Effect of Physical Training on Mitochondrial Function in Skeletal Muscle of Normal and Diabetic Rats

Adil el Midaoui, Gilles Tancrède, and André Nadeau

The study was designed to assess the impact of physical training on the oxidative phosphorylation rate (OPR) in mitochondria isolated from two different skeletal muscles of rats with or without chronic diabetes mellitus. Diabetes was induced by an intravenous injection of streptozotocin (50 mg/kg), and only animals with a blood glucose level between 14 and 22 mmol/L 1 week later were kept in the protocol. Exercise training was performed on a treadmill with a progressive 10-week program. Rats were killed by decapitation at the end of the training program, and mitochondria were isolated from the gastrocnemius and the red vastus lateralis muscles. When the data were expressed as per milligrams of protein, OPR was significantly depressed by diabetes mellitus in the mitochondria from each muscle; a similar negative impact also appeared to be produced by physical training in mitochondria isolated from the red vastus lateralis muscle. However, due to changes in mitochondrial protein yield between groups, the capacity to oxidize pyruvate and malate was also calculated per gram of muscle. Adenosine triphosphate (ATP) production rate appeared to be unaffected by diabetes but significantly increased by training in both muscles of diabetic and nondiabetic rats. This effect of training was not associated with any improvement in plasma glucose or insulin levels in diabetic animals. However, the large increase in plasma levels of β-hydroxybutyric acid in sedentary diabetic rats was partly reversed by training (1,079 ± 472 *v* 3,424 ± 618 μmol/L, $P < .001$). These results suggest that the training-induced increase in the capacity of skeletal muscles to oxidize substrates and generate energy may also contribute to reduce the elevated plasma β-hydroxybutyric acid levels observed in a state of insulin deficiency. This may have clinical relevance, since ketoacidosis remains a life-threatening event in insulin-dependent diabetic subjects.

CHRONIC UNTREATED experimental diabetes mellitus has detrimental effects on skeletal muscle mitochondria. Using mitochondria isolated from the muscles of the total hindleg of the rat, Gross et al[1] were probably the first to report that the oxidative phosphorylation rate (OPR) was significantly depressed by experimental diabetes mellitus of 19-day duration. Ultrastructural studies performed later by Chao et al[2] also showed alterations in skeletal muscle mitochondria of rats with diabetes mellitus of 70- to 80-day duration, with the nature of these changes varying depending on the fiber types examined. Depressed activity of different mitochondrial enzymes has also been observed in skeletal muscle homogenates from diabetic rats,[3,4] also with a divergence in the response between muscles of different fiber types. The primary role of mitochondria in skeletal muscle is to supply the cells with the high-energy phosphate compounds necessary for myofibrillar contraction, maintenance of ion homeostasis, protein synthesis, and other endergonic cellular functions.[5] The mitochondrial defects mentioned above certainly play a role in the altered contractile properties of skeletal muscle observed in diabetic animals.[6,7]

In contrast, exercise training is known to have beneficial effects on skeletal muscle mitochondrial function. For instance, it has been shown that the activity of many mitochondrial enzymes is increased by physical training, and this appears to lead to an enhanced capacity of skeletal muscle to generate high-energy phosphates.[8] Moreover, the training-induced adaptive changes in mitochondrial enzyme activity are known to develop in diabetic and nondiabetic rats.[9] However, the impact of training on the OPR of adenosine triphosphate (ATP) production rate in mitochondria isolated from skeletal muscle of diabetic animals has not yet been reported.

Recent studies in our laboratory have shown that physical training attenuates the decrease in phosphocreatine content in the heart of diabetic rats.[10] More importantly, physical training totally reversed the decreased capacity of heart mitochondria to produce ATP in this model.[11] This prompted us to examine if parallel changes could be observed at the skeletal muscle level. This study was thus designed to evaluate the OPR or ATP production rate in mitochondria obtained from skeletal muscle of control and diabetic rats, either trained or sedentary. Moreover, because diabetes mellitus appears to affect differently the mitochondria in skeletal muscles of different fiber types, these studies were performed in both the gastrocnemius muscle, which is rich in fast-twitch high-glycolytic (FG) fibers, and in the red portion of the vastus lateralis muscle, which is richer in fast-twitch high-oxidative and high-glycolytic (FOG) fibers than gastrocnemius muscle.[12]

MATERIALS AND METHODS

Male Wistar rats with a mean initial body weight of 223 ± 1 g were used for this study. Diabetes was induced after a 4-hour fast by intravenous injection of streptozotocin 50 mg/kg (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-hour fast, glucose concentration in the tail blood of streptozotocin-injected animals was assessed with a One-Touch I meter (LifeScan, Burnaby, British Columbia, Canada), and only animals with a value between 14 and 22 mmol/L were retained in the protocol. Control and diabetic

From the Diabetes Research Unit, Research Center of Laval University Medical Center, Ste-Foy, Quebec, Canada.

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Address reprint requests to André Nadeau, MD, Director, Diabetes Research Unit, Room S-24, Laval University Medical Center, 2705 Laurier Blvd, Ste-Foy, Quebec, Canada G1V 4G2.

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animals were then randomly distributed into sedentary and exercise-trained groups. Physical training was conducted on a rodent treadmill (model 42-15; Quinton Instruments, Seattle, WA) set at 8° over a 10-week period as previously reported.[13] In brief, animals were exercised in the morning and afternoon 4 hours apart 5 d/wk in a program made progressively more vigorous: each exercise bout consisted of 10 minutes of running at 22 m/min during the first 3 weeks, 40 minutes at 28 m/min during the next 3 weeks, and finally 60 minutes at 31 m/min during the last 4 weeks. Rats were individually housed at 23°C under standard lighting (lights on 6 AM to 8 PM), and weighed once per week.

Eleven weeks after induction of diabetes, in the morning 64 hours after the last exercise bout, the animals were transferred to a quiet room and, after 1 hour of rest, killed by decapitation. Blood was rapidly taken and immediately transferred into a chilled tube containing 1.25 mg/mL sodium EDTA. The plasma was separated from blood cells and kept frozen at −20°C for later determination of glucose,[14] β-hydroxybutyric acid,[15] and insulin.[16] The left gastrocnemius and the two deep vastus lateralis muscles were removed and placed in ice-cold isotonic KCl (154 mmol/L), and the mitochondria were isolated with the method of Makinen and Lee[17] with a few modifications.[18] All further procedures were performed at 2° to 4°C. The muscle tissue was freed of connective and fat tissues, weighed, minced finely with scissors, and rinsed thoroughly with isotonic KCl followed by several rinsings with a medium consisting of 100 mmol/L KCl, 50 mmol/L Tris hydrochloride, 1 mmol/L ATP, 5 mmol/L $MgCl_2$, and 2 mmol/L EDTA, pH 7.5. The minced tissue was suspended in 15 mL Tris-KCl medium to which protease XXIV 5 mg/g tissue was added (Sigma Chemical, St Louis, MO). The mixture was stirred frequently for 7 minutes, the optimal period of incubation for muscle tissue.[18] Ten milliliters of additional Tris-KCl medium was added to the mixture, and homogenization was performed in a glass Potter-Elvehjem homogenizer with a motor-driven Teflon pestle for 30 seconds. The homogenate was then centrifuged at 600× *g* for 10 minutes, and the supernatant was decanted and filtered through two thicknesses of cheesecloth. The pH was readjusted to 7.3 with 0.1N HCl, and the suspension was centrifuged at 14,000 × *g* for 10 minutes. The resultant supernatant was discarded, and the pellet was resuspended in a volume equal to that of the original homogenate of the following medium: 100 mmol/L KCl, 50 mmol/L Tris hydrochloride, 2 mmol/L ATP, 2 mmol/L $MgCl_2$, 1 mmol/L EDTA, and 1% bovine serum albumin (BSA), pH 7.5. The suspension was centrifuged at 7,000 × *g* for 10 minutes, followed by resuspension of the resultant pellet in half the volume of the Tris-KCl medium without BSA. This was centrifuged at 3,500 × *g* for 10 minutes. A pale fluffy layer was shaken loose, resuspended in a similar fashion (volume equal to one eighth of the original homogenate), and centrifuged again at 3,500 × *g* for 10 minutes, and the heavy mitochondrial pellet was saved. This pellet was then suspended in 0.5 mL incubation medium that consisted of 150 mmol/L sucrose, 25 mmol/L Tris hydrocholoride and 10 mmol/L KH_2PO_4, pH 7.5.

Oxygen consumption was measured polarographically with a Clark electrode[19] in an oxygraph chamber maintained at 25°C. The mitochondria were added to the respiratory incubation medium containing 8 mmol/L pyruvate and 8 mmol/L malate as substrates. Mitochondrial respiration was stimulated by adding 343 nmol adenosine diphosphate (ADP). State 3 and state 4 respiratory rates, respiratory control ratio (RCR), ratio between the nanomoles of ADP phosphorylated to ATP and the nanoatoms of oxygen consumed (ADP/O ratio), and OPR were determined as described by Estabrook.[19] Mitochondrial protein concentration was measured according to the method of Lowry et al,[20] using BSA as standard.

Statistical Analysis

The data are expressed as the mean ± SE. Significant between-group differences for each variable except β-hydroxybutyric acid levels were evaluated by a two-way ANOVA. When a significant F ratio was observed, post hoc multiple comparisons were performed with the Bonferroni/Dunn test, with four pairwise comparisons: sedentary controls versus the three other groups and trained diabetics versus sedentary diabetics. β-Hydroxybutyric acid levels were compared between groups with the Mann-Whitney *U* test. Significance was set at *P* less than .05, and *P* values are shown with Bonferroni correction when appropriate.

RESULTS

Table 1 presents basic characteristics for the four groups of rats. As expected, final body weight was lower in diabetic than in nondiabetic sedentary animals ($P < .001$) even if initial body weight was similar in the four groups. Trained nondiabetic rats had a lower final body weight than their sedentary counterparts ($P < .001$), but physical training did not alter the final body weight of diabetic animals. Plasma glucose values were much greater ($P < .001$) in diabetic than in nondiabetic sedentary rats. This parameter was not affected by training in diabetic or nondiabetic rats. Plasma insulin concentration was lower ($P < .001$) in sedentary diabetic than in sedentary control rats. Physical

Table 1. Body Weight, Plasma Characteristics, Muscle Weight, and Mitochondrial Protein Yield (mean ± SE)

Parameter	Control Rats		Diabetic Rats	
	Sedentary (n = 13)	Trained (n = 16)	Sedentary (n = 13)	Trained (n = 17)
Initial body weight (g)	227 ± 4	224 ± 2	222 ± 1	219 ± 1
Final body weight (g)	524 ± 9	416 ± 11†	348 ± 8†	333 ± 8†
Glucose (mmol/L)	8.0 ± 0.2	7.3 ± 0.2	33.4 ± 1.1†	32.1 ± 0.9†
β-Hydroxybutyric acid	373 ± 25	337 ± 26	3,424 ± 618†	1,079 ± 472†‡
Insulin (pmol/L)	606 ± 58	418 ± 50*	78 ± 8†	85 ± 7†
Gastrocnemius				
Weight (g)	2.61 ± 0.05	2.21 ± 0.06†	1.45 ± 0.04†	1.43 ± 0.05†‡
Protein yield (mg/g)	1.26 ± 0.09	1.69 ± 0.12	1.39 ± 0.10	2.24 ± 0.15†‡
Vastus lateralis				
Weight (g)	2.55 ± 0.07	2.16 ± 0.07†	1.40 ± 0.05†	1.48 ± 0.06†
Protein yield (mg/g)	1.52 ± 0.10	3.01 ± 0.19†	1.79 ± 0.08	3.97 ± 0.35†‡

*$P < .01$, †$P < .001$: *v* sedentary control rats,

‡$P < .001$ *v* sedentary diabetic rats.

training had no effect on insulin levels in diabetic rats, whereas it significantly decreased ($P < .01$) plasma insulin concentration in nondiabetic rats. Plasma β-hydroxybutyric acid levels were not modified by training in nondiabetic rats, but the great increase present in sedentary diabetic rats ($P < .001$ *v* sedentary control rats) was partially reversed by training ($P < .001$ *v* sedentary diabetic rats and $P < .001$ *v* sedentary control rats).

Gastrocnemius and deep red vastus lateralis muscle weights were also significantly lower ($P < .001$) in sedentary diabetic rats than in sedentary control rats. Physical training also significantly decreased ($P < .001$) the weight of these two muscles in nondiabetic animals. In diabetic rats, both the gastrocnemius and the deep vastus lateralis weights were not altered by training.

The mitochondrial protein yield was not modified by diabetes in the two muscles. In nondiabetic animals, training increased the protein yield by 98% in red vastus lateralis muscle ($P < .001$), whereas it increased it only by 34% ($P = .076$) in the gastrocnemius muscle. In diabetic rats, the mitochondrial protein yield was increased with training by 61% ($P < .001$) in the gastrocnemius and by 121% ($P < .001$) in the red vastus lateralis.

The effects of diabetes and training on gastrocnemius mitochondrial function are shown in Figs 1, 2, and 3. Oxygen consumption by mitochondria in the absence of ADP (state 4 respiratory rate) was not affected by diabetes or training. A similar finding was observed for the oxygen consumption stimulated by addition of ADP (state 3 respiratory rate) and for the RCR. ADP/O was slightly lower ($P < .05$) in trained diabetic than in control sedentary rats. When expressed as per milligram of mitochondrial protein, which allows evaluation of the intrinsic quality of mitochondria to yield energy on an individual basis, the OPR or ATP production rate was slightly lower ($P < .05$)

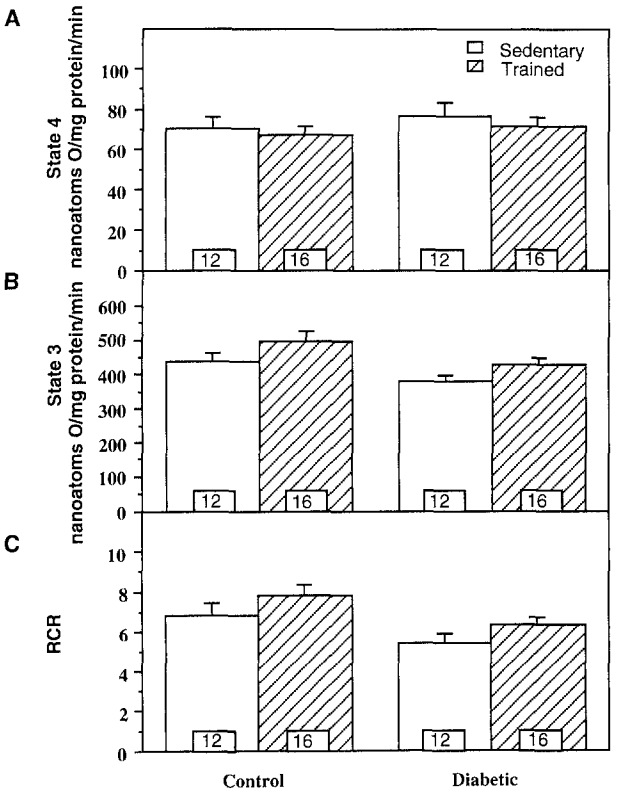

Fig 1. Effects of diabetes, physical training, or both on state 4 (A) and state 3 (B) respiratory rates and RCR (C) in gastrocnemius muscle. Data are the mean ± SE. Number of rats in each group is shown in each column.

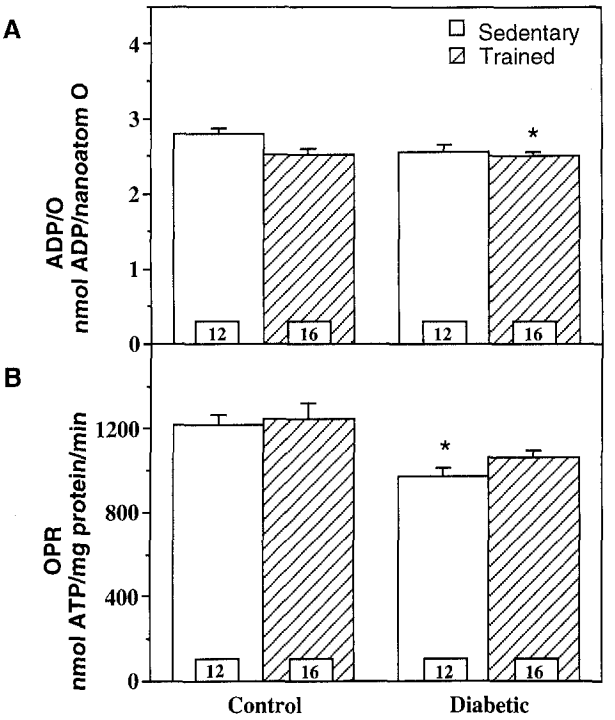

Fig 2. Effects of diabetes, physical training, or both on the ADP/O ratio (A) and OPR (B) in gastrocnemius muscle. Data are the mean ± SE. Number of rats in each group is shown in each column. *$P < .05$ *v* sedentary control rats.

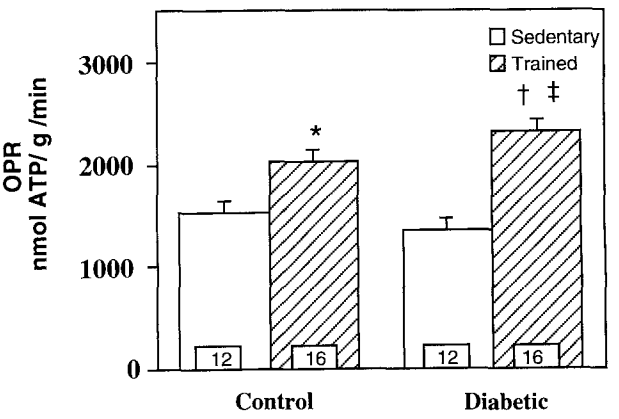

Fig 3. Effects of diabetes, physical training, or both on OPR in gastrocnemius muscle. Data are the mean ± SE. Number of rats in each group is shown in each column. *$P < .05$, †$P < .001$: *v* sedentary control rats. ‡$P < .001$ *v* sedentary diabetic rats.

in sedentary diabetic than in sedentary control rats, but was not different between trained diabetic rats and the two groups of sedentary rats. On the other hand, when expressed as per gram of muscle (Fig 3) to obtain information about the capacity of muscle tissue to provide energy, OPR was not modified by diabetes, but it was significantly higher in trained nondiabetic ($P < .05$) and diabetic ($P < .001$) rats than in their sedentary counterparts.

The effects of diabetes and training on deep vastus lateralis mitochondrial function are shown in Figs 4, 5, and 6. Oxygen consumption by mitochondria in the absence of ADP (state 4 respiratory rate) was not affected by diabetes, but it was slightly lower ($P < .05$) in trained control rats than in their sedentary counterparts. Mitochondrial respiration stimulated by ADP (state 3 respiratory rate) was not affected by diabetes or training. There also was no significant difference in RCR between the four groups of rats. A similar finding was observed for the ADP/O. When expressed as per milligram of mitochondrial protein, the OPR or energy production in the form of ATP per minute was diminished by 21% ($P < .01$) in mitochondria from sedentary diabetic rats in comparison to sedentary control rats. A similar decrease was observed in the two groups of trained rats. On the other hand, when expressed as per gram of muscle (Fig 6), the OPR was not modified by diabetes but was significantly higher in trained nondiabetic ($P < .01$) and diabetic ($P < .001$) rats than in their sedentary counterparts.

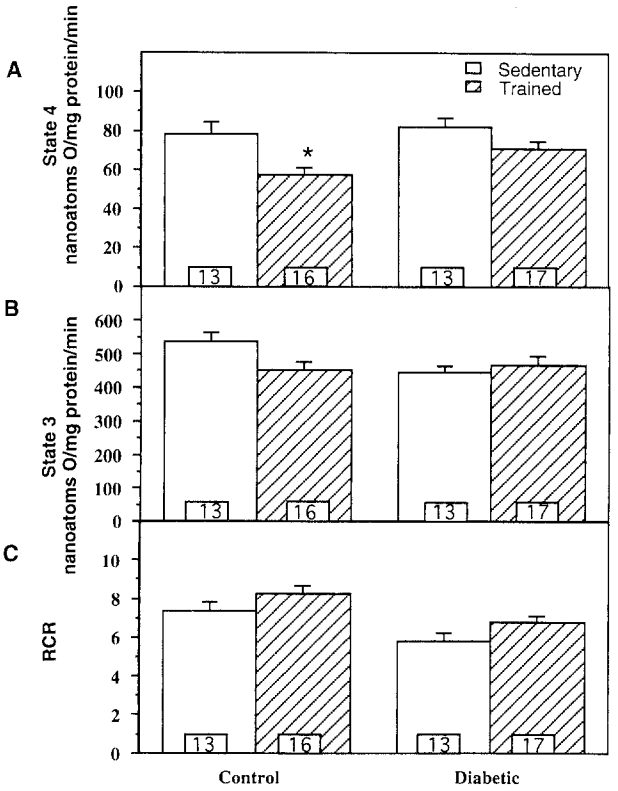

Fig 4. Effects of diabetes, physical training, or both on state 4 (A) and state 3 (B) respiratory rates and RCR (C) in red vastus lateralis muscle. Data are the mean ± SE. Number of rats in each group is shown in each column. *$P < .05$ *v* sedentary control rats.

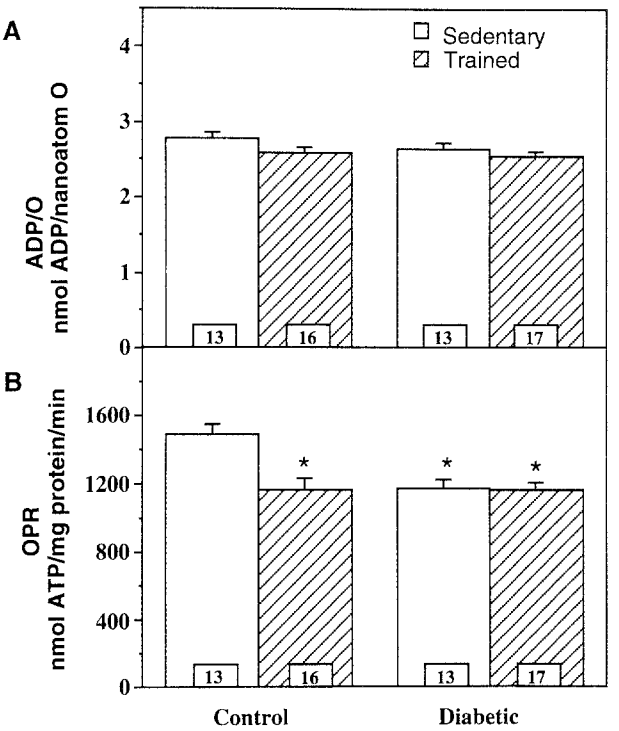

Fig 5. Effects of diabetes, physical training, or both on ADP/O ratio (A) and OPR (B) in red vastus lateralis muscle. Data are the mean ± SE. Number of rats in each group is shown in each column. *$P < .01$ *v* sedentary control rats.

DISCUSSION

Effect of Diabetes on Mitochondrial Function

This study is the first to report on the impact of chronic untreated experimental diabetes mellitus on the ATP production rate in mitochondria isolated from two different skeletal muscles in the rat. In comparison to the sedentary

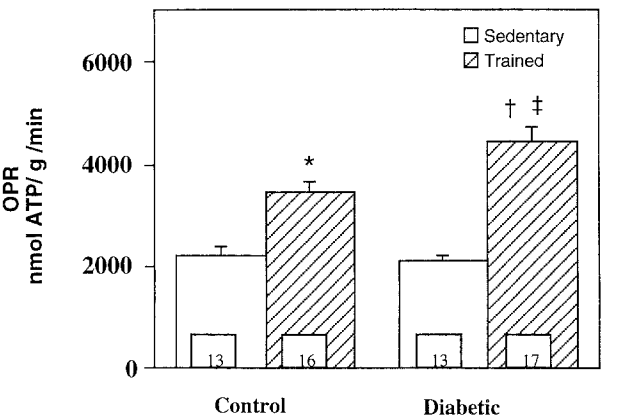

Fig 6. Effects of diabetes, physical training, or both on OPR in red vastus lateralis muscle. Data are the mean ± SE. Number of rats in each group is shown in each column. *$P < .01$, †$P < .001$: *v* sedentary control rats. ‡$P < .001$ *v* sedentary diabetic rats.

control group, sedentary rats with diabetes mellitus of 11 week duration exhibited a statistically significant approximately 20% decrease in ATP production rate in mitochondria isolated from either the gastrocnemius or the red vastus lateralis muscle. Gross et al[1] previously reported that state 3 respiration rate was diminished by 40% in mitochondria isolated from the total hindleg muscle of rats with experimental diabetes mellitus of 19-day duration, but they did not calculate the ATP production rate. Moreover, the values they reported for state 3 respiration rate and RCR in their control rats were lower than obtained in the present study, suggesting that the isolation procedure they used provided mitochondria of lesser intrinsic quality.

The fact that we were able to evaluate the function of mitochondria isolated from two different skeletal muscles also provides information that was not obtained in the studies by Gross et al.[1] Although not assessed in the present study, it has been established previously[12] that the relative composition in FG, FOG, and SO (slow-twitch high-oxidative) fibers in the gastrocnemius muscle is 74%, 20%, and 6%, respectively, as compared with 43%, 51%, and 6% in the red vastus lateralis muscle. Data obtained in ultrastructural studies[2] have previously suggested that chronic experimental diabetes of 70- to 80-day duration in the rat differentially affected the mitochondria in each fiber type: a decrease in the number of mitochondria was observed in FG fibers, but a loss of mitochondrial cristae was present in FOG and SO fibers. Because the oxidative enzymes are located in the mitochondrial cristae and the proportion of FOG and SO fibers is about two times greater in the red portion of the vastus lateralis muscle than in the gastrocnemius muscle, one would have predicted that the negative impact of diabetes mellitus on the ATP production rate is greater in the former muscle. However, we observed that ATP production was decreased by approximately 20% in both the gastrocnemius and the red vastus lateralis muscles, and this is interpreted as evidence that the ultrastructural changes in cristae reported by Chao et al[2] may not be predictive of functional changes at this level.

Effect of Physical Training on Mitochondrial Function

This study is also the first to provide direct information on the impact of physical training on the ATP production rate in mitochondria isolated from two different skeletal muscles in the rat. Obviously, the two muscles studied adapted differently to treadmill running, since the mitochondrial protein yield increased only by 34% in the gastrocnemius muscle, in comparison to a 98% increase in the red vastus lateralis muscle. This difference in response is particularly of significance if one takes into account that the weight of these two muscles diminished similarly by approximately 15% in trained animals, which also had a 21% lower body weight than their sedentary counterparts.

Physical training in nondiabetic rats significantly reduced the ATP production rate by 21% in red vastus lateralis when the data are expressed as per milligram of mitochondrial protein, whereas no effect of training occurred in the gastrocnemius muscle. The reasons for this apparent negative impact of training on the mitochondrial capacity to produce ATP in the red vastus lateralis muscle are not readily apparent. It can be hypothesized that the training-induced increase in mitochondrial proteins involved in ATP production from the substrates used (pyruvate and malate) was of a lesser magnitude than the increase in other mitochondrial proteins, but further study is needed to examine this possibility. Nonetheless, when the results are expressed as per gram of muscle, the ATP production rate increased with training by 33% ($P < .05$) in the gastrocnemius muscle and by 54% ($P < .01$) in the red vastus lateralis muscle, indicating that the overall capacity of skeletal muscle to produce energy from the substrates used was indeed increased by training. Using the same experimental model of treadmill running in control and diabetic rats, we have previously reported[21] that the activity of NAD-linked isocitrate deshydrogenase, one of the mitochondrial enzymes, was significantly increased by training in crude extracts of the gastrocnemius muscle. On the other hand, electron microscopic studies by Holloszy and Coyle[22] have shown that both the size and number of mitochondria are responsible for the increase in mitochondria protein in response to endurance training. It is thus not surprising that the ATP production rate is increased when the data are expressed as per gram of muscle.

Holloszy[8] has examined the impact of physical training on the function of mitochondria isolated from skeletal muscle. A 102% increase in the state 3 respiration rate was observed in mitochondria isolated from gastrocnemius muscle when the data were expressed as per gram of muscle. Holloszy[8] did not report the data on as per milligram of mitochondrial protein, but it is noteworthy that he found a 57% increase in mitochondrial protein yield in trained animals. If state 3 respiration rate is calculated in the present study with the approach used by Holloszy,[8] ie, as per gram of muscle, it increased highly significantly ($P < .001$) in both muscles: 47% in the gastrocnemius and 65% in the red vastus lateralis. The increase observed in the present study is nevertheless smaller than the one reported by Holloszy.[8] This is possibly due to differences in the training protocol with the exercise bouts in Holloszy's protocol being not only twice as long as in our protocol but also interspaced with 30-second sprints at 10-minute intervals, which was not the case in our training program. Krieger et al[23] have previously studied the effect of training on mitochondria isolated from the gastrocnemius muscle. Two distinct populations of mitochondria were compared. Although no significant difference was found in the more abundant intermyofibrillar mitochondria, a significant approximately 47% increase in state 3 respiration was observed in the less abundant subsarcolemmal mitochondria when the data are expressed as per milligram of protein. Their results contrast with the results reported herein. Although the reasons for such discrepancies are not apparent, it must be pointed out that Krieger et al[23] have performed their studies with genetically hypertensive female rats.

Effect of Training on Mitochondrial Function in Diabetic Rats

As indicated earlier, this study was mainly designed to examine if physical training could reverse some diabetes-induced alterations in the OPR or ATP production rate in mitochondria isolated from two skeletal muscles differing in fiber type. Such a possibility was raised by our previous observation that training had such an effect in mitochondria isolated from heart muscle.[11] Notably, when the data were expressed as per milligram of mitochondrial protein, as in our heart study,[11] there was a diabetes-associated decrease in the capacity of mitochondria to produce energy. However, this was not reversed by training in the red vastus lateralis muscle or in the gastrocnemius muscle. Nevertheless, as already discussed for nondiabetic rats, training in diabetic rats was associated with an increase in the mitochondrial protein yield, which was greater in the red vastus lateralis muscle than in the gastrocnemius muscle. The overall capacity of muscle to oxidize substrates and produce energy thus appeared to be greatly increased by training in diabetic animals, as in nondiabetic animals.

It could be hypothesized that such an increase in overall mitochondrial oxidative capacity might have resulted in an improvement in glucose homeostasis in diabetic animals. Indeed, previous studies[13,24,25] have shown that endurance exercise training improved glucose homeostasis in rats with mild experimental diabetes mellitus, although this may not be the case in rats with a more severe diabetic state.[26] In the present study, no improvement in plasma glucose levels was observed with training in diabetic animals. This suggests that the increased capacity of skeletal muscle mitochondria to oxidize substrates like pyruvate did not translate into a parallel change in glucose homeostasis. This is in keeping with the recent observations of Kainulainen et al[27] that the reduced level of GLUT4 in skeletal muscle of diabetic rats was not reversed by physical training, even if there was an increase in the activity of some enzymes directly involved in oxidative metabolism at the mitochondrial level. Glucose transport into skeletal muscle cells under the control of GLUT4 is obviously a step preceding its metabolism. It is thus not so surprising that the increased capacity of mitochondria to oxidize substrates is not necessarily associated with an improvement in glucose metabolism.

Of interest, a clearcut improvement in plasma β-hydroxybutyric acid levels was observed with training in diabetic rats. To our knowledge, this is the first time that a beneficial effect of physical training on plasma levels of this ketone body in diabetic rats is reported. Theoretically, this effect can be produced by a decrease in the production rate of ketone bodies by the liver or an increase in their utilization rate by peripheral tissues, mainly skeletal muscle, or both. Previous studies in nondiabetic rats suggested that training may indeed increase the uptake and oxidation of ketone bodies in skeletal muscle,[28,29] but we know of no such studies that have been conducted in diabetic animals, and further investigation will be necessary to examine this possibility more directly.

In conclusion, the present study showed that untreated chronic experimental diabetes mellitus in the rat is associated with approximately a 20% decrease in the capacity of skeletal muscle mitochondria to oxidize pyruvate and malate and to generate energy in the form of ATP. A similar negative impact also appears to be produced by physical training in mitochondria isolated from the red vastus lateralis muscle when the data are expressed as per milligram of mitochondrial protein. However, no effect of diabetes is present if the data are expressed as per gram of muscle. Moreover, due to the increase in mitochondrial protein yield observed in trained animals, the overall capacity to oxidize pyruvate and malate appeared to be increased by training in skeletal muscles of both diabetic and nondiabetic rats. This effect of training was not associated with an improvement in glucose homeostasis in diabetic animals. However, the increased plasma levels of β-hydroxybutyric acid in diabetic rats were significantly reduced by physical training. This may have clinical relevance, since the development of ketoacidosis remains a life-threatening event in insulin-dependent diabetic subjects.

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