Exercise Training Increases the Activity of Pyruvate Dehydrogenase Complex in Skeletal Muscle of Diabetic Rats

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Abstract. The effects of diabetes and exercise training on the activity of pyruvate dehydrogenase (PDH) complex in skeletal muscle were examined in rats. Male Sprague-Dawley rats were divided into four groups as follows: non-diabetic sedentary, non-diabetic trained, diabetic sedentary, and diabetic trained groups. Diabetic rats were prepared by a bolus injection of intravenous streptozotocin (50 mg/kg body weight). Exercise training was performed by having rats run on a treadmill at a speed of 25 m/min for 45 min/day, 6 days/wk for 4 wks. Exercise training decreased serum concentrations of glucose and non-esterified fatty acid in diabetic rats. GLUT4 content in skeletal muscle of sedentary rats was significantly decreased by diabetes; however, exercise training significantly increased the GLUT4 content in diabetic rats. The total and actual activities and the proportion of actual activity of the PDH complex were decreased in diabetic sedentary rats. Exercise training did not affect the total activity of the PDH complex in non-diabetic rats, whereas it increased the total activity in diabetic rats to the same level as that in non-diabetic rats. In diabetic rats, exercise training tended to increase the proportion of actual activity of the PDH complex from 2.7 ± 0.4% to 4.7 ± 0.8%, although the proportion of actual activity in non-diabetic rats was decreased by exercise training. The present study suggests that exercise training may improve glucose metabolism in the skeletal muscle of streptozotocin-induced diabetic rats probably through the mechanisms of increasing both GLUT4 content and the activity of the PDH complex.

Key words: GLUT4, Pyruvate dehydrogenase complex, Diabetes, Exercise training

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EXERCISE training has been reported to improve whole body glucose metabolism in man and experimental animals [1–4]. Insulin-mediated glucose uptake has been demonstrated to be significantly increased in skeletal muscle of exercise trained rats [5]. Glucose uptake is the rate-limiting step in muscle glucose utilization [6, 7] and is mediated by one or more membrane proteins [8]. The glucose transporter isoform responsible for insulin- and contraction-stimulated glucose uptake in skeletal muscle is termed GLUT4 [9]. It has been demonstrated that a correlation exists between GLUT4 concentration in sarcolemmal membrane and the capacity of glucose uptake in skeletal muscle in man [10] and experimental animals [11]. It has been reported that streptozotocin-induced diabetes decreased the concentration of GLUT4 in heart [12] and skeletal muscle [13], and that exercise training increased the GLUT4 concentration in skeletal muscle [3, 5] of rats.

Once taken up by muscle, glucose can be either converted to glycogen or metabolized through the glycolytic pathway. The pyruvate dehydrogenase (PDH) complex is a multienzyme complex located in the internal mitochondrial membrane, and is consi-
ordered to play a pivotal role in the regulation of intramuscular glucose metabolism, serving as the rate-limiting enzyme of aerobic glucose oxidation in the cells. Full oxidation of glucose to carbon dioxide is dependent on the activity of PDH complex, which catalyzes the stepwise conversion of pyruvate to acetyl-CoA [14]. The PDH complex is known to be subject to covalent modification; PDH kinase is responsible for phosphorylation and inactivation of the complex, and PDH phosphatase is responsible for dephosphorylation and activation of the complex [15]. Many studies have provided evidence of the role for PDH complex in long term regulatory mechanisms of glucose metabolism. Experimental diabetes [16–18], food deprivation [19, 20] and aging [21] have been shown to suppress PDH complex activities. These findings, together with the physiological roles of the PDH complex, suggest that the attenuation of PDH complex activities may contribute to the suppression of whole body glucose metabolism.

Since exercise training has been reported to stimulate the first step of glucose metabolism in the skeletal muscle by increasing the glucose uptake through the augmentation of GLUT4 protein synthesis [3, 5], it is important to determine whether or not exercise training also induces the stimulation of the second step or the intracellular step of glucose metabolism through the activation of PDH complex. Moreover, exercise training has been recommended as an important form of treatment for diabetic patients [22, 23]. In the present study, we investigated the long term effects of exercise training on the concentration of GLUT4 and the activity of PDH complex in streptozotocin-induced diabetic rat skeletal muscles. We reported here that exercise training improved the glucose metabolism in diabetic rats through the stimulation of both glucose uptake into cells and glucose oxidation in the cells, by increasing GLUT4 concentrations and the activity of PDH complex in skeletal muscles, respectively. In addition, the direct effect of muscle contractions on the activity of PDH complex of both diabetic and non-diabetic rats was assessed to study the possible relation between improved glucose metabolism and exercise training.

Materials and Methods

Materials

Male Sprague-Dawley rats aged 7 wk with weights of 220–240 g were obtained from CLEA Japan (Tokyo, Japan). [1-14C]Pyruvic acid (sodium salt) was purchased from Amersham Japan (Tokyo). A broad-specificity phosphoprotein phosphatase was prepared from bovine heart by a method reported previously using column chromatography [24].

Induction of diabetes mellitus

Rats were fed laboratory chow (CE-2, CLEA Japan) and tap water ad libitum and housed in an animal room with controlled temperature (23±1°C) and light (12-h cycles) conditions. All procedures involving animals were approved by the Experimental Animal Care Committee of Nagoya Institute of Technology. After one week, rats were randomly divided into non-diabetic (n = 14) and diabetic (n = 14) groups. Diabetes was induced by a bolus injection of intravenous streptozotocin (50 mg/kg body weight). Streptozotocin was dissolved (25 mg/ml) in a 10 mM citrate buffer (pH 4.5) just prior to injection. Non-diabetic group of rats received an equivalent amount of the citrate buffer. Non-diabetic and diabetic groups of rats were subdivided into sedentary and exercise trained groups (7 animals each). Blood samples were obtained from the tail vein 4 days after the injection of streptozotocin and the glucose level was determined to verify the induction of diabetes.

Exercise training protocol

Rats in trained groups were run on a rodent motor-driven treadmill set up a 6° incline 6 days/wk for 4 weeks. The intensity of exercise was gradually increased during the first week of the program from a speed of 15 to 25 m/min. Rats were run at 25 m/min, 45 min/day for the subsequent 3 weeks. The exercise training was started 4 days after the injection of streptozotocin and after the confirmation of diabetes mellitus.
**Electrical stimulation protocol**

The experimental procedures of electrical stimulation for muscle contraction were as described previously [25]. On the last day of the experiment, rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The sciatic nerve complex was exposed via a lateral incision in the left thigh for connecting the electrodes. The left hindlimb muscles were electrically stimulated for 1 min with one 10-ms train/sec to investigate the direct effect of electrical stimulation on the PDH activities in the muscles. Each train consisted of a series of 6-V pulses of 0.1-ms duration delivered at 100 Hz. Immediately after the end of the 1 min stimulation, the gastrocnemius muscles of both hindlimbs were retrieved and then immediately freeze-clamped with liquid nitrogen and stored at −80°C until analysis. The contralateral unstimulated hindlimb muscle was used as a control. Blood samplings were also performed from the portal vein after the electrical stimulation. The trained rats were killed 24 h after the final treadmill exercise bout.

**Blood analysis**

Serum concentrations of glucose and non-esterified fatty acids (NEFA) were determined by colorimetric methods using commercial kits (Wako Pure Chemical Industries, Osaka, Japan). Serum concentration of insulin was determined by radioimmunoassay [26].

**Measurement of GLUT4 in skeletal muscle**

Total GLUT4 content of skeletal muscle was determined in preparations of total cellular muscle. Total cellular membrane of gastrocnemius muscle was prepared according to the method reported previously [11]. Protein concentrations of the membrane preparations were determined using bicinchoninic acid reagent (Pierce Chemical, Rockford, IL). GLUT4 concentrations in the membrane were determined immunologically using 80 μg of the membrane preparations with Western blot analysis described previously [3]. GLUT4 protein levels were expressed as an arbitrary unit/mg protein on the basis of readings of relative absorbance.

**Activity of PDH complex in skeletal muscle**

Activity of PDH complex was determined as reported previously [27]. Activity of PDH complex was assessed by measuring the total activity (dephosphorylated form) and the actual activity of the complex by determining the radioactivity released from [1-14C] pyruvate at 30°C. One unit of each activity was defined as the enzyme produces 1 μmol of CO2/min using [1-14C] pyruvate as a substrate [28]. For determination of the total activity, the complex was fully activated by adding a broad-specificity phosphoprotein phosphatase [24] in the presence of 10 mmol/l MgCl2. The percentage of actual activity of the PDH complex was calculated by dividing the actual activity by the total activity multiplied by 100.

**Statistical analysis**

Data are expressed as mean ± SE. Data for body weight and glucose concentration before starting exercise training were analyzed using unpaired Student’s t test. Differences were considered significant when P<0.05. Other data were analyzed using two way analysis of variance (ANOVA). Differences between individual groups of data were further established by Bonferroni/Dunn test. Differences were considered significant when P<0.0125.

**Results**

**Body weight and serum concentrations of glucose, insulin and NEFA**

Injection of streptozotocin significantly (P<0.01) decreased the body weight of rats before starting exercise training; 256 ± 4 g for diabetic rats (n = 14) and 276 ± 3 g for non-diabetic rats (n = 14). Induction of diabetic condition by the streptozotocin injection was confirmed by a significant (P<0.01) increase in the serum concentration of glucose before starting the exercise training; 22.4 ± 0.6 mmol/l for diabetic rats (n = 14) and 7.9 ± 0.2 mmol/l for non-diabetic rats (n = 14). At the end of the four-week experimental period, body weight of the diabetic sedentary group was significantly (P = 0.0022) less than that of the non-diabetic sedentary group (Table 1). Four weeks of exercise training did not affect the body weight
Table 1. Effects of exercise training on body weight and serum concentrations of glucose, insulin and NEFA in non-diabetic and diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic Sedentary</th>
<th>Non-diabetic Trained</th>
<th>Diabetic Sedentary</th>
<th>Diabetic Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>424 ± 8</td>
<td>392 ± 11</td>
<td>348 ± 12*</td>
<td>365 ± 16</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>10.4 ± 0.4</td>
<td>9.6 ± 0.5</td>
<td>35.6 ± 1.6*</td>
<td>28.3 ± 2.7**</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>10.4 ± 2.8</td>
<td>7.2 ± 1.2</td>
<td>0.8 ± 0.1*</td>
<td>1.8 ± 0.5*</td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>0.18 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>0.28 ± 0.04*</td>
<td>0.19 ± 0.02*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 rats in each group. *P < 0.0125 vs. non-diabetic corresponding group; †P < 0.0125 vs. sedentary rats in the same group.

either in non-diabetic or diabetic groups. It should be noted that exercise training significantly (P = 0.0031) decreased the glucose concentration in diabetic rats, although exercise training did not affect the glucose concentration in non-diabetic rats. Serum concentrations of insulin in diabetic rats was significantly lower (P = 0.0002) than that in non-diabetic rats (Table 1). Exercise training did not affect the insulin concentration both in diabetic and non-diabetic rats. The serum concentration of NEFA was significantly (P = 0.0089) higher in diabetic sedentary rats than in non-diabetic sedentary rats (Table 1). Exercise training significantly (P = 0.0093) lowered the NEFA concentration in diabetic rats to a level comparable to that observed in non-diabetic sedentary rats, whereas exercise training did not lower the NEFA concentration in non-diabetic rats.

**Effect of exercise training on GLUT4 concentrations in skeletal muscle**

Total amounts of the membrane protein recovered from 100 mg of muscle ranged between 1.97 mg to 2.04 mg/100 mg muscle which was not different among the four groups. Immunoreactive GLUT4 concentration in the skeletal muscle membranes of diabetic sedentary rats was significantly (P = 0.0006) lower than that of non-diabetic sedentary rats by 48% (Fig. 1). The 4 weeks of exercise training significantly (P = 0.0093) increased GLUT4 concentration in diabetic rats by 66%, the concentration of which was comparable to that observed in the skeletal muscle membranes prepared from non-diabetic sedentary rats. Exercise training did not induce a significant increase in GLUT4 in non-diabetic rats. In contrast to the effect of exercise training, the 1 min of the electrical muscle contractions did not affect the concentration of GLUT4 in either non-diabetic or diabetic rats (data not shown).

**Effect of exercise training on the activity of PDH complex (Table 2)**

Diabetes induced a significant (P = 0.0001) decrease in the total activity of PDH complex in the skeletal muscle in sedentary rats. It is noteworthy here that exercise training significantly (P < 0.0001) increased the total activity in diabetic rats, showing a preventive effect against the decrease in the total activity caused by diabetes. In the non-diabetic rats, however, this favorable effect of exercise was not observed. Diabetes also induced a significant (P = 0.0002) decrease in the actual activity of PDH complex in sedentary rats. Exercise training induced a
PDH COMPLEX IN TRAINED DIABETIC RATS

Table 2. Effects of exercise training on the total and actual activities and the percentage of actual activity of the PDH complex in skeletal muscle of non-diabetic and diabetic rats.

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic Sedentary</th>
<th>Non-diabetic Trained</th>
<th>Diabetic Sedentary</th>
<th>Diabetic Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total activity (U/g)</td>
<td>1.35 ± 0.04</td>
<td>1.51 ± 0.04</td>
<td>1.04 ± 0.04*</td>
<td>1.42 ± 0.03</td>
</tr>
<tr>
<td>Actual activity (U/g)</td>
<td>0.18 ± 0.02</td>
<td>0.12 ± 0.01*</td>
<td>0.03 ± 0.01*</td>
<td>0.07 ± 0.01*</td>
</tr>
<tr>
<td>% of actual activity</td>
<td>13.6 ± 1.7</td>
<td>8.1 ± 1.0*</td>
<td>2.7 ± 0.4*</td>
<td>4.7 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 rats in each group. *P<0.0125 vs. non-diabetic corresponding group; **P<0.0125 vs. sedentary rats in the same group.

2-fold increase in the actual activity of complex in diabetic rats. In contrast to the effect on diabetic rats, exercise training significantly (P = 0.0049) decreased the actual activity of the PDH complex in non-diabetic rats. Diabetes induced a significant (P<0.0001) decrease in percentage of the actual activity in the PDH complex in sedentary diabetic rats compared with sedentary non-diabetic rats. Exercise training tended to induce an increase in the percentage of actual activity in diabetic rats, whereas the training induced a significant (P = 0.0013) decrease in that in non-diabetic rats.

Effect of electrical muscle contractions on the activity of PDH complex (Table 3)

One minute of electrically-induced muscle contractions did not affect the total activity of PDH complex either in non-diabetic or diabetic groups. The muscle contractions increased both the actual activity and the percentage of the actual activity in the complex in all groups as compared with those without the contractions. When the median values of the actual activity were compared, the magnitude of increases in response to the contractions was higher in diabetic rats than in non-diabetic rats.

Discussion

Exercise training is known to improve glucose tolerance and whole-body insulin sensitivity in human patients with type 2 diabetes [29] and experimental animals [3, 5]. The mechanism(s) of the improvement has been reported to be derived from the increased uptake of glucose mediated by the increased GLUT4 [3, 30]; however, the details of intracellular mechanisms of the improvement of glucose metabolism still remain to be elucidated. In the present study, we used streptozotocin-induced diabetic rats that have been considered to be an animal model of type 1 diabetes. Application of exercise to type 1 diabetic patients must be assessed carefully because exercise triggers an increase in counterregulatory hormones and endocrine response enhancing glucose production and inhibiting glucose uptake [31]. On the other hand, muscle contraction during exercise increases the glucose uptake without pres-

Table 3. Effects of exercise training on the total and actual activities and the percentage of actual activity of the PDH complex in skeletal muscle of non-diabetic and diabetic rats after muscle contractions.

<table>
<thead>
<tr>
<th></th>
<th>Non-diabetic Sedentary</th>
<th>Non-diabetic Trained</th>
<th>Diabetic Sedentary</th>
<th>Diabetic Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>After contraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total activity (U/g)</td>
<td>1.43 ± 0.07</td>
<td>1.53 ± 0.05</td>
<td>1.16 ± 0.04*</td>
<td>1.49 ± 0.05*</td>
</tr>
<tr>
<td>Actual activity (U/g)</td>
<td>0.40 ± 0.06</td>
<td>0.32 ± 0.05</td>
<td>0.15 ± 0.03*</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>% of actual activity</td>
<td>28.6 ± 4.4</td>
<td>20.6 ± 2.9</td>
<td>13.2 ± 3.3*</td>
<td>15.8 ± 2.9</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 rats in each group. *P<0.0125 vs. non-diabetic corresponding group; **P<0.0125 vs. sedentary rats in the same group.
ence of insulin. To clarify the effect of exercise on the glucose metabolism in skeletal muscle under insulin deficient state, the activity of PDH complex, the rate-limiting enzyme of aerobic glucose oxidation, was examined.

Previous studies have demonstrated that experimentally-induced diabetes in rat decreases the actual activity and the percentage of actual activity of PDH complex without changing the total activity in liver [18], heart [16] and skeletal muscle [17]. In the present study, we found that exercise training increased the actual activity and the percentage of actual activity of the PDH complex in rat skeletal muscles, which may play important roles in the oxidation of glucose. Another new finding in the present study is that the total activity of the complex was also decreased in the skeletal muscle of the diabetic rats, the result of which contradicts some previous findings [16–18]. These discrepancies could be explained by the fact that the degree of insulin deficiency and duration of diabetic conditions were different between the present study and the previous ones; the rats had been diabetic for a much longer period (4 weeks) in our study than other studies (<13 days) [16–18]. Another possible source of discrepancy lies in the methodological differences for the determination of the total activity of PDH complex; we added exogenous protein phosphatase to induce complete activation of the complex by dephosphorylation, whereas the others did not add phosphatase to the activation mixture. The observed decrease in the total activity of PDH complex in skeletal muscle of the diabetic rats was in accordance with the observation in rat heart [32]. It is noteworthy here that exercise training in diabetic rats increased the total activity of PDH complex to levels comparable to those observed in non-diabetic rats.

An interesting finding in the present study was that exercise training sometimes showed different effects on PDH complex between non-diabetic and diabetic rats. Exercise training significantly increased the total activity of PDH complex in diabetic rats but did not affect that in non-diabetic rats; it doubled the actual activity of the complex in diabetic rats but significantly decreased that in non-diabetic rats; and it did not affect the percentage of actual activity of the complex in diabetic rats, but significantly decreased that in non-diabetic rats. The observed decrease in the actual activity and the percentage of actual activity of PDH complex in non-diabetic trained rats could be explained as follows. Because exercise training increases fatty acid utilization by skeletal muscle [33], increased fatty acid oxidation elevates the acetyl-CoA/CoA and NADH/NAD ratios, which in turn induces the activation of PDH kinase, resulting in the phosphorylation of PDH complex or and the inactivation of PDH phosphatase resulting in diminished dephosphorylation. The induced activation of kinase and inactivation of phosphatase may be reflected as the decrease in the actual activity and the percentage of actual activity of PDH complex observed in the present study in non-diabetic rats [16, 34, 35]. On the other hand, the activity of PDH complex was increased in trained diabetic rats. Further investigation is necessary to clarify the different response of the PDH complex to exercise training between diabetic and non-diabetic rats.

Exercise training increased GLUT4 concentrations in the skeletal muscle in diabetic rats, an observation which is in agreement with the report of Osborn et al. [12] in rat heart. Because the amount of GLUT4 in skeletal muscle has been demonstrated to be correlated with the muscles' glucose uptake capacity [10, 11], the skeletal muscle of the trained diabetic rats in this study were theoretically assumed to have a higher capacity for glucose uptake than those in sedentary diabetic rats. Furthermore, the glucose uptake has been reported to be increased by exercise even in streptozotocin-induced diabetic rats [36]. Accordingly the increased glucose uptake to the skeletal muscles induced by exercise would stimulate intracellular enzymatic reactions to increase the actual activity of the complex through activation of PDH phosphatase or and inhibition of PDH kinase. In addition to this, it was reported that the PDH complex is activated by muscle contractions [37]. The actual activity and the percentage of actual activity of PDH complex were significantly higher in gastrocnemius muscle with the electrical contractions than those without the contractions both in non-diabetic and diabetic rats. Judging from these results, the observed increases in the actual activity of the PDH complex in trained diabetic rats may be induced, at least in part, by repeated activation of the complex generated by muscle contractions evoked during bouts of exercise training.

In conclusion, the beneficial effect of exercise training on glucose metabolism in streptozotocin-induced
diabetic rats has been shown to be result of the decrease in serum glucose level through two putative mechanisms; one is the enhancement of GLUT4 content and the other is the activation of PDH complex in skeletal muscle, part of which may be induced by repeated muscle contractions generated by prolonged periods of exercise training.

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