Insulin Activation of Pyruvate Dehydrogenase Complex Is Enhanced by Exercise Training

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We studied the effects of exercise training on the activity of the pyruvate dehydrogenase (PDH) complex in rat gastrocnemius muscle (experiment 1) and the response of the complex to glucose and insulin infusion (euglycemic clamp) in trained and sedentary rats (experiment 2). In experiment 1, half of the rats were randomly allocated as sedentary animals and the other half were trained by voluntary running exercise for 8 weeks. The total activity of the PDH complex was not affected by exercise training, and the activity state (proportion of the active form) of the PDH complex was decreased from 15.0% ± 2.4% to 7.5% ± 1.1% by exercise training. The activity of 3-hydroxyacyl-coenzyme A (CoA) dehydrogenase ([3-HADH] an enzyme in β-oxidation) was significantly higher in trained versus sedentary rats. In experiment 2, sedentary and trained rats were starved for 24 hours before performing the euglycemic clamp. Glucose and insulin infusion was performed by a euglycemic clamp (insulin infusion rate, 6 mU/kg/min) for 90 minutes. The PDH complex was inactivated to less than 1% in both sedentary and trained rats after 24 hours of starvation. The glucose infusion rate (GIR) during the euglycemic clamp was higher in trained versus sedentary rats. The euglycemic clamp resulted in activation of the PDH complex in both sedentary and trained rats, but the response of the PDH complex to the euglycemic clamp was significantly higher in trained rats (5.8% ± 0.5%) than in sedentary rats (2.9% ± 0.5%). These results suggest that exercise training promotes fatty acid oxidation in association with suppression of glucose oxidation in skeletal muscle under resting conditions, but increases the rate of carbohydrate oxidation when glucose flux into muscle cells is stimulated by insulin.

THE PYRUVATE DEHYDROGENASE (PDH) complex is believed to play a pivotal role in the regulation of intramuscular fuel metabolism. Full oxidation of glucose to carbon dioxide is dependent on the activity of the PDH complex, which catalyzes the conversion of pyruvate to acetyl-coenzyme A (CoA).[1] The PDH complex is subject to covalent modification: PDH kinase bound to the complex is responsible for inactivation of the complex by phosphorylation, and PDH phosphatase is responsible for activation of the complex by dephosphorylation.[2] Many studies on the regulation of the PDH complex have been performed under a variety of conditions in vivo. Inactivation of the PDH complex in rat skeletal muscle was demonstrated by experimental diabetes[3,4] and food deprivation.[5] On the other hand, refeeding,[6] muscle contractions,[7] and insulin infusion[8] activate the PDH complex in skeletal muscle. However, the effect of exercise training on the activity of the PDH complex has not been intensively studied, even though exercise training is known to alter intramuscular fuel oxidation.

Exercise training has been reported to improve whole-body insulin sensitivity in both the human and the rat.[9-11] Skeletal muscle from exercise-trained rats exhibits a significant increase in insulin-mediated glucose uptake.[12] In addition to the beneficial effect of exercise training on glucose metabolism, training also increases fatty acid utilization in skeletal muscle.[13] Increased fatty acid oxidation elevates the ratios of acetyl-CoA/CoA and $NADH/NAD^+$, which favor the phosphorylation and inactivation of the PDH complex.[14,15] From these effects on glucose and fatty acid metabolism, it is of interest to investigate whether the activity of the PDH complex is altered by exercise training.

In the present study, we examined the effects of exercise training on the activity of the PDH complex in rat skeletal muscle and the response of the PDH complex to the euglycemic clamp in exercise-trained and sedentary (untrained) rats.

MATERIALS AND METHODS

Materials

Female Wistar rats aged 3 weeks were obtained from CLEA Japan (Tokyo, Japan). [1-^{14}C]pyruvic acid (sodium salt) was purchased from Amersham Japan (Tokyo, Japan). The broad-specificity phosphoprotein phosphatase was prepared by a method reported previously.[16] All other reagents were of biochemical grade as described previously.[17]

Animal Care and Experimental Design

Rats were fed laboratory chow (CE-2; CLEA Japan) and tap water ad libitum and housed individually in a room with controlled temperature and light (12-hour light/dark cycle, lights on from 5 AM to 5 PM) conditions. All procedures involving animals were approved by the Experimental Animal Care Committee of Nagoya Institute of Technology.

Experiment 1 was designed to compare the activities of the PDH complex and its kinase in skeletal muscle of sedentary and trained rats. One week after arrival, animals (4 weeks of age) were randomly assigned to sedentary (n = 8) and trained (n = 8) groups. Each rat in the trained group was housed in a cage with a running wheel throughout the experimental period (8 weeks), which allowed the animals to run voluntarily in the wheel. Daily running distance for the trained group was recorded as wheel revolutions (1.16 m per revolution). On the final day of the experiment (12 weeks of age), rats were anesthetized at 3 PM with sodium pentobarbital (60 mg intraperitoneal injection/kg body weight) under the fed condition, and gastrocnemius muscle and blood were obtained. The muscle was immediately freeze-clamped at liquid nitrogen temperature and stored at −80°C until use. Serum was prepared from the blood and stored at −80°C until analysis. The trained rats were transferred to a regular cage (without the wheel) approximately 32 hours before killing, to minimize the effect of acute exercise.

Experiment 2 was designed to investigate the effect of glucose and insulin infusion (euglycemic clamp) on the activity of the PDH complex in sedentary and trained rats. Rats were trained for 8 weeks as described in experiment 1. All of the rats were starved for 24 hours before a

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euglycemic clamp study. On the final day, half of the rats in the sedentary and trained groups were randomly allocated to the euglycemic clamp study, and the other half were killed just before starting the euglycemic clamp study. Muscle samples and blood were collected from the rats as described in experiment 1:

Euglycemic Clamp Studies

The euglycemic clamp was performed after starving the rats for 24 hours as described previously.[18] At the end of the euglycemic clamp for 90 minutes (6 mU insulin infused/kg body weight/min), gastrocnemius muscles were removed, freeze-clamped, and stored at −80°C for subsequent analyses. The glucose infusion rate (GIR) was obtained during the last 30 minutes of the insulin infusion period (70, 80, and 90 minutes from initiation of infusion) when the steady state was attained.

Blood Analyses

Blood glucose was determined using a glucose analyzer (model 23A; Yellow Springs Instruments, Yellow Springs, OH). Serum nonesterified fatty acids (NEFAs) were determined with a commercial kit (Wako Pure Chemical Industries, Osaka, Japan). Serum insulin during the euglycemic clamp was determined by radioimmunoassay.[19]

Metabolite Analyses

Lactate and pyruvate in skeletal muscle were determined with a commercial kit (Wako Pure Chemical Industries). Gastrocnemius muscle was homogenized in 20-vol of 6% perchloric acid. After centrifugation at 10,000 × *g*, the supernatant obtained was neutralized with NaOH and used for spectrophotometric analysis. A reduced form of coenzyme A (CoASH) and acetyl-CoA in skeletal muscle were determined fluorimetrically.[20]

Enzyme Activity Analyses

The activity of the PDH complex was determined by the method of Nakai et al[21] with a slight modification. Frozen muscle was powdered at liquid nitrogen temperature, and approximately 50 mg powdered muscle was weighed and homogenized in an extraction buffer containing 50 mmol/L HEPES (pH 7.4 with KOH), 3% Triton X-100, 2 mmol/L EDTA, 5 mmol/L dithiothreitol, 0.5 mmol/L thiamine pyrophosphate, 2 mmol/L dichloroacetate (PDH kinase inhibitor), 2% bovine serum, 0.1 mmol/L *N*-tosyl-L-phenylalanine chloromethyl ketone, 0.1 mg/mL trypsin inhibitor, and 0.02 mg/mL leupeptin. The homogenate was divided into two portions for assay of actual (active/dephosphorylated enzyme) and total (fully activated/dephosphorylated enzyme) activities. For determination of the actual activity, 50 mmol/L potassium fluoride (phosphatase inhibitor) was immediately added and then centrifuged at 12,000 × *g* for 10 minutes. The supernatant obtained was used for the assay of actual activity. For measurement of total activity, the complex in the supernatant without potassium fluoride was fully activated using the broad-specificity phosphoprotein phosphatase[16] in the presence of 10 mmol/L $MgCl_2$ at 30°C for 20 minutes. The activity of the PDH complex was measured radiochemically at 30°C using [1-^{14}C]pyruvate as a substrate.[22] One unit of PDH complex catalyzed the formation of 1 μmol CO_2/min. The activity state of the PDH complex was expressed as a percentage of the active form of the enzyme relative to the total activity.

The activity of PDH kinase was assayed by measuring the rate of adenosine triphosphate (ATP)-dependent inactivation of the PDH complex.[17] Frozen muscle was homogenized and activated as described before, except using the extraction buffer without thiamine pyrophosphate and dichloroacetate, and then the extract was made to 6.5% (wt/vol) in polyethylene glycol and allowed to stand on ice for 20 minutes before centrifugation at 12,000 × *g* for 10 minutes. The pellet was suspended in a kinase assay buffer containing 20 mmol/L HEPES (pH 7.3 with KOH), 50 mmol/L potassium hydrogen phosphate, 1.5 mmol/L $MgCl_2$, 2 mmol/L dithiothreitol, and 20% glycerol. The samples containing PDH activity at approximately 1 U/mL were preincubated for about 3 minutes at 30°C, and then the reaction was initiated with the addition of 0.5 mmol/L ATP. Aliquots of the mixture were removed as a function of time (1 to 6 minutes) for the radiochemical assay of residual dehydrogenase activity. First-order rate constants were calculated from the data to compare the relative kinase activity. No inactivation of the PDH complex occurred in the absence of ATP during incubation for the assay of kinase activity.

The 3-hydroxyacyl-CoA dehydrogenase (3-HADH) in skeletal muscle was assayed by the method of Bradshaw and Noyes.[23] One unit of 3-HADH activity catalyzed 1 μmol NADH oxidized/min.

Statistics

Data are expressed as the mean ± SE. In experiment 1, data were analyzed by unpaired Student *t* test. In experiment 2, data were analyzed using two-way ANOVA. When significance was established, differences between individual groups of data were tested for significance using Fisher's paired least-significant difference test. A P value less than .05 was considered significant in both experiments.

RESULTS

Effect of Exercise Training on the Activity of PDH Complex and Its Kinase in Skeletal Muscle

The mean running distance for trained rats was 5.2 ± 0.7 km/d. Body weight on the final day of the experiment was not different between sedentary and trained groups. Blood glucose and serum NEFA levels were significantly lower in the trained group versus the sedentary group. The concentration of pyruvate was significantly lower in the trained group than in the sedentary group. The concentration of lactate in skeletal muscle was not different between sedentary and trained groups. CoASH and acetyl-CoA levels were significantly higher in the trained group versus the sedentary group, although the acetyl-CoA/CoASH ratio was not significantly different between the groups (Table 1).

The actual activity of the PDH complex was significantly lower in the trained group versus the sedentary group, although total activity of the PDH complex in skeletal muscle was not different between the groups. Since the total activity of the PDH complex was not different, the activity state showed the same

Table 1. Body Weight, Blood Substrates, Muscle Metabolites, and Enzyme Activities in Sedentary and Trained Rats

Parameter	Sedentary	Trained
Body weight (g)	237 ± 6	244 ± 7
Serum glucose (mmol/L)	8.77 ± 0.29	7.92 ± 0.23*
Serum NEFA (mmol/L)	0.136 ± 0.005	0.106 ± 0.007*
Pyruvate (μmol/g tissue)	0.130 ± 0.013	0.079 ± 0.012*
Lactate (μmol/g tissue)	1.58 ± 0.26	1.42 ± 0.19
CoASH (nmol/g tissue)	7.61 ± 0.59	9.72 ± 0.51*
Acetyl-CoA (nmol/g tissue)	0.80 ± 0.08	1.14 ± 0.09*
Acetyl-CoA/CoASH ratio	0.10 ± 0.01	0.12 ± 0.01
PDH complex		
Actual activity (U/g tissue)	0.18 ± 0.02	0.10 ± 0.01*
Total activity (U/g tissue)	1.21 ± 0.05	1.31 ± 0.07
Activity state (%)	15.0 ± 2.4	7.5 ± 1.1*
PDH kinase activity (min^{-1})	0.16 ± 0.01	0.20 ± 0.01*
3-HADH activity (U/g tissue)	4.09 ± 0.15	5.62 ± 0.22*

NOTE. Values are the mean ± SE for 8 rats.
*$P < .05$ *v* sedentary rats.

trend as the actual activity: the activity state of the PDH complex was significantly lower in the trained group versus the sedentary group (Table 1).

The activity of PDH kinase was significantly higher in the trained group than in the sedentary group (Table 1).

The activity of 3-HADH was significantly higher in the trained group versus the sedentary group (Table 1).

Effect of Euglycemic Clamp on the Activity of PDH Complex in Sedentary and Trained Rats

Body weight on the final day of the experiment was not different between sedentary and trained groups. The concentration of blood glucose was not different between sedentary and trained groups before or after the euglycemic clamp. The concentration of serum NEFA before the euglycemic clamp was lower in the trained group than in the sedentary group, and the euglycemic clamp markedly decreased the concentration in both sedentary and trained groups, resulting in no difference between the groups (Table 2).

The concentration of serum insulin was increased to approximately 150 μU/mL during the euglycemic clamp and was not different between sedentary and trained groups. The GIR was significantly higher in the trained group versus the sedentary group (14.10 ± 0.67 *v* 8.15 ± 0.38 mg/kg/min).

Pyruvate and lactate levels in skeletal muscle were not different between sedentary and trained groups before or after the euglycemic clamp. The euglycemic clamp decreased pyruvate and lactate concentrations in skeletal muscle. CoASH and acetyl-CoA levels in skeletal muscle were not different between sedentary and trained groups before or after the euglycemic clamp. The euglycemic clamp significantly decreased the concentration of acetyl-CoA and the acetyl-CoA/CoASH ratio in both sedentary and trained groups (Table 2).

The actual activity of the PDH complex in skeletal muscle before the euglycemic clamp was not different between sedentary and trained groups, but after the euglycemic clamp, it was twofold higher in the trained group versus the sedentary group. The total activity of the PDH complex in skeletal muscle before or after the euglycemic clamp was not different between sedentary and trained groups; the euglycemic clamp did not affect the total activity of the PDH complex. The activity state of the PDH complex before and after the euglycemic clamp showed the same trend as the actual activity (Table 2).

DISCUSSION

The present study demonstrates that the activity state (percentage of the active/dephosphorylated form) of the PDH complex in rat skeletal muscle was decreased by endurance training, although the total activity of the complex was not affected by the training. It is generally believed that the PDH complex is associated with intrinsic PDH kinase.[24] PDH kinase activity in skeletal muscle was elevated by exercise training, suggesting that the increased kinase activity is involved in the mechanisms responsible for reducing the activity state of the PDH complex in trained rats.

Endurance training has been suggested to promote fatty acid utilization in skeletal muscle.[13] The results obtained in the present study support this finding, because voluntary wheel-running increased the activity of 3-HADH, an enzyme in β-oxidation, in skeletal muscle. In addition, the concentration of acetyl-CoA in skeletal muscle was increased by exercise training, although the ratio of acetyl-CoA/CoASH was not affected. Since it has been shown that products of β-oxidation (acetyl-CoA and an increased ratio of NADH/NAD^+) stimulate PDH kinase,[15] promotion of β-oxidation is suggested to be involved in the mechanisms responsible for the decrease in the activity state of the PDH complex in skeletal muscle, as proposed in the glucose–fatty acid cycle.[25,26] In contrast, there was a discrepancy between the concentration of serum NEFA and the activity state of the PDH complex in the present study: both the activity state of the PDH complex and serum NEFA are lower in the trained group. The concentration of serum NEFA is the result of lipolysis from adipose tissue and utilization by other tissues. Collier et al[27] reported that inhibition of fatty acid oxidation with etomoxir (a specific carnitine palmitoyltransferase inhibitor) increased PDH activity without changing serum NEFA and triglyceride levels. These findings suggest that the utilization of fatty acids rather than the concentration of serum NEFA is more important to determine the activity state of the PDH complex. Pyruvate is also known as an activator of the

Table 2. Body Weight, Blood Substrates, Muscle Metabolites, and PDH Complex Activities in Rats Before and After the Euglycemic Clamp

Parameter	Before Clamp		After Clamp	
	Sedentary	Trained	Sedentary	Trained
Body weight (g)	230 ± 4	224 ± 3	226 ± 5	234 ± 4
Serum glucose (mmol/L)	3.72 ± 0.11	3.53 ± 0.07	3.57 ± 0.25	3.93 ± 0.16
Serum NEFA (mmol/L)	0.252 ± 0.005	0.218 ± 0.012*	0.075 ± 0.009†	0.069 ± 0.011†
Pyruvate (μmol/g tissue)	0.227 ± 0.030	0.234 ± 0.029	0.107 ± 0.017†	0.060 ± 0.009†
Lactate (μmol/g tissue)	7.15 ± 1.46	6.41 ± 1.07	1.18 ± 0.24†	0.68 ± 0.08†
CoASH (nmol/g tissue)	7.83 ± 0.56	8.84 ± 0.53	8.38 ± 0.26	9.47 ± 0.66
Acetyl-CoA (nmol/g tissue)	2.25 ± 0.20	2.32 ± 0.12	0.77 ± 0.09†	0.94 ± 0.11†
Acetyl-CoA/CoASH ratio	0.29 ± 0.03	0.27 ± 0.02	0.09 ± 0.01†	0.10 ± 0.01†
PDH complex				
Actual activity (U/g tissue)	0.012 ± 0.004	0.013 ± 0.004	0.037 ± 0.006†	0.072 ± 0.005*†
Total activity (U/g tissue)	1.14 ± 0.05	1.23 ± 0.03	1.31 ± 0.04	1.28 ± 0.06
Activity state (%)	0.8 ± 0.4	0.8 ± 0.2	2.9 ± 0.5†	5.8 ± 0.5*†

NOTE. Values are the mean ± SE for 8 rats.

*$P < .05$ *v* sedentary rats in the same group.

†$P < .05$ *v* before the euglycemic clamp.

PDH complex. It has been reported that formation of the active form of the PDH complex is increased by perfusion with pyruvate.[14] In the present study, the concentration of pyruvate in skeletal muscle was lower in trained rats than in sedentary rats, which may be associated with a lower activity state of the PDH complex in trained rats.

Since the activity state of the PDH complex reflects glucose oxidation,[1] the findings obtained in the present study suggest that exercise training decreased glucose oxidation under the fed condition. However, the present study and previous studies[10,18] found that exercise training increased insulin-stimulated whole-body glucose uptake. To solve this discrepancy, we examined the alteration of PDH complex activity caused by the euglycemic clamp in this study. For the euglycemic clamp study, rats were starved for about 24 hours, resulting in a very low activity state of the enzyme complex, as reported previously.[28-30] This starvation-induced inactivation of the enzyme complex was explained by promotion of fatty acid oxidation in association with elevation of circulating NEFA concentrations.[31,32] In fact, the concentration of acetyl-CoA and the acetyl-CoA/CoASH ratio in the present study were increased by starvation.

The euglycemic clamp did not affect the total activity of the PDH complex, but the activity state of the enzyme complex after the euglycemic clamp was twofold higher in trained rats versus sedentary rats. The GIR during the euglycemic clamp was significantly higher in trained rats versus sedentary rats. It has been reported that during the euglycemic clamp, greater than 85% of glucose uptake occurs in skeletal muscle.[33] These findings suggest that glucose flux into skeletal muscle during the euglycemic clamp was greater in trained rats than in sedentary rats. Greater than 60% of glucose was phosphorylated into glycogen during the euglycemic clamp,[33] and the remaining glucose might be degraded by the glycolytic pathway and oxidized through the PDH complex. The higher activity state of the PDH complex in trained rats after the euglycemic clamp may result from greater uptake of glucose into skeletal muscle. However, we did not measure substrate oxidation such as glucose, pyruvate, and lactate. To evaluate the regulation of energy metabolism by the PDH complex in vivo, measurement of substrate oxidation rates may be needed in future studies. Another possible explanation for the higher activity state of the PDH complex in trained rats after the euglycemic clamp is that exercise training might increase PDH phosphatase, which is activated by insulin.[8,34]

In conclusion, exercise training decreased the activity state of the PDH complex under the fed condition and increased the activity state of the PDH complex in response to insulin and glucose infusion. These findings suggest that under the fed condition, exercise training results in glucose sparing by inactivation of the PDH complex. However, exercise training increases the capability for glucose oxidation in skeletal muscle when glucose flux into skeletal muscle is stimulated.

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