Physical training reverses the increased activity of the hepatic ketone body synthesis pathway in chronically diabetic rats

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Physical training reverses the increased activity of the hepatic ketone body synthesis pathway in chronically diabetic rats. Am J Physiol Endocrinol Metab 290: E207–E212, 2006; doi:10.1152/ajpendo.00608.2004.—This study was designed to examine whether the training-induced improvement in the plasma concentration of ketone bodies in experimental diabetes mellitus could be explained by changes in the activity of the hepatic ketone body synthesis pathway and/or the plasma free fatty acid levels. Diabetes mellitus was induced by an intravenous injection of streptozotocin (50 mg/kg), and training was carried out on a treadmill. The plasma concentration of β-hydroxybutyric acid was increased (P < 0.001) in sedentary diabetic rats, and this was partly reversed by training (P < 0.001). The plasma concentration of free fatty acids was increased (P < 0.001) in sedentary diabetic rats, and this was reversed to normal by training (P < 0.001). Diabetes was also associated with an increased activity of the hepatic ketone body synthesis pathway. When the data are expressed as per total liver, physical training decreased the activity of the hepatic ketone body synthesis pathway by 18% in nondiabetic rats (P < 0.05) and by 22% in diabetic rats (P < 0.01), the activity present in trained diabetic rats being not statistically different from that of sedentary control rats. These data suggest that the beneficial effects of physical training on the plasma β-hydroxybutyric acid levels in the diabetic state are probably explained in part by a decrease in the activity of the hepatic ketone body synthesis pathway and in part by a decrease in plasma free fatty acid levels.

β-hydroxybutyric acid; streptozotocin; diabetes

HUMAN STUDIES INDICATE that physical training is beneficial to diabetic subjects (8, 22). Physical conditioning is also known to be beneficial in animal models with diabetes mellitus. For instance, exercise training improves glucose homeostasis in rats with mild streptozotocin (STZ)-induced diabetes mellitus (19, 24, 25). However, in the case of a more severe diabetic state, physical training does not improve glucose homeostasis (5, 14, 27), although it is effective in lowering free fatty acid (FFA) and serum triglyceride concentrations (5, 17). This training-induced reduction in plasma FFA and triglyceride concentrations could be a possible explanation for the decreased plasma levels of ketone bodies that we observed in trained diabetic rats in the absence of any improvement in plasma glucose or insulin levels (4).

However, a decrease in the circulating levels of ketone bodies could also be due to a decrease in the capacity of the liver to synthesize them from circulating FFA. Ketone bodies are synthesized in liver mitochondria by a four-step enzymatic pathway that involves acetoacetyl-CoA thiolase, 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, hydroxymethylglutaryl-CoA lyase, and 3-hydroxybutyrate dehydrogenase (13). Although all four enzymes have been found in extrapatic tissues, such as heart, kidney, and intestine, the enzyme generally considered rate limiting for the sequence, hydroxymethylglutaryl-CoA synthase, is present in large quantities only in liver, which makes this tissue the primary site of ketogenesis (12). Ketoadidosis in humans remains associated with excess morbidity and mortality (2). Investigations from our laboratory have recently shown that type 1 diabetes was associated with a decreased activity of skeletal muscle 3-keto-acid-CoA transferase (3), a rate-limiting step in the utilization of ketone bodies by peripheral tissues (9). Animal studies have demonstrated that the HMG-CoA synthase activity is increased in the livers of alloxan diabetic rats (30). In addition, investigators have demonstrated that STZ-induced diabetes increased the expression of the mitochondrial HMG-CoA synthase gene, the key regulatory enzyme in the ketone bodies production pathway (26). Therefore, the aim of the present study was twofold: 1) to evaluate the effect of training on plasma FFA and its relation to ketogenesis in diabetic and nondiabetic rats; and 2) to examine in the same animals whether training could decrease the production of β-hydroxybutyric acid by decreasing the capacity of the liver to generate ketone bodies. To our knowledge, no such studies have yet been conducted in either normal or diabetic animals.

MATERIALS AND METHODS

Male Wistar rats with an average initial body weight of 218 ± 2 g were used for these experiments. All experiments conformed to the guidelines of the Canadian Council of Animal Care and were approved by the Animal Care Committee at Laval University. Rats were individually housed at 23°C under standard lighting (0600–2000) and had free access to Purina rat chow and tap water ad libitum. Body weight was measured once per week. Experimental diabetes was induced with the intravenous injection of STZ (50 mg/kg, kindly provided by Upjohn Laboratories, Kalamazoo, MI) freshly dissolved in citrate buffer in 4-h-fasted rats. Control rats received an equivalent amount of citrate buffer. One week later, after a 4-h fast, the glucose concentration in the tail blood of the STZ-injected animals was evaluated with a One-Touch I glucose meter (LifeScan, Burnaby, BC, Canada), and selection was made to retain in the protocol only the animals with a value between 14 and 22 mM. The reason for using STZ at a dose of 50 mg/kg and selecting the animals within a specific...
range of plasma glucose was to be able to induce a severe diabetic state without necessitating insulin injections to maintain the animals alive. Control and diabetic rats were then randomly assigned to a sedentary or an exercise training program. Exercise training began 10 days after STZ injection and was achieved by having the rats run on a motor-driven treadmill (model 42-15; Quinton Instruments, Seattle, WA) set at 8° incline, according to a program previously described (25). In brief, the rats were trained twice/day, 4 h apart, 5 days/wk; they initially ran for 10 min at 22 m/min for 3 wk, then for 40 min at 28 m/min during the next 3 wk, and, finally, for 60 min at 31 m/min for the remaining 4 wk.

Eleven weeks after the induction of diabetes, 64 h after the last bout of exercise, in the morning, the animals were conducted into a quiet room and killed by decapitation after a 4-h fast. Blood was taken and immediately transferred into a chilled tube containing 1.25 mg/ml EDTA. The plasma was rapidly separated from blood cells by centrifugation and kept frozen at −20°C for later measurement of glucose (20), β-hydroxybutyric acid (29), FFA (NEFA C test; Wako Chemicals, Neuss, Germany), and insulin (1). The liver was removed and cut into three pieces; each piece was weighed and submersed in liquid N2 for 2 min and then kept frozen at −80°C.

Assay of ketone body synthesis pathway. Ketone bodies are synthesized in liver mitochondria by the four-step enzymatic pathway shown in Fig. 1. From the enzymatic reactions in this pathway, it can be seen that for each molecule of β-hydroxybutyrate generated from acetocetate, one molecule of NADH (nicotinamide adenine dinucleotide, reduced form) is transformed into NAD (nicotinamide adenine dinucleotide). The activity of the hepatic ketone body synthesis pathway was assessed by measuring the change in the optical density of NADH after the addition of acetyl-CoA to a medium containing the experimental groups of rats were studied through a covariance analysis (31).

RESULTS

As shown in Table 1, even though the initial body weight was similar in the four groups of rats, the final body weight was lower in diabetic than in nondiabetic sedentary animals (P < 0.001). Physical training also had a lowering effect on weight gain in nondiabetic rats (P < 0.001), but it did not statistically alter the final weight of the diabetic animals. The plasma

![Fig. 1. Hepatic ketone body synthesis pathway.](http://ajpendo.physiology.org/)}
The liver weight was significantly less (statistically different from those in the sedentary control rats. Diabetic rats such that the levels observed in these rats were not butyric acid levels were significantly (P < 0.001) in sedentary diabetic than in sedentary control rats. Physical training did not modify plasma FFA concentration of nondiabetic animals, but it significantly decreased (P < 0.001) plasma FFA concentration in diabetic rats such that the levels observed in these rats were not statistically different from those in the sedentary control rats. The liver weight was significantly less (P < 0.05) in sedentary diabetic rats than in sedentary control rats. Physical training also decreased significantly the liver weight in both control (P < 0.001) and diabetic animals (P < 0.001).

The effects of diabetes and training on plasma β-hydroxybutyric acid levels are shown in Fig. 2. 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. The marked increase observed in sedentary diabetic rats was partly reversed by training (P < 0.001 vs. both sedentary control and sedentary diabetic rats).

The effects of diabetes and training on the activity of the hepatic ketone body synthesis pathway are shown in Figs. 3 and 4. When expressed per gram of liver, the activity of the hepatic ketone body synthesis pathway was not affected by training, but it was significantly higher (30%, P < 0.01) in sedentary diabetic rats than in their sedentary counterparts (Fig. 3). Because the liver weight was significantly affected by training and diabetes, it appears pertinent to calculate the total capacity of liver to generate ketone bodies. When expressed as per total liver, the activity of hepatic ketone body synthesis pathway was significantly higher (18%, P < 0.05) in sedentary diabetic rats than in sedentary control rats (Fig. 4). Physical training decreased the activity of the hepatic ketone body synthesis pathway by 18% in nondiabetic rats (P < 0.05) and by 22% in diabetic rats (P < 0.01) such that the activity in trained diabetic rats was similar to that of sedentary control rats (Fig. 4).

The relationships between FFA and β-hydroxybutyryc acid levels, as well as between the activity of the hepatic ketone body synthesis pathway and the β-hydroxybutyric acid levels in each group of rats, are shown in Figs. 5 and 6, respectively. The linear regression of these relationships was significantly (P < 0.001) higher in sedentary diabetic rats than in sedentary control rats. There were no significant differences in the linear regressions between trained and sedentary control rats, whereas the linear regressions were significantly (P < 0.001) lower in trained diabetic rats compared with sedentary diabetic rats.

**DISCUSSION**

This study shows that chronic insulin deficiency induced by STZ is associated with a significant increase in plasma FFA and β-hydroxybutyric acid levels in sedentary rats. It also shows that exercise training will normalize plasma FFA levels.
and markedly reduce β-hydroxybutyric acid levels in these diabetic rats. Furthermore, exercise training also decreases hepatic ketone body synthesis measured as the activity of the HMG-CoA, a rate-limiting enzyme in hepatic ketogenesis (10).

It has been demonstrated by hepatic catheterization in severe uncontrolled diabetes that up to 80–90% of the FFA taken up by the liver is converted to ketone bodies (16, 28). STZ-induced diabetes in the rat was associated with an 80% increase in FFA levels and a 700% increase in β-hydroxybutyrate. This major difference between the rises in FFA and β-hydroxybutyrate is most likely explained by the more efficient liver in synthesizing ketone bodies from FFA (Fig. 4).

The sedentary diabetic rats had a significant increase in HMG-CoA synthase activity compared with the sedentary control group, whether it is expressed per gram of liver (30%) or per total liver (18%). It has to be pointed out that the methodology used to extract mitochondrial HMG-CoA synthase is contaminated with cytosolic HMG-CoA synthase. However, the mitochondrial and cytosolic HMG-CoA synthases are regulated differently. The cytosolic HMG-CoA synthase is repressed by fasting and cholesterol feeding (21). In contrast, the mitochondrial HMG-CoA synthase is increased by fasting (7). Moreover, Mg²⁺ has opposite effects on these enzymes because it inhibits the mitochondrial HMG-CoA synthase and activates the cytosolic HMG-CoA synthase (7). Therefore, it is very likely that the differences we observed in HMG-CoA synthase activity are those from the mitochondria and thus the ketogenesis pathway.

Similar observations have been made by Quant et al. (18), who reported that the activity of HMG-CoA synthase from rat liver extracts doubled in alloxan-induced diabetes. Williamson et al. (30) have also demonstrated that the HMG-CoA synthase activity was increased by 50% in whole homogenate of liver from alloxan-diabetic rats. The same authors have reported that HMG-CoA lyase and acetoacetyl-CoA thiolase were increased by 35 and 4%, respectively, in whole homogenate of livers from alloxan-diabetic rats. The present study evaluated the impact of long-term untreated experimental diabetes mellitus on the overall activity of the hepatic ketone body synthesis pathway, as measured by HMG-CoA synthase in relation to the production of β-hydroxybutyric acid from acetyl-CoA. In the present study, the reduced body weight gain was associated with a smaller liver weight in sedentary diabetic and trained nondiabetic rats. Oscai and Holloszy (15) have also observed in trained animals a reduced body weight gain that was attributed to both the increased caloric expenditure and the suppression of appetite. Under such conditions, it appeared important to express the results on both a per-gram and a per-liver basis. Compared with the sedentary control group, sedentary rats with diabetes mellitus of 11-wk duration exhibited a statistically significant 30 and 18% increase in the activity of the hepatic ketone body synthesis pathway when expressed per gram of liver or per total liver, respectively. Furthermore, the linear regression of the relationship between the activity of HMG-CoA synthase and the β-hydroxybutyric acid levels was significantly higher (P < 0.001) in sedentary diabetic than in sedentary control rats. These observations strongly suggest that the increase in plasma ketone body levels present in sedentary diabetic rats could be partly explained by the increased activity of the hepatic ketone body synthesis pathway.

The data obtained in trained diabetic rats confirm our recent observations that physical training can attenuate the increase in plasma levels of β-hydroxybutyric acid in diabetic rats (4). This happens without any evidence of an improvement in glucose and insulin levels because they were similarly altered in both sedentary and trained diabetic rats. The lowering effect of physical training on plasma ketone body levels could be explained by either a decrease in their production by the liver or an increase in their utilization by peripheral tissues. We have recently reported (3) that the beneficial effects of training on plasma ketone bodies in diabetic rats are explained, at least in part, by an increase in ketone body utilization and mediated by an increase in skeletal muscle 3-ketoacid CoA-transferase activity. In the present study, physical training decreased the activity of the total liver ketone body synthesis pathway by 18% in nondiabetic rats (P < 0.05) and by 22% in diabetic rats (P < 0.05). The decrease in the activity of HMG-CoA synthase could be due to a decrease in the enzyme per se, a decrease in the concentration of the enzyme, or both. Furthermore, STZ-induced diabetes in the rat has been shown to be associated with an increase in the activity of hepatic carni-

Fig. 6. Relationship between the activity of hepatic ketone body synthesis pathway activity and the log of β-hydroxybutyric acid levels in each group of rats.
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tine palmitoyltransferase (CPT I), which is a key enzyme in the regulation of FFA transport into the mitochondria for oxidation (6). It is also associated with an increase in the concentration required to produce 50% inhibition of the enzyme for its inhibitor malonyl-CoA (6). The decreased fatty acid levels observed in trained diabetic rats would be expected to produce lower rates of ketone body production and decrease ketone body levels. It is therefore suggested that the decrease in β-hydroxybutyrate is explained in part by the decrease in plasma FFA levels and in part by the decrease in HMG-CoA synthase activity (Fig. 4). This would also be compatible with observations that physical training is associated with an increase in the sensitivity of CPT I to malonyl-CoA (23), which would be expected to decrease the oxidation of FFA and the production of ketone bodies. In the present study, physical training resulted in a 65% reduction in β-hydroxybutyrate but only a 35% in FFA concentrations (Table 1 and Fig. 2), which suggests that the decrease in ketone body synthesis pathway activity not only is due to the lower FFA levels but could also be due to some other regulatory factors, such as the HMG-CoA synthase expression. Moreover, we (3) have already shown that physical training increases the metabolism of ketone bodies, resulting in an overall lower concentration. Above and beyond the reduction of the substrate for ketogenesis (FFA), physical training is associated with a reduction in HGM-CoA synthase, resulting in further reduction in ketone body production. Therefore, the lower ketone body concentration in trained diabetic rats is due to a combination of those three mechanisms. Finally, the linear regression of the relationship between FFA levels and β-hydroxybutyrate was significantly higher ($P < 0.001$) in sedentary diabetic rats than in sedentary control rats. More importantly, this linear regression was significantly ($P < 0.001$) lower in trained diabetic rats compared with sedentary diabetic rats, which suggests that the decrease in ketone body production was in part due to the decrease in FFA in response to training. Furthermore, the linear regression of the relationship between the activity of the hepatic ketone body synthesis pathway, as measured by the enzyme HGM-CoA synthase, and the β-hydroxybutyric acid levels was significantly ($P < 0.001$) lower in trained diabetic rats compared with sedentary diabetic rats. Therefore, the decreased activity of the hepatic ketone body synthesis pathway might partly explain the lower plasma β-hydroxybutyric acid levels observed with exercise training in diabetic rats.

In conclusion, the results of the present study indicate that physical training attenuated the severity of the hyperketonemia that developed in rats rendered chronically insulin deficient by an injection of STZ. These beneficial effects of physical training are probably explained in part by a decrease in the activity of the hepatic ketone body synthesis pathway and in part by the lower plasma FFA levels. How such changes can be translated into the clinical setting remains to be established.

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