Exercise training alleviates MCT1 and MCT4 reductions in heart and skeletal muscles of STZ-induced diabetic rats

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Enoki, Taisuke, Yuko Yoshida, Hideo Hatta, and Arend Bonen. Exercise training alleviates MCT1 and MCT4 reductions in heart and skeletal muscles of STZ-induced diabetic rats. J Appl Physiol 94: 2433–2438, 2003. —We compared the changes in monocarboxylate transporter 1 (MCT1) and 4 (MCT4) proteins in heart and skeletal muscles in sedentary control and streptozotocin (STZ)-induced diabetic rats (3 wk) and in trained (3 wk) control and STZ-induced diabetic animals. In nondiabetic animals, training increased MCT1 in the plantaris (+51%; P < 0.01) but not in the soleus (+9%) or the heart (+14%). MCT4 was increased in the plantaris (+48%; P < 0.01) but not in the soleus muscles of trained nondiabetic animals. In sedentary diabetic animals, MCT1 was reduced in the heart (−30%), and in the plantaris (−31%; P < 0.01) and soleus (−26%) muscles. MCT4 content was also reduced in sedentary diabetic animals in the plantaris (−52%; P < 0.01) and soleus (−25%) muscles. In contrast, in trained diabetic animals, MCT1 and MCT4 in heart and/or muscle were similar to those of sedentary, nondiabetic animals (P > 0.05) but were markedly greater than in the sedentary diabetic animals [MCT1: plantaris (+63%, soleus (+51%, heart +51% (P > 0.05); MCT4: plantaris +107%, soleus +17% (P > 0.05)]. These studies have shown that 1) with STZ-induced diabetes, MCT1 and MCT4 are reduced in skeletal muscle and/or the heart and 2) exercise training alleviated these diabetes-induced reductions.

Type 1 diabetes; plantaris; soleus; lactate transport; monocarboxylate transporter; streptozotocin

SKELETAL MUSCLE IS THE PRIMARY site of lactate production, but this tissue, as well as the heart, can also oxidize this substrate (8, 12). The transfer of lactate from the site of production to sites of oxidation is facilitated by the movement of lactate between muscle cells and by delivery of lactate, via the circulation, to other muscles and tissues. The dynamic movement of lactate across the plasma membrane is closely associated with a lactate-proton cotransport system (16). In the past few years, a family of monocarboxylate transporter (MCT) isoforms have been identified that regulate the rate of flux of monocarboxylates, such as lactate as well as pyruvate and acetooacetate (16). MCT1 is ubiquitously expressed in many tissues, whereas MCT4 is present in skeletal muscle (2, 3). In rat skeletal muscle, MCT1 expression is highly correlated with indexes of oxidative metabolism, whereas MCT4 expression is highly correlated with indexes of muscle glycolytic metabolism (5, 20, 26).

A number of studies have shown that skeletal muscle lactate transport can be increased when muscle activity is chronically increased, either by training (1, 10, 30, 32) or by electrical stimulation (6, 25, 27). In contrast, when muscle activity is decreased by denervation (24, 31) or hindlimb suspension (11), lactate transport is decreased (31). With exercise training (1, 10, 30) and denervation (36), these changes in lactate transport have been accompanied by concomitant changes in MCT1 and MCT4, although the transport capacities of these proteins are quite different (7, 9).

With streptozotocin (STZ)-induced diabetes, there are many changes in substrate metabolism, which include reductions in the glucose transporter GLUT-4 (13, 17, 21) and an increase in the long-chain fatty acid transporters in the heart and skeletal muscles (23). With STZ-induced diabetes, circulating concentrations of lactate are increased at times (22, 29) but not always (33, 34). At the tissue level, it has been observed that with STZ-induced diabetes there is an increased carrier-mediated lactate uptake into hepatocytes (28), whereas lactate transport into adipocytes (15) and skeletal muscles was decreased (33, 34). These reductions in adipocytes were accompanied by concurrent reductions in MCT1 (15). Unexpectedly, the skeletal muscle reductions in lactate transport were not accompanied by concurrent reductions in either MCT1 or MCT4 (33, 34). Collectively, these studies appear to indicate that there may be tissue-specific responses in MCT expression with STZ-induced diabetes.

However, it seemed quite unusual that a severe metabolic disorder such as STZ-induced diabetes did not alter MCT expression (34), when many other transport proteins such as GLUT-4 (17, 21) and fatty acid transporters (23) are altered in STZ-induced diabetes. Therefore, we have examined the changes of MCT1 and MCT4 proteins in skeletal muscles of STZ-induced diabetic rats. These proteins were also examined in the heart of these animals, because the heart is also an...
important site for lactate oxidation, which is reduced with STZ-induced diabetes (8). Finally, we also examined whether exercise training might alter the responses to STZ-induced diabetes in the heart and muscle, because exercise training is known to increase MCT in these tissues in healthy animals (1, 4) and humans (10, 30).

METHODS

Male Wistar rats (aged 4 wk) were obtained (Nihon Seibutsu Zairyou Center, Tokyo, Japan) and housed in an air-conditioned room on a 12:12-h light-dark cycle. All rats were fed a diet of Purina rat chow and water. Their body weights were checked daily. Ethical approval for these studies was obtained from the Committee on Animal Care at the University of Tokyo.

Body weights were determined in week 4, the week before the onset of the study. At 5 wk of age, the animals were randomly assigned to the treatment groups: control, trained, STZ-induced diabetes, and STZ-induced diabetes plus training. Diabetes was induced by injecting STZ (50 mg/kg ip) in a 0.1 M citrate buffer (pH 4.5). Two days after the STZ injection, nonfasting glucose concentrations were determined in a blood sample taken from the tail vein. Diabetes was defined when blood glucose concentrations were ≥30 mM. At 8 wk of age, after 3 wk of training and/or diabetes, nonfasting circulating lactate and glucose concentrations were determined in a tail vein blood sample.

Exercise training was started at 5 wk of age. Treadmill exercise was performed for 30 min/day, 7 days/wk, for 3 wk. Initially, the trained nondiabetic and diabetic groups were familiarized with a motor-driven treadmill running at low speeds (20–25 m/min) for 30 min/day for the first 5 days. Thereafter, the speed was increased progressively over the 3-wk period, until the animals were running 30 m/min for 30 min for the last 7 days. The control and sedentary diabetic animals remained sedentary in their cages for the duration of the 3-wk training program. Over the 3-wk period, deaths occurred in the sedentary diabetic (n = 3) and trained diabetic (n = 3) groups. After 3 wk, the surviving animals were anesthetized (pentobarbital sodium, ip), and the soleus and plantaris muscles and hearts were excised rapidly. These tissues were frozen in liquid nitrogen and stored at −80°C until analyzed for MCT1 and MCT4 protein.

Proteins from the plantaris and soleus muscles and heart were isolated and separated electrophoretically as previously described (1, 4, 18, 25, 26, 35). MCT1 and MCT4 were detected by using Western blotting, as previously described in detail (1, 4, 18, 25, 35). For each set of Western blots, data from muscles and heart from each of the four treatment groups were included. Also, a standard was included with each Western blot. This permitted normalization of the data to the standard across the different blots. Densities of the MCT1 and MCT4 protein bands were quantified by scanning the resultant films on a densitometer (Epson, Nagano, Japan) connected to a computer with appropriate software (NIH Image 1.62, National Institutes of Health, Bethesda, MD).

ANOVA for repeated measures (plantaris, soleus, heart) was used to analyze the data. Post hoc analyses were performed by using Student’s t-test. Significance was accepted at P < 0.05. All data are reported as means ± SE.

RESULTS

Body Weight. At the start of the study, there were no differences in body weight among the four groups of animals (Fig. 1). Thereafter, rats in the control and trained groups gained ~40 g/wk during the next 3 wk (P < 0.01). In contrast, the body weights of the sedentary diabetic animals decreased in the first few days after the onset of diabetes, and then body weight remained stable from week 6 to week 8. At the end of the 3-wk study, the body weight in the sedentary diabetic group was 25–40% lower than in the other three groups (P < 0.01). The trained diabetic animals gained weight during the 3-wk training program, although the body weights remained below that observed in the sedentary and trained nondiabetic animals (Fig. 1).

Blood glucose and lactate concentration at rest. As expected, at the end of the 3-wk study, the diabetic rats, both sedentary and trained, had significantly higher blood glucose concentrations than the sedentary and trained nondiabetic animals (P < 0.01; Fig. 2). Compared with the sedentary diabetic group, blood
glucose concentration in the trained diabetic group was significantly reduced (−19%; P < 0.05). No differences in nonfasting glucose were observed between the sedentary and trained nondiabetic animals (P > 0.05; Fig. 2).

Blood lactate concentrations, measured at rest at the end of the 3-wk study did not differ between the nondiabetic sedentary and trained animals (Fig. 3). In contrast, in the sedentary diabetic animals, the resting lactate concentrations were increased (+55%). However, with training, the lactate concentrations at rest were lowered to the levels observed in the nondiabetic animals (Fig. 3).

**MCT1 and MCT4 in heart and skeletal muscles.** With Western blotting, a single MCT1 band was detected in the heart and skeletal muscles (plantaris and soleus). There were differences in MCT1 and MCT4 among the tissues examined. MCT1 content was greater in heart than in soleus muscle, and the lowest MCT1 content was present in the plantaris muscle (Table 1). A single band for MCT4 was also detected in skeletal muscles, whereas MCT4 was not detected in the heart. MCT4 content in the plantaris muscle was greater than in the soleus muscle (Table 1). For the analyses below, the data in the sedentary control group have been set to 100% for each tissue.

**Effects of STZ-induced diabetes on MCT1 and MCT4.** After 3 wk of STZ-induced diabetes, the MCT1 content of the plantaris (−31%; P < 0.01), soleus (−26%), and heart (−30%) were reduced (Fig. 4). In the same skeletal muscles, MCT4 content was also reduced (plantaris −52%, P < 0.01; soleus −25%) (Fig. 5).

**Effects of training on MCT1 and MCT4.** In the nondiabetic animals, there was an increase in MCT1 with training in plantaris muscle (+51%; P < 0.05) but not in the soleus muscle (+9%; P > 0.05) and in the heart (+14%; P > 0.05) (Fig. 4). There was also a training-induced increase in MCT4 in the plantaris (+48%; P < 0.01) but not in the soleus muscles (+1%; P > 0.05) in the nondiabetic animals (Fig. 5).

In the trained diabetic animals, compared with sedentary diabetic animals, MCT1 was increased in skeletal muscles (plantaris +64%, soleus +51%; P < 0.01 for both) and in the heart (+51%; P < 0.01) (Fig. 4). The MCT1 levels in the heart and muscles of the trained diabetic group were similar to those observed in

Table 1. MCT1 and MCT4 protein in heart and skeletal muscles of sedentary control animals

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MCT1 Content</th>
<th>MCT4 Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>542 ± 64 †</td>
<td>ND</td>
</tr>
<tr>
<td>Soleus</td>
<td>152 ± 12</td>
<td>170 ± 24</td>
</tr>
<tr>
<td>Plantaris</td>
<td>141 ± 18</td>
<td>170 ± 13 †</td>
</tr>
</tbody>
</table>

Values are means ± SE given in arbitrary optical density units. MCT1 and MCT4, monocarboxylate transporter 1 and 4, respectively; ND, not detectable. *P < 0.05 compared with soleus and plantaris muscles. †P < 0.05 plantaris vs. soleus muscle.

Fig. 3. Lactate concentrations at the end of 3 wk of training and/or streptozotocin-induced diabetes. Values are means ± SE. C, n = 5; Tr, n = 7; D, n = 8; D+T, n = 13. **P < 0.01; C vs. D, D vs. D+T.

Fig. 4. Monocarboxylate transporter 1 (MCT1) protein in muscles and heart at the end of 3 wk of training and/or streptozotocin-induced diabetes. Values are means ± SE. OD, optical density. Nondiabetic control group was set to 100%. C, n = 5; Tr, n = 6; D, n = 5; D+T, n = 10. **P < 0.01. *P < 0.05.

Fig. 5. Monocarboxylate transporter 4 (MCT4) protein in muscles and heart at the end of 3 wk of training and/or streptozotocin-induced diabetes. Values are means ± SE. Nondiabetic control group was set to 100%. C, n = 5; Tr, n = 6; D, n = 5; D+T, n = 10. **P < 0.01. *P < 0.05.
the sedentary nondiabetic group ($P > 0.05$) but lower than those in the nondiabetic, trained group (Fig. 4).

The MCT4 levels were also increased with training in the diabetic animals, relative to the sedentary diabetic rats (plantaris $+105\%$, $P < 0.01$; soleus $+17\%$) (Fig. 5). The MCT4 content of the plantaris and soleus muscles in the trained diabetic group did not differ from those observed in the sedentary nondiabetic group ($P > 0.05$; Fig. 5).

**DISCUSSION**

We have examined the effects of STZ-induced diabetes on MCT1 and MCT4 in heart and skeletal muscles, when animals remained sedentary or trained for 3 wk. The novel results in this study are as follows: 1) STZ-induced diabetes markedly reduced MCT1 in the heart, and MCT1 and MCT4 in skeletal muscles, and 2) treadmill training alleviated these reductions in heart MCT1 and skeletal muscle MCT1 and MCT4 in diabetic animals.

Induction of diabetes was confirmed by the very high concentrations of glucose ($>30$ mM) in the STZ-treated animals, compared with the nondiabetic animals ($10$ mM). During the course of this study, the body weights of the animals in each of the four groups were markedly changed. In particular, the nondiabetic animals gained weight at a normal rate, as reported elsewhere (13, 14, 21, 27, 28), and the STZ-induced diabetic animals gained weight at a much lower rate, as has been shown previously (13, 14). Goodyear et al. (13) have reported that food consumption of STZ-induced diabetic rats was significantly lower than that of control rats.

Although not a principal area of inquiry in this study, we found that blood glucose levels in the trained, diabetic animals were much lower than in the sedentary animals. This suggested that there was a positive effect of exercise training on glucose homeostasis in this model of Type 1 diabetes. A previous study found that 4 wk of exercise training could improve skeletal muscle glucose uptake in STZ-induced diabetic (13).

Circulating lactate concentrations were increased in the sedentary diabetic animals at rest. This confirms several previous observations (22, 29), although others have failed to observe any increase in circulating lactate with STZ-induced diabetes (33, 34). The normalization of the circulating lactate concentrations at rest in trained diabetic animals has not been reported previously. The underlying mechanism of this normalization is not known, but we speculate that this may be due to reductions in circulating glucose concentrations in combination with the concomitant restorations of MCT levels in the trained diabetic animals.

Other studies have determined that lactate uptake in skeletal muscles of STZ-induced diabetic rats was significantly decreased (33), and Chatham et al. (8) have reported that cardiac lactate oxidation is decreased in STZ-induced diabetic rats but that MCT1 is not. However, in the present study, there were marked reductions in MCT1 in the heart and skeletal muscles and in MCT4 in skeletal muscle of STZ-induced diabetic animals. The magnitude of the MCT1 reductions were quite similar in the heart and these muscles (−31 to −26\%), whereas the MCT4 reductions were greater in plantaris, a muscle that expresses considerable quantities of MCT4 (i.e., plantaris −52\%), compared with soleus muscle (−25\%), which expresses lower quantities of MCT4. This suggests that differences in muscle fiber composition and differences in oxidative capacities in the heart and muscles did not influence the diabetes-induced reductions in MCT1, whereas fast-twitch muscle appeared to be particularly susceptible to the diabetes-induced reductions in MCT4. We cannot state whether these observed changes in MCTs were due to pretranslational or posttranscriptional mechanisms, because the required measurements to establish these mechanisms were not made. Previous studies in which changes in MCT1 and/or MCT4 have been induced in heart and/or skeletal muscle have attributed changes in protein expression of MCTs to posttranscriptional mechanisms (5, 6). There are as yet no studies that have examined the mechanisms regulating MCT expression in STZ-induced diabetes. We do not believe that the MCT reductions in STZ-induced diabetes represent a general reduction in all skeletal muscle proteins, because it has recently been shown that with STZ-induced diabetes, fatty acid transport proteins are increased (23).

Only a few other studies have examined the effects of STZ-induced diabetes on MCT proteins. After only 4 days of STZ-induced diabetes (65 mg/kg, glucose $=15$ mM), MCT1 protein content was reduced by $80\%$ in adipose tissue, which does not express MCT4 (15). A concomitant reduction in adipocyte lactate transport (−64\%) was also observed (15). Thus our present work and that of Hajduch et al. (15) are in agreement that STZ-induced diabetes reduces the content of MCT proteins. Although we did not measure lactate transport in the present studies, the study of Hajduch et al. and a number of our laboratory’s previous studies (1, 5, 6, 26, 27) have shown that lactate transport into adipocytes (15) and skeletal muscles (1, 5, 6, 26, 27) parallels closely the changes in MCT1 proteins.

Py et al. (33, 34) found that after 15 days of STZ-induced diabetes (65 mg/kg, glucose $35.4$ mM), $V_{\text{max}}$ of lactate was modestly reduced (−15\%) (33). However, this occurred in the absence of changes in plasma membrane MCT1 and MCT4 (33), or total MCT1 content in the heart, and MCT1 and MCT4 content in a number of muscles (soleus, extensor digitorum longus, red tibialis anterior) (34). These observations by Py et al. are difficult to reconcile with those of Hajduch et al. (15) and those in the present study. On the basis of the circulating glucose concentrations in our animals (33.8 mM) and those in the studies by Py et al. (33.5 mM) (33, 34), it appears that a different severity of diabetes cannot account for the discrepant results in our present study and those of Py et al. Although the body weights of the animals in the study by Py et al. (33, 34) (−371 g) were greater than those in our studies (−305
of which are linked to insulin insufficiency in this milieu (glucose, insulin, lactate) and by increased content in heart and/or skeletal muscle. Our studies showed that when diabetic rats are exposed to exercise training, there was an increase in MCT1 in the heart, and in MCT1 and MCT4 in muscles, compared with the sedentary diabetic rats. However, the MCT content in these tissues of the trained diabetic animals did not differ from those observed in the trained sedentary animals. Thus we cannot state whether exercise training induced an increase in MCT1 and MCT4 in the diabetic rats or whether exercise training of 30 min/day prevented the decrease in MCT1 and MCT4 observed in the sedentary diabetic animals. Time course studies are required to answer this question. Nevertheless, whatever the mechanisms involved, it is clear that exercise training provided a substantial prophylactic effect on MCT1 and MCT4 content in heart and/or skeletal muscle.

Our studies have shown that MCT content in muscle and heart is regulated both by the ambient metabolic milieu (glucose, insulin, lactate) and by increased contractile activity (exercise training). A number of studies have already shown that contractile activity or altered metabolic demand by muscle regulates MCT expression (1, 6, 10, 11, 27, 30, 31). An entirely other level of regulation of MCTs is suggested in the studies of STZ-induced diabetes. It is well known that this perturbation markedly alters substrate metabolism, many of which are linked to insulin insufficiency in this model. This, however, does not prove that insulin necessarily regulates MCT expression; some other metabolic factors that are concomitantly altered may also be involved. Whether circulating lactate concentrations regulate the expression of MCTs is not known. It is tempting to suggest that increased concentrations of lactate in the sedentary diabetic animals contributed to the downregulation of MCT1 and MCT4. However, this would seem unlikely given that in situations in which lactate is increased (i.e., exercise training) these proteins are increased. Nevertheless, the present studies demonstrate that the substrate and/or endocrine milieu can alter MCT1 and MCT4 in heart and muscle. However, it appears that muscle contractile activity can override these endocrine and/or metabolic effects that contribute to the repression of MCT1 and MCT4.

In summary, we have shown that STZ-induced diabetes reduced MCT1 in heart and MCT1 and MCT4 in several skeletal muscles. We also found that the exercise training 1) increased these proteins in nondiabetic animals and 2) served to prevent the diabetes-induced reductions in MCT1 and MCT4 in muscle and heart.

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REFERENCES


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