Physical training reverses defect in 3-ketoacid CoA-transferase activity in skeletal muscle of diabetic rats

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El Midaoui, Adil, Jean Louis Chiasson, Gilles Tancrède, and André Nadeau. Physical training reverses defect in 3-ketoacid CoA-transferase activity in skeletal muscle of diabetic rats. Am J Physiol Endocrinol Metab 288:E748–E752, 2005; doi:10.1152/ajpendo.00515.2004.—To investigate one potential mechanism whereby physical training improves the plasma concentration of ketone bodies in experimental diabetes mellitus, we measured the activity of 3-ketoacid CoA-transferase, the key enzyme in the peripheral utilization of ketone bodies. Diabetes was induced with streptozotocin (50 mg/kg) and training carried out on a treadmill with a progressive 10-wk program. Diabetes resulted in an increase (P < 0.001) in plasma concentration of β-hydroxybutyric acid in sedentary rats, which was partly reversed by training (P < 0.001). Diabetes was also associated with a decreased activity of 3-ketoacid CoA-transferase in gastrocnemius muscle. When expressed per total gastrocnemius, training increased the activity of 3-ketoacid CoA-transferase by 66% in nondiabetic rats (P < 0.001) and by 150% in diabetic rats (P < 0.001), the decrease present in diabetic rats being fully reversed by training. Simple linear regression between the log of 3-ketoacid CoA-transferase activity and the log of plasma β-hydroxybutyric acid levels showed a statistically significant (r = 0.563, P < 0.001) negative correlation. The beneficial effects of training on plasma ketone bodies in diabetic rats are probably explained, at least in part, by an increase in ketone body utilization, mediated by an increase in skeletal muscle 3-ketoacid CoA-transferase activity.

β-hydroxybutyric acid; gastrocnemius; streptozotocin diabetes; free fatty acids

Physical training has long been known to play a crucial role in the management of subjects with diabetes mellitus (1, 16). Physical conditioning is also known to be beneficial in animal models with diabetes mellitus. Previous studies have shown that endurance exercise training improves glucose homeostasis in rats with mild experimental diabetes mellitus (28, 31, 32). Studies from our laboratory (22) have shown that the increased death rate of chronically diabetic rats submitted to acute experimental myocardial infarction can be reversed by a previous program of physical training even in the absence of an improvement in plasma glucose or insulin levels (10, 21). Further studies have demonstrated that physical training reversed the defect in mitochondrial energy production in the heart of chronically diabetic rats (19).

Recent studies from our laboratory (11) have suggested that physical training can attenuate the increase in plasma concentration of β-hydroxybutyric acid in streptozotocin (STZ)-diabetic rats without any improvement in plasma glucose or insulin levels. The diminished circulating levels of ketone bodies may be due to a decrease in their production rate by the liver, an increase in their utilization by peripheral tissues, in particular the skeletal muscle, or both. It is noteworthy that ketoacidosis remains dangerous in patients with deficiencies in ketone body utilization enzymes (18). Ketone bodies are oxidized in the mitochondria by a three-step enzymatic pathway, which involves β-hydroxybutyrate dehydrogenase, succinyl-CoA:3-ketoacid CoA-transferase (also known as 3-ketoacid CoA-transferase), and acetoacetyl-CoA thiolase (17). The 3-ketoacid CoA-transferase is known to be the rate-limiting step in the utilization of ketone bodies by peripheral tissues (16). During periods of limited availability of carbohydrates or when carbohydrates cannot be used effectively, ketolysis enables fat-derived energy to be used by such organs as the heart, kidney, skeletal muscle, and brain. Studies have demonstrated an impaired utilization of ketone bodies in diabetes (15, 25) and decreased 3-ketoacid CoA-transferase activity (13, 14).

To examine whether the improvement in plasma β-hydroxybutyric acid levels observed in trained diabetic rats depends on an increased capacity to utilize ketone bodies, we measured the activity of 3-ketoacid CoA-transferase in the gastrocnemius muscle. We focused our studies on skeletal muscle, because this tissue is the major ketone body-consuming tissue (6) and because the uptake and oxidation of ketone bodies are increased by physical training in skeletal muscle of nondiabetic animals (24, 36). The gastrocnemius muscle was selected because it is mainly composed of fast glycolytic and fast oxidative glycolytic fibers, which show good adaptive response to physical training (4).

Materials and Methods

Animals and training. Male Wistar rats with an average initial body weight of 223 ± 1 g were used for this study. All experiments conformed to guidelines of the Canadian Council of Animal Care and were approved by the Animal Care Committee of Laval University. Diabetes was induced after a 4-h fast by the intravenous injection of 50 mg/kg STZ (kindly provided by Upjohn Laboratories, Kalamazoo, MI) freshly dissolved in an acidified citrate buffer. Control rats were injected with citrate buffer only. One week later, after a 4-h fast, the glucose concentration in the tail blood of the STZ-injected animals was assessed with a One-Touch I meter (LifeScan, Burnaby, BC, Canada), and only the animals with a value between 14 and 22 mM were retained in the protocol. Control and diabetic animals were then randomly distributed into sedentary and exercise-trained groups. Physical training was conducted on a rodent treadmill (Quinton Instruments, model 42–15, Seattle, WA) set at 8° over a 10-wk period, as previously reported (32). In brief, the animals were exercised in the
morning and afternoon, 4 h apart, 5 days/wk, in a program made progressively more vigorous: each exercise bout consisted of 10 min of running at 22 m/min during the first 3 wk, 40 min at 28 m/min during the next 3 wk, and finally 60 min at 31 m/min during the last 4 wk. Rats were individually housed at 23°C under standard lighting (lights on 0600–2000) and weighed once a week.

Procedures. Eleven weeks after induction of diabetes, in the morning, 64 h after the last exercise bout, the animals were transferred into a quiet room, and after 1 h of rest the rats were killed by decapitation. Blood was rapidly taken and immediately transferred into a chilled tube containing 1.25 mg/ml EDTA. The plasma was separated from blood cells and kept frozen at −20°C for later measurement of glucose (29), β-hydroxybutyric acid (35), free fatty acids (NEFA C test; Wako Chemicals, Neuss, Germany), and insulin (9). The right gastrocnemius muscle was removed, weighed, and kept at −80°C for later measurement of the 3-ketoacid CoA-transferase activity. The gastrocnemius muscle 3-ketoacid CoA-transferase activity was determined spectrophotometrically according to the method of Stern et al. (30), as modified by Williamson et al. (34). All further procedures were carried out at 2−4°C. The thawed gastrocnemius muscle was placed in cold extraction buffer [Tris-(hydroxymethyl)-aminomethane-sucrose] and finely minced with scissors. The gastrocnemius muscle was homogenized in a Potter-Elvehjem with a motor-driven teflon pestle for 30 s. The homogenate, cooled in ice water, was immediately exposed to ultrasonic vibrations for exactly 45 s (3 x 15 s interspaced by 15 s) at 60 kHz. The ultrasonically treated homogenate was then centrifuged for 20 min at 30 000 g and the supernatant decanted and saved. The supernatant was used for enzymatic activity determination. The activity of 3-ketoacid CoA-transferase was measured with a Beckman Spectrophotometer DU-8/UV. The assay is based on the determination of the rate of acetooacetyl-CoA formation from succinyl-CoA and acetooacetate. The homogenate sample (0.1 ml) was added, and the rate of increase in extinction at 313 nm, temperature 25°C, light path 1 cm, was measured for 2 min.

Statistical analysis. The experimental data for each group of rats are presented as means ± SE. The effect of diabetes and training was tested with a two-way analysis of variance. The statistical significances of the differences between groups were further established by the Bonferroni-Dunn multiple comparison test when there was a significant F-ratio. Because of heterogeneity of variances, comparison between groups for the β-hydroxybutyric acid levels was done with the Kruskal-Wallis test, with post hoc comparisons between groups performed with the Mann-Whitney U-test. Association between the log 3-ketoacid CoA-transferase activity and the log of β-hydroxybutyric acid level at was assessed with the Pearson correlation coefficient. Significance was set at P < 0.05, and values are interpreted with Bonferroni’s correction when appropriate, with four pairwise comparisons considered of interest: sedentary control rats vs. the three other groups and trained diabetic rats vs. sedentary diabetic rats.

Table 1. Body weight, plasma characteristics, and gastrocnemius muscle weight

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sedentary Control (n = 15)</th>
<th>Trained Control (n = 20)</th>
<th>Sedentary Diabetic (n = 15)</th>
<th>Trained Diabetic (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt, g</td>
<td>227±4</td>
<td>224±2</td>
<td>222±1</td>
<td>219±1</td>
</tr>
<tr>
<td>Final body wt, g</td>
<td>524±9</td>
<td>416±11*</td>
<td>348±8*</td>
<td>333±8*</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>8.0±0.2</td>
<td>7.3±0.1</td>
<td>33.3±1.0*</td>
<td>32.1±0.8*</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>609±49</td>
<td>419±42*</td>
<td>77±6*</td>
<td>93±8*</td>
</tr>
<tr>
<td>Free fatty acid, µM</td>
<td>578±39</td>
<td>495±50</td>
<td>1018±73*</td>
<td>620±37§</td>
</tr>
<tr>
<td>Gastrocnemius wt, g</td>
<td>2.64±0.06</td>
<td>2.31±0.05*</td>
<td>1.51±0.04*</td>
<td>1.56±0.05*</td>
</tr>
<tr>
<td>Muscle wt/body wt, mg/g</td>
<td>5.07±0.10</td>
<td>5.45±0.08‡</td>
<td>4.35±0.09*</td>
<td>4.61±0.11‡</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.001 vs. sedentary control rats; †P < 0.05 vs. sedentary control rats; ‡P < 0.01 vs. sedentary control rats; §P < 0.001 vs. sedentary diabetic rats.

RESULTS

Body weight, plasma characteristics, and gastrocnemius muscle weight. Table 1 presents some basic characteristics for the four groups of rats. As expected, the final body weight was less in diabetic than in nondiabetic sedentary animals (P < 0.001), even if the initial body weight was similar in the four groups of rats. Trained nondiabetic rats had smaller final body weight than their sedentary counterparts (P < 0.001), but physical training did not alter the final body weight of diabetic animals. The plasma glucose values were much greater (P < 0.001) in diabetic than in nondiabetic sedentary rats. This variable was not affected by training in both diabetic and nondiabetic rats. The plasma insulin concentration was lower (P < 0.001) in sedentary diabetic than in sedentary control rats. Physical training had no effect on insulin levels in diabetic rats, whereas it significantly decreased (P < 0.001) plasma insulin concentration in nondiabetic rats. The plasma free fatty acid levels were significantly higher (P < 0.001) in sedentary diabetic rats than in sedentary control rats. The plasma free fatty acid levels were not significantly modified by training in nondiabetic animals, whereas the increase present in sedentary diabetic rats was reversed by training (P < 0.001 vs. sedentary diabetic rats, P = 0.56 vs. sedentary control rats). The gastrocnemius muscle weight was significantly less (P < 0.001) in sedentary diabetic rats than in sedentary control rats. The physical training also significantly decreased (P < 0.001) the weight of this muscle in nondiabetic animals. In diabetic rats, the gastrocnemius muscle weight was not altered by training. The ratio of muscle weight over body weight showed a slight increase with training in both nondiabetic and diabetic animals, although this did not reach statistical significance for diabetic rats in the Bonferroni-Dunn analysis.

Plasma β-hydroxybutyric acid concentration. The effects of diabetes and training on plasma β-hydroxybutyric acid levels are shown in Fig. 1. The plasma β-hydroxybutyric acid levels were significantly (P < 0.001) higher in diabetic than in control sedentary rats. The plasma β-hydroxybutyric acid levels were not modified by training in nondiabetic rats, but the great increase present in sedentary diabetic rats was partly reversed by training (P < 0.001 vs. sedentary diabetic rats, P < 0.001 vs. sedentary control rats).

3-Ketoacid CoA-transferase activity. The effects of diabetes and training on the activity of 3-ketoacid CoA-transferase in gastrocnemius muscle are shown in Figs. 2 and 3. When expressed per gram of gastrocnemius muscle (Fig. 2), the activity of 3-ketoacid CoA-transferase transferease was de-
creased by 32% in sedentary diabetic rats compared with their sedentary counterparts ($P < 0.05$). Physical training increased the activity of 3-ketoacid CoA-transferase by 88% in nondiabetic rats ($P < 0.001$) and by 148% in diabetic rats ($P < 0.001$), the activity present in trained diabetic rats being even higher ($P < 0.001$) than in sedentary control rats. When expressed per total gastrocnemius muscle (Fig. 3), the activity of 3-ketoacid CoA-transferase was decreased by 61% in sedentary diabetic rats compared with their sedentary counterparts ($P < 0.001$). Physical training increased the activity of 3-ketoacid CoA-transferase by 66% in nondiabetic rats ($P < 0.001$) and by 150% in diabetic rats ($P < 0.001$), the activity present in trained diabetic rats being not statistically different from that in sedentary control rats.

Relation between activity of 3-ketoacid CoA-transferase and β-hydroxybutyric acid. To evaluate the relation between the activity of 3-ketoacid CoA-transferase and β-hydroxybutyric acid levels in four groups of rats, simple linear regression between these parameters was calculated. As shown in Fig. 4, there was a statistically significant ($P < 0.0001$) negative correlation between the log of 3-ketoacid CoA-transferase activity and the log of plasma β-hydroxybutyric acid levels.

DISCUSSION

In this study, insulin deficiency induced by STZ was associated with a significant increase in plasma β-hydroxybutyric acid and free fatty acid levels in sedentary rats. It is well known that, under condition of carbohydrate deprivation or insulin lack, there is an increase in plasma free fatty acid and ketone body concentrations resulting from increased lipolysis to compensate the fall in available carbohydrates (3). The concentration of ketone bodies in the blood represents the balance between their production and utilization rates by tissues. In-creased ketone body levels thus result from either their increased synthesis or their impaired removal. Ketone bodies are catabolized in the mitochondria by a three-step enzymatic pathway that involves β-hydroxybutyrate dehydrogenase, which catalyzes the conversion of β-hydroxybutyrate to acetoacetate, succinyl-CoA:3-ketoacid CoA-transferase, which cata-

![Fig. 1. Effects of diabetes or physical training or both on plasma β-hydroxybutyric acid levels. Data are means ± SE. No. of rats in each group is shown in each column. *$P < 0.001$ vs. sedentary control rats; †$P < 0.001$ vs. sedentary diabetic rats.](image1)

![Fig. 2. Effects of diabetes or physical training or both on 3-ketoacid CoA-transferase activity expressed per gram of muscle. Data are means ± SE. No. of rats in each groups is shown in each column. *$P < 0.05$, †$P < 0.001$ vs. sedentary control rats; §$P < 0.001$ vs. sedentary diabetic rats.](image2)

![Fig. 3. Effects of diabetes or physical training or both on 3-ketoacid CoA-transferase activity expressed per total muscle. Data are means ± SE. No. of rats in each group is shown in each column. *$P < 0.001$ vs. sedentary control rats; †$P < 0.001$ vs. sedentary diabetic rats.](image3)

![Fig. 4. Relation between the log of 3-ketoacid CoA-transferase activity expressed per gram of muscle and the log of β-hydroxybutyric acid levels in all groups of rats. ○, Sedentary control rats values; ●, trained control rats values; △, sedentary diabetic rats values; ■, trained diabetic rats values.](image4)
lyzes the transformation of acetoacate to acetoacetyl-CoA, and acetoacetyl-CoA thiolase, which catalyzes the conversion of acetoacetyl-CoA into two molecules of acetyl-CoA (34).

In the present study, the impact of long-term untreated experimental diabetes mellitus on the activity of 3-ketoacid CoA-transferase, the key enzyme in the peripheral utilization of ketone bodies (23), was evaluated at the level of the gastrocnemius muscle. Using in vivo intravenous infusion of ketone bodies, Bässler et al. (5) observed that the blood levels of ketone bodies rise exponentially with the infusion rate in diabetic rats comparatively with the linear rise observed in normal rats, suggesting that the same rate of ketone body production by the liver would lead to much higher blood levels of ketone bodies in diabetic than in normal rats. Beatty et al. (7) previously showed that the uptake of acetoacetate by muscle preparations in vitro was lower in diabetic than in normal rat. Furthermore, Bässler et al. (6) showed that the 3-ketoacid CoA-transferase activity was decreased by 40% (units/g) in skeletal muscle of diabetic rats. Overall, these observations suggest that the increase in plasma ketone body levels observed in diabetic animals is explained, at least in part, by a decrease in their removal, presumably due to a decrease in the activity of 3-ketoacid CoA-transferase in the muscle.

Winder et al. (36) previously demonstrated that homogenates of gastrocnemius muscles from endurance-trained rats oxidized 3-β-hydroxybutyrate two to three times as rapidly as homogenates from sedentary rats. Also, Beattie and Winder (8) showed that trained rats exhibited lower plasma β-hydroxybutyric acid levels during and after a bout of exercise than their sedentary counterparts. Ohmori et al. (24) showed that trained rats had lower circulating levels of β-hydroxybutyric acid during exercise than sedentary rats. Ohmori et al. also demonstrated that ketone body uptake by perfused hindlimb was greater in trained than in sedentary rats. Studies conducted in skeletal muscle of nondiabetic animals have demonstrated that 3-β-hydroxybutyrate dehydrogenase, 3-ketoacid CoA-transferase, and acetoacetyl-CoA thiolase activities were increased by training (37). However, no study has yet examined the effect of training in ketone body metabolism in diabetic rats.

The data obtained in trained diabetic rats are in agreement with recent studies, also carried out in our laboratory (11), which showed that physical training attenuated the increase in plasma levels of hydroxybutyric acid observed in STZ-diabetic rats. This lowering effect of physical training on plasma ketone body level could theoretically be explained by a decrease in their production rate by the liver, an increase in their utilization rate by peripheral tissues, or both. When expressed on a per gram basis, training increased the activity of 3-ketoacid CoA-transferase in the gastrocnemius muscle by 88% in nondiabetic rats (P < 0.001) and by 148% in diabetic rats (P < 0.001). Moreover, the activity was 68% higher in trained diabetic rats than in sedentary control rats, and this supports the hypothesis that the lower levels of β-hydroxybutyric acid in trained diabetic rats is partly explained by their increased utilization in skeletal muscle. However, when interpreting these data, one should take into consideration the fact that the muscle mass was modified by both training and diabetes mellitus. It is well known that training causes a reduction in body weight in nondiabetic male rats, a phenomenon which has been attributed to both an increase in caloric expenditure and the suppression of appetite (26). A loss in muscle mass is also present but not proportional, as shown by an increase in the ratio of muscle weight to body weight. On the other hand, the loss of body weight caused by diabetes is much greater, and this is associated with a decrease in the ratio of muscle weight to body weight. Although the body weight and the gastrocnemius muscle weight were similar between trained and sedentary diabetic rats, it appears of interest to express the 3-ketoacid CoA-transferase activity on a per muscle basis. It was found that physical training reversed the decrease in the activity of 3-ketoacid CoA-transferase observed in diabetic rats. Furthermore, a highly negative correlation between plasma β-hydroxybutyric acid levels and the 3-ketoacid CoA-transferase activity was observed in all groups of rats. These observations suggest that the reduction in plasma β-hydroxybutyric acid levels observed in trained diabetic rats is probably explained, in part, by the reversal of the decrease in 3-ketoacid CoA-transferase activity present in sedentary diabetic rats. However, because complete reversal of the decreased activity of the 3-ketoacid CoA-transferase was not associated with normalization of the β-hydroxybutyric acid levels, other mechanisms must also be involved to explain the increased level of β-hydroxybutyric acid levels found in trained diabetic rats. For instance, it is well known that, in sedentary diabetic rats, up to 80–90% of free fatty acids taken up by the liver are converted to ketone bodies (27, 33). The lower levels of free fatty acids in trained diabetic rats observed in the present study could thus also partly explain their lower levels of β-hydroxybutyric acid.

On the other hand, studies performed in rats (2) have demonstrated that the ketone body production is elevated in isolated hepatocytes from nonobese diabetic animals. More importantly, we have demonstrated that physical training reverses the increased activity of the hepatic ketone body synthesis pathway in chronically diabetic rats (12).

In conclusion, the results of the present study confirm our previous observation that physical training attenuated the severity of the hyperketonemia that developed in rats rendered insulin deficient by an injection of STZ. These beneficial effects of physical training are explained, at least in part, by an increase in ketone body utilization, mediated by an increase in skeletal muscle 3-ketoacid CoA-transferase activity.

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GRANTS

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