Increased expression of transferrin receptor on membrane of erythroblasts in strenuously exercised rats

ZHONG MING QIAN, DE SHENG XIAO, PAK LAI TANG, FIONA YAN DONG YAO, AND QING KUI LIAO
Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Kowloon, Hong Kong

Increased expression of transferrin receptor on membrane of erythroblasts in strenuously exercised rats. J. Appl. Physiol. 87(2): 523–529, 1999.—This study investigated the effects of strenuous exercise on transferrin (Tf)-receptor (TfR) expression and Tf-bound iron (Tf-Fe) uptake in erythroblasts of rat bone marrow. Female Sprague-Dawley rats were randomly assigned to either an exercise or sedentary group. Animals in the exercise group swam 2 h/day for 3 mo in a glass swimming basin. Both groups received the same amount of handling. At the end of 3 mo, the bone marrow erythroblasts were freshly isolated for Tf-binding assay and determination of Tf-Fe uptake in vitro. Tissue nonheme iron and hematological iron indexes were measured. The number of Tf-binding sites found in erythroblasts was 5'674,500 ± 132,766 and 1,270,011 ± 235,321 molecules/cell in control and exercised rats, respectively (P < 0.05). Total Fe and Tf uptake by the cells was also significantly increased in the exercised rats after 30 min of incubation. Rates of cellular Fe accumulation were 5.68 and 2.58 fmol·10^6 cells^-1·min^-1 in the exercised and control rats, respectively (P < 0.05). Tf recycling time and TfR affinity were not different in exercised and control rats. Increased cellular Fe was mainly located in the stromal fraction, suggesting that most of accumulated Fe was transported to the mitochondria for heme synthesis. The findings demonstrated that the increased cellular Fe uptake in exercised rats was a consequence of the increased TfR expression rather than the changes in TfR affinity and Tf recycling time. The increase in TfR expression and cellular Fe accumulation, as well as the decreased serum Fe concentration and nonheme Fe in the liver and the spleen induced by exercise, probably represented the early signs of Fe deficiency.

iron is the trace mineral that has been studied most extensively with respect to exercise. It has been reported that strenuous exercise may adversely affect iron metabolism and contribute to low-iron status characterized by reduced blood hemoglobin, serum iron, and ferritin concentration in both animals and human athletes (5, 24, 33, 36, 40). However, it is still unknown whether the observed low-iron status indicates a true iron deficiency or a false impression of the iron-deficient status (24, 40). In addition, the mechanism by which strenuous exercise produces this change in iron status is not well determined. Several explanations for this low-iron status induced by exercise have been suggested, such as intravascular hemolysis due to the increased mechanical destruction of red blood cells; significant increase in plasma volume relative to red cell mass (blood dilution); negative iron balance between gain and loss (increased iron loss in urine, sweat, and feces and decreased intestinal iron absorption); iron redistribution from the reticuloendothelial system to the hepatocytes due to hemolysis; or suppression of erythropoiesis due to the decrease in erythropoietin synthesis (6, 24, 37, 40). However, these explanations do not appear to be valid, at least in some cases. For example, it was reported that mean serum ferritin decreased to 32 µg/l in elite male distance runners (10). However, median serum ferritin for adult men is 94 µg/l (7). If this low plasma ferritin is due to an expanded plasma volume, then this would require a threefold expansion of the plasma volume, which is highly unlikely (10). Magazanik et al. (22) also reported that the decrease in serum iron levels of subjects after the second week of training cannot be related to plasma volume expansion.

Until now, however, almost all research on the relationship between exercise and iron status has been focused on observations of the changes in the iron balance between gain and loss. The data generated were mainly obtained from the measurement of tissue iron and hematological iron indexes (5, 8, 10, 22, 24, 27, 33, 34, 36, 39, 40), and little is known about the effect of strenuous exercise on the mechanism of iron uptake by some important cells in iron utilization and metabolism, such as bone marrow cells. Therefore, it is necessary to investigate further the effect of strenuous exercise on iron metabolism, including studies at cellular and/or molecular levels to elucidate the precise mechanisms of strenuous-exercise-induced low-iron status. As in other iron-utilizing cells, the mechanism of iron uptake by the bone marrow cells also involves the processes of transferrin-bound iron and non-transferrin-bound iron uptake. It is generally believed that transferrin and transferrin-receptor-mediated iron delivery is the main route for cellular iron accumulation in vivo. This process is initiated with the binding of transferrin-iron to receptors on the cell membrane, and then transferrin-iron enters into the cell by endocytosis of the receptor-transferrin-iron complex (30, 31). The number of transferrin receptors on cell membrane is an important factor affecting the ability of cells to take up iron from transferrin (12). By controlling the level of transferrin-receptor expression, cells can determine the amount of iron acquired. Transferrin-receptor expression, in turn, is regulated by in-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
tracellular iron level. When the intracellular iron level falls, the transferrin-receptor level rises.

Bone marrow cells are the major iron-transporting and -utilizing cells in the body. Hence, this cell type was used in the present study to investigate the effect of strenuous exercise on the expression of transferrin receptor and the mechanism of transferrin-receptor-mediated iron uptake in rats.

**METHODS**

Materials. 
$^{59}$Fe ($\text{FeCl}_3$, 5 µCi/µg) and $^{125}$I Na (carrier free) were purchased from Radiochemical Center (Amersham). Rat apotransferrin, bovine serum albumin, HEPES, Sephadex G50, hemoglobin assay kit, dibutyl phthalate, and Histopaque were obtained from Sigma Chemical (St. Louis, MO). Transferrin was labeled with $^{125}$I and $^{59}$Fe as previously described (29). Pronase was purchased from Boehringer-Mannheim (Mannheim, Germany).

Animals and exercise protocol. Female Sprague-Dawley rats (weighing 190 ± 20 g), 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 12:12-h dark-light periods. After being kept under the standard laboratory conditions for 1 wk, the animals were randomly assigned to either an exercise group ($n = 8$) or sedentary control group ($n = 6$). Laboratory rodent diet for rats (PMI Nutrition, the Richmond Standard) and distilled water were provided ad libitum throughout the experimental period. Swimming was performed by a modification of the method of Ruckman and Sherman (34) and Prasad and Pratt (27).

The rats in the exercise group swim in groups of three in a 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/wk. The daily training lasted for 30 min in the first week and 1 h in the second week. The 2-wk swimming period was considered as a training period (34) so that increased exercise could be tolerated later. After the training period, 2 h of exercise per day were given and lasted for 3 mo. The rats in the control group remained sedentary in their cages and received an approximately the same amount of handling as the exercised animals throughout the entire experiment.

Blood, tissue samples, and preparation of bone marrow mononuclear cells. At the end of the 3-mo experiment, animals were fasted for 24 h after the last exercise regimen and then heparinized and anesthetized with diethyl ether. Fast-is blood samples were collected after death, and aliquots were taken immediately for hemoglobin, iron, and hematocrit determination. The liver, spleen, kidney, heart, and adrenal gland were removed, weighed, and stored in a freezing chamber at −70°C for subsequent determination of tissue nonheme iron concentrations. The bone marrow cells were isolated from both femurs and tibiae by rapidly splitting scraped bones and were suspended in ice-cold saline buffered with 20 mM HEPES (pH 7.4) at 4°C. The crude cell suspension was washed three times with ice-cold saline buffered with 20 mM HEPES, pH 7.4, containing 2% BSA. Subsequently, the cells were suspended in the same buffer to a final concentration of $1 \times 10^6$ cells/ml. The cell suspension was kept at 4°C for no longer than 3 h before use. It was found that $\sim 1.8 \times 10^6$ mononuclear cells were obtained in each rat. To determine the percentage of erythroblasts in cell suspensions, cell counts were carried out on dried cell smears stained with May-Grunwald-Giemsa stains.

Transferrin-binding assay. Binding assay was performed as described by Muta et al. (23). Briefly, $2 \times 10^6$ mononuclear cells were incubated with labeled transferrin in various amounts, ranging from 0.1- to 70 nM in a total incubation volume of 0.1 ml HBSS buffered with HEPES (pH 7.4) containing 2 mg/ml of BSA at 4°C for 60 min. For nonspecific binding, 7 µM of unlabeled ferric transferrin were added. All assays were done in triplicate. At the end of the incubation period, the cell samples were placed on top of 120 µl of dibuty1 phthalate-toluene, 4.5:1, in a 400-µl Eppendorf centrifuge tube and then centrifuged at 5,000 g for 1 min at 4°C. The bottom of the tube containing the cell pellet was cut off, and radioactivity was counted by a gamma counter. The transferrin-binding data were transformed to Scatchard plots. The number of transferrin receptors per cell and the apparent dissociation constants were calculated from Scatchard plot analysis.

Transferrin-mediated iron uptake. The cell suspensions were prewarmed in a shaking water bath for 10 min at 37°C. Labeled transferrin ($^{59}$Fe, $^{125}$I) was then added at a final concentration of 0.5 µM in a total volume of 3.2 ml of HBSS containing 2 mg/ml BSA. After the desired incubation periods, 100 µl of incubation suspension containing $2 \times 10^6$ mononuclear cells were removed. The cellular transferrin and iron uptakes were immediately stopped by diluting the cell suspension with 40 vol of ice-cold PBS (16). After being washed three times with 2 ml of cold PBS, the cells were incubated with 200 µl of Pronase (1 mg/ml) in ice-cold PBS for 30 min at 4°C. This led to the release of receptor-bound transferrin on the outer cell membrane and allowed separation of membrane-bound and intracellular radioactive transferrin and iron. For the measurement of transferrin and iron in the cytosolic and stromal fractions, the cell suspension was washed three times with ice-cold saline buffered to pH 7.4 with 20 mM HEPES, transferred to a new tube, and hemolyzed with buffer-detergent solution (Tris-buffered saline, pH 7.2, containing 0.1% Triton X-200). The cytosolic and stromal fractions were then separated by a centrifuge as described earlier (28). Each fraction was counted for radioactivity.

Analytic methods. Cell counts were made by using a hemocytometer. Hemoglobin concentration was determined by cyanmethemoglobin method (4), and hematocrit was measured by the microhematocrit centrifuge. Tissue nonheme iron concentrations were measured according to Kaldor (17). Plasma iron and total iron-binding capacity were determined by using commercial kits (Sigma Chemical).

Both $^{125}$I transferrin and $^{59}$Fe transferrin radioactivity were measured in a three-channel gamma counter (Packard 5003 COBRA Q). Fractal analysis was applied to calculate the rates of intracellular transferrin according to the method described previously (32). The data were expressed as means ± SE. The statistical calculation was performed by using Student's t-test.

**RESULTS**

Effect of strenuous exercise on body and organ weights, hematologic indexes, and tissue nonheme iron concentrations. The average body weights and most of the organ weights in the two groups did not differ significantly at the end of the 3-mo period (Table 1). Heart weight in the exercised rats was significantly higher ($P < 0.01$) than that in the control animals. This result confirmed the observations in several studies using swimming for exercising rats (8, 15). It indicated that exercised rats might have an adaptation resulting in an increase in
the capacity of the heart to deliver more blood to the muscle and other tissues. Liver and spleen weights were not affected by exercise, and this is in keeping with the observations of Gagne et al. (8) and Ruckman and Sherman (34).

Blood hemoglobin concentration and hematocrit in the two groups were not significantly different (P > 0.05) (Table 2). However, plasma iron and transferrin saturation (%) were significantly decreased in exercised rats compared with control animals (P < 0.05). No significant difference was found in total iron-binding capacity in the two groups, indicating no marked effect of exercise on the concentration of plasma transferrin (Table 2). Table 3 reports the mean nonheme iron concentrations in the liver, spleen, kidney, heart, and brain. Liver, spleen, kidney, and heart iron concentrations were significantly lower in exercised rats than in control animals. Exercise resulted in a reduction of iron stores in these organs. The above results showed that exercise could induce a low-iron status in rats without anemia.

Binding of [125I]transferrin to erythroblasts. The bone marrow mononuclear cells were incubated with radioactively iodinated transferrin at 4°C, a temperature at which only receptor binding takes place on the cell surface (16, 19). Our preliminary experiments showed that saturation of specific transferrin-binding sites was found to occur at a transferrin concentration of 35 nM. This concentration was similar to that obtained previously from human erythroblasts (23). Scatchard analysis of the transferrin-binding data was performed to determine the apparent dissociation constant and the number of specific transferrin receptors per cell (Fig. 1).

Table 1. Body and organ weights of strenuously exercised and control rats at the end of 3-mo experimental period

<table>
<thead>
<tr>
<th></th>
<th>Exercised (n = 8)</th>
<th>Control (n = 6)</th>
<th>P (vs. Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body</td>
<td>291.5 ± 7.1</td>
<td>283.4 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Liver</td>
<td>10.1 ± 0.39</td>
<td>10.4 ± 4.3</td>
<td>NS</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.501 ± 0.017</td>
<td>0.504 ± 0.021</td>
<td>NS</td>
</tr>
<tr>
<td>Heart</td>
<td>1.066 ± 0.027</td>
<td>0.960 ± 0.026</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Lung</td>
<td>1.606 ± 0.172</td>
<td>1.895 ± 0.370</td>
<td>NS</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.130 ± 0.03</td>
<td>2.090 ± 0.061</td>
<td>NS</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.259 ± 0.022</td>
<td>0.218 ± 0.032</td>
<td>NS</td>
</tr>
<tr>
<td>Brain</td>
<td>1.976 ± 0.022</td>
<td>1.917 ± 0.040</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats; NS, not significant.

Table 2. Hematological indexes of iron status of strenuously exercised and control rats at the end of 3-mo experimental period

<table>
<thead>
<tr>
<th></th>
<th>Exercised (n = 8)</th>
<th>Control (n = 6)</th>
<th>P (vs. Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/dl</td>
<td>14.2 ± 1.3</td>
<td>15.3 ± 0.5</td>
<td>NS</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>39.2 ± 0.64</td>
<td>41.3 ± 1.06</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma iron, µg/dl</td>
<td>119.9 ± 13.05</td>
<td>217.8 ± 21.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TIBC, µg/dl</td>
<td>415.9 ± 5.23</td>
<td>400.8 ± 14.3</td>
<td>NS</td>
</tr>
<tr>
<td>TS, %</td>
<td>28.8 ± 1.51</td>
<td>54.5 ± 2.53</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats; TIBC, total iron-binding capacity; TS, transferrin saturation (%) = plasma iron/TIBC.

Table 3. Measurements of tissue nonheme iron in strenuously exercised and control rats at the end of 3-mo experimental period

<table>
<thead>
<tr>
<th></th>
<th>Exercised (n = 8)</th>
<th>Control (n = 6)</th>
<th>P (vs. Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.174 ± 0.006</td>
<td>0.236 ± 0.011</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.193 ± 0.080</td>
<td>1.832 ± 0.259</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.055 ± 0.003</td>
<td>0.076 ± 0.006</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>0.019 ± 0.001</td>
<td>0.022 ± 0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Brain</td>
<td>0.010 ± 0.001</td>
<td>0.011 ± 0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD. n, No. of rats.

The apparent dissociation constants were 12.7 ± 0.27 nM in the exercised rats and 11.7 ± 1.10 nM in the sedentary rats. Neither constant differed significantly from the other (P > 0.05), indicating no effects of exercise on the binding affinity of receptors for transferrin.

Morphological or functional studies about transferrin receptors (16, 23, 26) have indicated that almost all transferrin receptors in bone marrow cells are present on the erythroblast. In this study, the receptor numbers per cell were estimated by dividing the total number of receptors by the amount of total erythroid cells in the

Fig. 1. A: specific [125I]transferrin (Tf) binding to rat erythroblasts of strenuously exercised rats (○) and control animals (●). Saturation concentration of binding is ~35 nM of transferrin. For assay condition, see METHODS. Symbols represent means of assays in triplicate. B: Scatchard plots of saturation results shown in A.
cell suspension. Because erythroblast populations in the samples used were heterogeneous with regard to the stage of their development, the cells in different stages of development had different numbers of transferrin receptors. Therefore, the receptor level obtained was an average value for all types of erythroblasts present. In the sedentary rats, the average number of surface transferrin-binding sites was 674,500 ± 132,766 molecules/erythroblast. This average value was found to be slightly higher than that for human erythroblasts reported by Muta et al. (23) and was in good agreement with the result obtained from rat erythroblasts reported by Intragumtornchai et al. (14). Compared with those in control rats, erythroblasts in exercised rats had a significantly higher level of specific surface transferrin-binding sites (P < 0.05), reaching an average value of 1,270,011 ± 235,321 molecules/erythroblast. This value was about twofold higher than that in control rats. It indicated that strenuous exercise could significantly increase the expression of transferrin receptors on the surface of bone marrow erythroblasts.

Time course of \(^{59}\)Fe transferrin internalization and receptor-mediated iron uptake. The time courses of internalization of labeled transferrin and receptor-mediated iron uptake in erythroblasts were observed in the exercised and sedentary rats. The cells were incubated with 0.5 µM of labeled transferrin (\(^{59}\)Fe, \(^{125}\)I) for the various periods at 37°C. The approaches employed by Karin and Mintz (18) were used here to release cell-surface-bound ligand with Pronase digestion and to determine the internalization of transferrin. Transferrin internalization increased with the incubation time in a linear manner during the initial incubation period and then reached a steady state (Fig. 2). After 30 min of incubation, intracellular transferrin approached a level of 8.40 ± 1.94 fmol/10^6 cells in the erythroblasts of exercised rats. This was significantly different from the control level of 2.70 ± 0.38 fmol/10^6 cells (P < 0.05). At other time points (i.e., 5, 10, 20 min) it was also observed that transferrin endocytosis in the erythroblasts of exercised rats was significantly higher than that in the sedentary animals (Fig. 2A). This indicated that the rate of transferrin internalization in the exercised rats increased in proportion to the increase in transferrin-receptor numbers on the membrane of the cells. By contrast, no significant difference was found between the mean transferrin endocytotic cycle time in the exercised rats (2.91 ± 1.16 min) and that in the control animals (2.60 ± 0.49 min). The mean cycle time of transferrin was calculated from the following relationship as described previously (29): mean cycle time (min) = transferrin uptake (fmol·10^6 cells)/iron uptake rate (fmol·10^6 cells·min⁻¹). This calculation is based on the assumption that each molecule of diferric transferrin, which is endocytosed by a cell, donates both iron atoms to the cell (29).

The intracellular radioactive iron accumulation increased in a linear fashion with the increase in incubation time. Figure 2B shows the profiles of intracellular iron accumulation in the erythroblasts of exercised and sedentary rats. After 30 min of incubation, the total radioactive iron accumulation in the erythroblasts of exercised rats was ~1.9-fold higher than that in sedentary rats. Comparison of the slope of regression lines indicated that the rate of iron accumulation in both groups was significantly different (P < 0.05). In erythroblasts of sedentary rats, the rates of intracellular iron accumulation from transferrin-bound iron were 2.58 fmol·10^6 cells⁻¹·min⁻¹, whereas the exercised rats had a higher rate of 5.68 fmol·10^6 cells⁻¹·min⁻¹. Changes in radioactive iron in cytosolic and stromal fractions. After entering into the cell, iron is transported into the mitochondria for heme synthesis or stored in ferritin. Therefore, we further investigated the subcellular distribution of radioactive iron in two fractions, cytosolic and stromal. The latter consists of outer cell membrane plus intracellular organelles including mitochondria. The cells were preincubated with Pronase, and this treatment has been shown to give maximal release of surface-bound transferrin and iron. Thus it is reasonable to believe that the majority of radioactive iron in the stromal fraction reflects the radioactivity incorporated into heme (38). Figure 3 shows the time course of iron accumulation in both
In the present study, we compared the numbers of specific transferrin receptors on the membrane of bone marrow erythroblasts in strenuously exercised and control rats. It was found that the average number of surface transferrin receptors on erythroblasts was significantly increased in the strenuously exercised rats, reaching a level of about twofold more than that in the control animals.

The results also showed that the rate of radioactive transferrin internalization increased in the erythroblasts of the strenuously exercised rats proportional to the increase in the number of surface transferrin receptors. The parallel significant increase in the rate of radioactive iron uptake by the erythroblasts of exercised rats was also observed. Scatchard analysis demonstrated that the affinity of transferrin receptor to transferrin was not affected by exercise because the apparent dissociation constants of transferrin receptors were not found to have a significant difference between the strenuously exercised and the control rats. The mean recycling time of transferrin was 2.5–3 min in the erythroblasts of either strenuously exercised or control rats. This value was in good agreement with the previous results in several different types of immature erythroid cells (12, 13, 15, 29). All of these results demonstrated clearly that this increased iron uptake by the cells in exercised rats resulted directly from the increased expression of transferrin receptor on the cellular membrane rather than the changes in affinity of transferrin receptor and transferrin recycling time.

Bridges and Cudkowicz (2) demonstrated that the intracellular low iron availability might result in a parallel increase in surface and intracellular transferrin receptors. This increase is due to the increased biosynthesis of new receptors. The molecular mechanism of intracellular iron homeostasis has recently been well characterized (9). The amount of iron acquired by mammalian cells is determined by the level of transferrin-receptor expression on the cellular membrane, whereas the level of transferrin-receptor expression is mainly controlled by intracellular iron at the posttranscriptional level. Recent studies have established the existence and structures of iron response elements (IREs) and of iron regulatory proteins (IRPs) or IRE-binding proteins, which specifically bind and modify expression of the mRNAs of which the IREs are a part (1, 3, 20, 21). The IREs are sequences of RNA found in the 5′-untranslated regions of the mRNAs for ferritin and in the 3′-untranslated regions of the mRNAs for transferrin receptor. They are bound by IRPs that lack one of four possible iron atoms in a cubic iron sulfur cluster. When IRPs bind IREs tightly, they inhibit translation of mRNAs of ferritin and also inhibit the degradation of the mRNAs of transferrin receptor. Thus, under the conditions of cellular iron deficiency, cells make less ferritin, whereas the number of transferrin receptor is increased (1). Hence, iron deficiency is compensated for by the increased transferrin-receptor levels, permitting a cell to absorb more iron by endocytosis of transferrin. Apparently, like iron storage, iron uptake is adjusted by a feedback-control loop, in which intracellular iron controls its own size (11). The increased transferrin-receptor expression, found in the erythroblasts of strenuously exercised rats in this study, implied that strenuous exercise could lead to a decrease in intracellular iron level as well as an in-

**DISCUSSION**

In the present study, we compared the numbers of specific transferrin receptors on the membrane of bone marrow erythroblasts in strenuously exercised and control rats. It was found that the average number of surface transferrin receptors on erythroblasts was sign-

![Fig. 3. Time course of radioactive iron accumulation in stromal (A) and cytosolic (B) fractions of erythroblasts in strenuously exercised rats (\(\bullet\), \(n = 8\)) and control animals (\(\circ\), \(n = 6\)). After incubation, cells were fractionated and radioactivity was counted. Equations of regression lines are: for stromal (A) and cytosolic (B) fractions, respectively: \(y = 2.73x + 93.75, r^2 = 0.675\) (top line), and \(y = 1.11x + 23.31, r^2 = 0.788\) (bottom line); for stromal (A) and cytosolic (B) fractions, respectively: \(y = 1.63x + 31.33, r^2 = 0.925\) (top line), and \(y = 0.67x + 16.78, r^2 = 0.864\) (bottom line). *P < 0.05 vs. control.
crease in cellular iron demand. The low intracellular iron level stimulates the expression of transferrin receptor on the cellular membrane, which, in turn, increases cellular iron uptake. Probably, the increased iron acquired by the cells is mainly transported to the mitochondria for heme synthesis. This possibility was supported by the finding on the subcellular distribution of iron in cytosolic and stromal fractions in strenuously exercised rats. In addition, the increased cellular iron accumulation might be one of the causes of the low plasma iron concentration found in the strenuously exercised rats.

Iron deficiency progresses in three stages: 1) iron stores in the bone marrow, liver, and spleen are depleted; 2) erythropoiesis diminishes as the iron supply to the erythroid marrow is reduced; and 3) hemoglobin production falls, resulting in anemia (40). Our findings did not support the viewpoint that strenuous exercise could produce a "sport anemia" because no significant difference was found in hemoglobin concentrations in the strenuously exercised and the control rats. In the study on effects of exercise on iron metabolism in rats, Strause et al. (36) obtained similar results. They found that strenuous exercise (swimming) did not reduce hemoglobin concentration but did increase iron absorption. However, our results did show that strenuous exercise could induce a low-iron status, indicated by a low plasma iron concentration, transferrin saturation, and the marked decrease in nonheme iron concentrations of the liver and spleen in addition to the reduced intracellular iron level and enhanced transferrin-receptor expression. These results were in agreement with some previous results observed by others (8, 27, 36). Prasad and Pratt (27) and Strause et al. (36) observed lower iron stores in the liver and spleen of exercising compared with sedentary rats. Gagne et al. (8) reported that serum iron and transferrin saturation were significantly lower in exercised animals. Because all the measurements in the present study were made at the end of the 3-mo experimental period rather than at the earlier stage of the exercise, changes in the above indexes, including the increased transferrin-receptor expression and iron accumulation, might not be transitory physiological adaptations. They probably represented the early signs (the first stage) of iron deficiency. However, it should be pointed out that it is premature to conclude from these results that strenuous exercise can lead to iron deficiency in rats. More investigations are needed.

Our study demonstrated that 3 mo of strenuous exercise could not result in a true iron deficiency or sport anemia. However, it is unknown whether it could be developed after longer periods (6 mo or more) of strenuous exercise and/or of greater exercise intensity (3 h of swimming or more per day). It has been well documented that exercise increases erythropoiesis and erythrocyte turnover (35, 39), namely, iron turnover, which was supported by our results. It is reasonable to believe that, if iron turnover increased above the available iron provided from the body's iron stores or the diet, true iron deficiency might occur. Exercise intensity and length of exercise period should be considered as two important factors in determining the magnitude of the increase in iron turnover. On the basis of these considerations, therefore, it is worthy to investigate further the effect of longer periods and/or greater intensity of strenuous exercise on transferrin-receptor expression and cellular iron accumulation.

The research in this laboratory was supported by Competitive Emarked Grants of The Hong Kong Research Grants Council (A/C: 357/026-B-Q151 and 354/117-B-Q164) and The Hong Kong Polytechnic University Grants (A/C: 0350/539-V274, 353/105-P136, 350/814-V541, A-PA79, G-V739, and G-S966).

Address for reprint requests and other correspondence: Z. M. Qian, Dept. of Applied Biology and Chemistry Technology, The Hong Kong Polytechnic Univ., Kowloon, Hong Kong (E-mail: bczmqian@hkpucc.polyu.edu.hk).

Received 13 November 1998; accepted in final form 21 April 1999.

REFERENCES


