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Exercise Decreases Cytosolic Aconitase Activity in the Liver, Spleen, and Bone Marrow in Rats

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Effects of strenuous exercise on cytosolic aconitase activity (CAA) were investigated in this study. Female Sprague-Dawley rats were randomly assigned to four groups: S1 (Sedentary), S2 (Sedentary + L-NAME [*N*-nitro-L-arginine methyl ester]), E1 (Exercise), and E2 (Exercise + L-NAME). Rats in the E1 and E2 groups swam for 2 h/day for 3 months. L-NAME (an inhibitor of NOS) in drinking water (1 mg/ml) was administered to rats in the S2 and E2 groups for the same period. At the end of the third month, the CAA in the liver, spleen, and bone marrow cells was measured. In the exercise group (E1), CAA in the liver, spleen, and bone marrow cells was 19.99 ± 1.49, 1.61 ± 0.13, and 0.59 ± 0.09 mU/mg protein, respectively. These values were significantly lower than the corresponding sedentary values in the S1 group (33.96 ± 1.38, 3.96 ± 0.19, and 3.20 ± 0.18 mU/mg protein) ($P < 0.01$, 0.001, and 0.001, respectively). The treatment of L-NAME led to a significant increase in tissue CAA in the sedentary rats (S2). Also, the significantly higher CAA in the liver, spleen, and bone marrow cells was found in the exercised rats treated with L-NAME (E2) (29.50 ± 1.27, 2.89 ± 0.25, and 1.34 ± 0.20 mU/mg) than without L-NAME (E1) ($P < 0.01$, 0.01, 0.05, respectively). However, the values in the E2 group were still significantly lower than those in the S1 group ($P < 0.05$, 0.01, and 0.001, respectively). This indicates that L-NAME treatment can partly recover the decreased CA in tissues in the exercised rats. These results provide evidence for the existence of the increased activity of IRP1 (iron regulatory protein 1) that is probably induced by the increased nitric oxide production in the strenuously exercised rats. © 2001 Academic Press

Key Words: **strenuous exercise; cytosolic aconitase activity (CAA); iron-regulatory protein 1; iron metabolism; nitric oxide (NO); L-NAME (*N*-nitro-L-arginine methylester).**

In a previous study (1), we found that strenuous exercise could lead to a significant increase in transferrin receptor (TfR) expression and transferrin-bound iron (Tf-Fe) accumulation in bone marrow erythroblasts in rats. In addition, plasma iron concentration and non-heme iron contents in the liver, spleen and some other tissues were lower in the exercised rats than in the sedentary animals. Further study (2) showed that the exercised rats had a significantly increased plasma nitric oxide (NO) which was negatively correlative with the decreased plasma iron level. Thereafter, we investigated the effects of strenuous exercise and L-NAME (*N*-nitro-L-arginine methylester), an inhibitor of NOS (nitric oxide synthase), on tissue NO concentrations as well as non-heme iron contents (3). The results indicate that exercise may lead to a significant increase in NO level as well as a decrease in iron contents in the liver, spleen, and bone marrow cells. The effects of exercise on NO level and iron content in these tissues could be inhibited completely (NO) and partly (iron) by L-NAME treatment respectively. These data and the recent findings on interaction of NO with iron metabolism (4–7) suggest that the increased NO might be one of the causes leading to the changes of iron metabolism we found in the exercised rats (3).

The exercised rats treated with L-NAME have a significantly lower level of NO as well as a higher iron content in the tissues and plasma than those of the exercised rats treated without L-NAME (3). It implies that strenuous exercise might stimulate the activity of NOS and hence increase NO synthesis, while L-NAME, probably via inhibition of NOS activity, could reduce the effect of exercise on NO and hence partly recover the decreased plasma and tissue iron levels in the exercised rats. NO regulates iron metabolism by interaction with iron-regulatory protein 1 (IRP1) (4, 5). IRP1 is a [Fe-S] protein. IRP1 binding to IRE (iron responsive element) of both mRNAs of TfR and ferritin is regulated in response to the status of the iron sulfur cluster located near the center of IRP1 (5, 8, 9). In iron-abundant cells, IRP1 contains a cubane

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4Fe-4S cluster that prevents IRE binding and displays aconitase activity. In iron-poor cells, such a Fe-S cluster does not exist and IRP1 functions as an IRE-binding protein (5). In other words, IPR1 can function as a cytoplasmic aconitase as well as IRE-binding protein or cytosolic aconitase is a two-faced protein: enzyme and IRP1 (10–12). In addition to the effect of cellular iron, NO may target iron atoms in [Fe-S] cluster in the IRP1 molecules and result in an increased IRP1 binding activity. Therefore it is highly likely that the increased NO-induced by exercise may lead to an increased binding of IRP1 to IRE of both mRNAs of TfR and ferritin (or the decreased activity of cytosolic aconitase) and then the changes of iron metabolism in the exercised rats. Because the changes in cytosolic aconitase activity may reflect the IRP1 binding activity, therefore, we investigated the effect of exercise and L-NAME on cytosolic aconitase activity in the present study. The aims were to find the evidence for the existence of an increased IRP1 activity and to address the possible roles of the increased NO in the changes of cytosolic aconitase activity and iron metabolism in the exercised rats.

MATERIALS AND METHODS

Animals and exercise protocol. The Department of Health of Hong Kong Government and the Animal Ethics Committee of the Hong Kong Polytechnic University approved the use of animals for this study. Female Sprague-Dawley (SD) rats (aged 2 months), supplied by the Animal House of the Hong Kong Polytechnic University, were housed in pairs in stainless steel rust-free cages at 21 ± 2°C, relative humidity of 60–65% with alternating 12-h periods of light and dark. After being kept under the standard laboratory conditions for one week, the animals were randomly assigned to the following four groups: S1 (n = 5), Sedentary; S2 (n = 5), Sedentary + L-NAME (*N*-nitro-L-arginine methyl ester); E1 (n = 4), Exercise; E2 (n = 6), Exercise + L-NAME. Laboratory rodent diets for rats (PMI Nutrition International, the Richmond Standard) and distilled water were given free access to throughout the experimental period.

Swimming exercise was performed according to the procedure described previously (1). In brief, rats in the exercise groups (E1 and E2) swam in groups of two or three in glass swimming basin (45 cm width × 80 cm length × 80 cm height) filled with tap water to a depth of 50 cm. The water temperature was maintained at 35 ± 1°C. The rats swam 5 days per week. The daily training lasted for 30 min in the first week and 1 h in the second week. The two-weeks' swimming period was considered as a training period so that increased exercise could be tolerated later. After the training period, 2 h exercise per day (9.00–11.00 am) was given, lasting for 3 months. L-NAME (Calbiochem Co., USA) in the drinking water (1 mg/ml) was freshly prepared and orally administered to rats in the S2 and E2 groups for 3 months. The rats of the control groups (S1 and S2) remained sedentary in their cages and received approximately the same amount of handling as the exercised animals throughout the entire experiment.

Measurement of cytosolic aconitase activity in the liver, spleen, and bone marrow cells. At the end of the three-months experiment, animals were not fed for 24 h following the last exercise regimen. The rats were anesthetized with diethyl ether and the liver and spleen were removed and stored in a freezing chamber below −70°C. The blood in the liver and spleen was washed out by a perfusion of 0.9% saline (iron free) before the samples were stored. The bone marrow cells were isolated from both femora and tibiae by rapidly splitting scraped bones according to the method described previously (1). The tissue and cells were homogenized in 0.27 M sucrose buffered to pH 7.4 with Hepes and then centrifuged at 10,000*g* at 4°C for 15 min. The supernatant obtained was recentrifuged at 35,000*g* at 4°C for 30 min and used as "cytosol." The diluted supernatant was added into 20 mM aconitate substrate solution (pH 7.4) containing 0.02% BSA. Cytosolic aconitase activity was determined by the measurement of changes in absorbance of 240 nm at intervals of 15 s at 25°C for a few minutes. Bovine heart aconitase (purified) was run in parallel to samples and applied to establish standard curve. On the basis of the standard curve, cytosolic aconitase activity was calculated. The results in enzymatic activity were expressed miliunits (mU) per mg of protein.

Analytical methods. Protein contents of tissue and cell homogenates were assayed by Lowry's method using commercially prepared kits (Sigma Co., St. Louis, MO). The statistical calculation was performed using the Student's t test. The data were expressed as means ± standard error (SE).

RESULTS

Effects of exercise on cytosolic aconitase activities in the liver, spleen, and bone marrow. The results showed that strenuous exercise led to a significant decrease in cytosolic aconitase activities in the liver, spleen and bone marrow cells (Fig. 1). The cytosolic aconitase activities were 33.96 ± 1.38, 3.96 ± 0.19, and 3.20 ± 0.18 mU/mg protein in the liver, spleen, and bone marrow cells in the sedentary group (S1), respectively. As compared to sedentary group, the corresponding values in the exercise group (S1) were significantly lower, being 19.99 ± 1.49, 1.61 ± 0.13, and 0.59 ± 0.09 mU/mg protein in the liver, spleen, and bone marrow cells ($P < 0.01$, 0.001, and 0.001), respectively. The strenuous exercise produced 41.1 (liver), 59.3 (spleen), and 81.6% (bone marrow cells) decrease of cytosolic aconitoase activity respectively.

Effects of L-NAME on cytosolic aconitase activity in the liver, spleen, and bone marrow. To determine the role of NO in regulation of aconitase activity during exercise, L-NAME was administered into the exercised (E2) and the sedentary rat (S2). The sedentary rats treated with L-NAME (S2) had significantly higher activities of cytosolic aconitase in the liver (38.81 ± 1.12), spleen (4.56 ± 0.17), and bone marrow cells (4.00 ± 0.22 mU/mg protein) than those of the sedentary rat treated without L-NAME (S1) (all $P < 0.05$) (Fig. 1). The degree of increase was 14.3, 15.2, and 25.0% in the liver, spleen, and bone marrow cells, respectively. Also, significantly higher values of cytosolic aconitase activities in the liver, spleen and bone marrow cells were observed in the exercised rats treated with L-NAME (E2) than without L-NAME (E1). The cytosolic aconitase activities were 29.50 ± 1.27, 2.89 ± 0.25, and 1.34 ± 0.20 mU/mg protein in the liver, spleen, and bone marrow cells, respectively, in the exercised rats treated with L-NAME (E2). These values were significantly higher than their corresponding control values in the exercised rats treated without

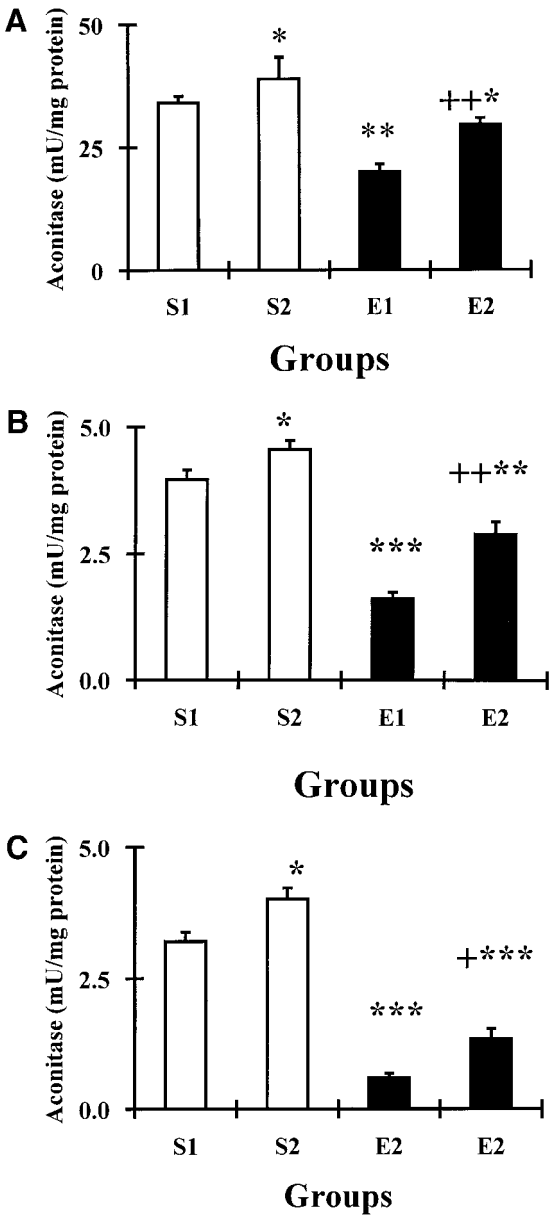

FIG. 1. Effects of strenuous exercise and L-NAME on cytosolic aconitase activities in the liver (A), spleen (B), and bone marrow cells (C) of rats. Female SD rats were assigned to the four groups: S1 (Sedentary); S2 (Sedentary + L-NAME); E1 (Exercise); E2 (Exercise + L-NAME). The exercise procedure and L-NAME treatment were detailed in the text. The data were means ± SE. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs S1 and $^{+}P < 0.05$, $^{++}P < 0.01$ vs E1.

L-NAME (E1) ($P < 0.01$, 0.01, 0.05, respectively). The degree of increase was 47.6, 79.5, and 127.1% in the liver, spleen, and bone marrow cells, respectively. However, these increased values in the exercised rats treated with L-NAME (E2) were still significantly lower than those in the sedentary rats treated without L-NAME (S1), being about 86.7, 72.9, and 41.9% of the corresponding sedentary values. *P* values were smaller than 0.05, 0.01, and 0.001 in the liver, spleen, and bone marrow cells, respectively (Fig. 1).

DISCUSSION

Cytosolic RNA-binding proteins known as iron-regulatory proteins (IRP1 and IRP2) control regulation of cellular iron homeostasis. Although IRP1 and IRP2 are similar proteins, they are regulated by different mechanisms. IRP2 does not contain a 4Fe-4S cluster similar to the cluster in IRP1 (13). As mentioned in Introduction, IRP1 and cytosolic aconitase are two forms of one protein. The determination of cytosolic aconitase activity has quantitatively been applied to observe cytosolic IRP1 activity and the molecular regulatory mechanism of iron metabolism in vivo and vitro (14–16). In this study, we demonstrated that strenuous exercise could lead to a significant decrease in activity of cytosolic aconitase in the liver, spleen and bone marrow cells. It provided evidence for the existence of the increased IRP1 activity and hence IRE-binding by IRP1 in these tissues and cells in the exercised rats. This result is in good agreement with our previous findings; namely, a significantly increase in TfR expression and Tf-Fe uptake in bone marrow cells in the strenuously exercised rats (1). The increased NO production might play a critical role in the exercise induced-decrease in cytosolic aconitase activity or increase in IRP1 binding activity with IRE. It is supported by the findings obtained in this study as well as our previous investigation. In the present study, results showed that L-NAME (NOS inhibitor) treatment led to a significant increase in cytosolic aconitase activity in the exercised rats when compared with the exercised rats treated without L-NAME. Our previous study (3) found that the exercised rats treated with L-NAME had a significantly lower NO level as well as a higher iron content in the liver, spleen, and bone marrow cells than the exercised rats treated without L-NAME. These data showed that the higher cytosolic aconitase activity, found in the exercised rats treated with L-NAME, might be due to the inhibition of L-NAME on NOS activity. Therefore NO level in the tissues decreases and accordingly cytosolic aconitase activity increases. The increased cytosolic aconitase or the decreased IRP1 activity in the exercised rats treated with L-NAME would lead to the lower TfR expression and Tf-Fe uptake as well as the higher ferritin expression. The decreased NO in the exercised rats induced by L-NAME treatment could also reduce the amount of iron removal from ferritin (17). It was probably one of causes for the increased iron contents found in the exercised rats treated with L-NAME.

However, L-NAME treatment did not lead to the completed recoveral of cytosolic aconitase activities and iron contents in these tissues or cells to the sedentary level. The cytosolic aconitase activities and iron

contents in the exercised rats treated with L-NAME, although higher than those in the exercise control rats, were still significantly lower than those found in the sedentary rats (3). Obviously, it was not due to the dosage of L-NAME used because tissue NO level did fully return to sedentary level by treatment of L-NAME, no difference being found in NO levels between the exercised rats treated with L-NAME and the sedentary rats. Therefore the difference in the effects of L-NAME on aconitase activities, iron contents and NO levels might imply that NO was not the only factor in the determination of cytosolic aconitase activities in the exercised rats. Other factors, including oxidative stress (18), phosphorylation (19), and hypoxia (20), might be also involved in the control of the cytosolic aconitase (or IRP1) activities and iron contents in these tissues and cells. Further investigation is needed.

In summary, the results obtained in this study indicated that strenuous exercise could lead to the decreased cytosolic aconitase activities in the liver, spleen, and bone marrow cells. The data on L-NAME treatment showed that the decreased cytosolic aconitase activities (or the increased IRP binding activities) might result from the increased NO production-induced by exercise. The results of this and our previous studies demonstrated that there was difference in the effects of L-NAME on cytosolic aconitase, iron contents and NO levels in these tissues. The difference suggests that the increased NO was important but not the only cause led to the decreased cytosolic aconitase activities and iron contents in the tissues and cells examined in the exercised rats.

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