship because, without markedly low values, these parameters would have remained within a narrow normal range and failed to exhibit enough variability to obtain a significant correlation. In contrast, in our study as well as that of Sahlin et al. [29], there was a wide range of variation in these parameters, which may explain why we found clear evidence of a relationship where Nordby et al. did not.

Finally, in our study, we found an improvement in lipid oxidation with training with no changes in the levels of FFA during exercise. This suggests that the main effect of our short and moderate-intensity exercise protocol targeted at the LIPOX$_{max}$ is probably to improve the use of intramuscular lipids that are reportedly increased in T2D [2]. But, because we failed to observe any significant changes in body composition or in parameters of glycaemic control after training, we conclude that our training protocol may be simply the first step of a rehabilitation program to improve muscle metabolism in T2D that will need to be continued over a longer period of time to achieve beneficial effects on body composition and glycaemic control.

In conclusion, the present study shows that a moderately intense exercise-training protocol targeted at the LIPOX$_{max}$ can improve the ability to oxidize lipids during exercise in T2D patients, and that this improvement is associated with enhanced muscle oxidative capacity.

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References


