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Effect of different durations of exercise on transferrin-bound iron uptake by rat erythroblast

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Abstract

This study evaluated effects of different durations of exercise on transferrin receptor (TfR) expression on the membrane of rat erythroblasts. Female rats were assigned to six groups: 3, 6 and 12 months of strenuous exercise (swimming 2 h/day, 5 days/wk) groups and their corresponding controls. At the end of experiments, the erythroblasts were isolated for Tf binding assay and transferrin-bound iron (Tf-Fe) uptake. Tissue non-heme iron and hematological iron indices were also measured. The TfR number on the cells was about 603,189 ± 107,562, 890,150 ± 164,849 and 384,695 ± 46,295 molecules/cell in three control groups (3, 6, 12 months) respectively. Exercise groups had significantly higher levels of TfR than those of the control groups, being 1,374,137 ± 243,677, 2,175,360 ± 462,737 and 1,012,759 ± 249,423 molecules/cell in 3, 6 and 12 months of exercise groups respectively ($p < 0.05$). After 30 min of incubation, cellular Tf approached to levels of 8.28 ± 1.94, 10.73 ± 3.30 and 6.60 ± 0.93 fmole/10^6 cells in 3, 6 and 12 months of exercise groups, while the corresponding control values were 3.09 ± 0.36, 5.03 ± 1.01 and 2.51 ± 0.88 fmole/10^6 cells respectively (all $P < 0.05$). The rates of cellular iron accumulation were 7.07 (3), 8.79 (6) and 5.96 (12 month) fmole/10^6 cells/min in the exercised rats and 2.91, 3.85, and 2.03 fmole/10^6 cells/min in their corresponding controls (all $p < 0.05$). However, no significant difference was observed in the ratios (Exercise/Corresponding control) of the increased TfR expression, Tf-Fe uptake and Tf endocytosis as well as of the decreased plasma iron and tissue non-heme iron levels induced by different periods of exercise. Furthermore, the increase in the length of exercise (6 or 12 month) did not induce a remarkable decrease in plasma hemoglobin and hematocrit. These results indicate that a true iron deficiency or 'sport anemia' can not develop even if under longer periods (6 or 12 month) of strenuous exercise.

Keywords: Transferrin-bound iron (Tf-Fe); Transferrin (Tf) internalization; Transferrin receptor (TfR) expression; Iron deficiency; Length of strenuous exercise

1. Introduction

Normal iron status in the athletes is especially important because of the central role of this mineral in oxygen transport and the synthesis of hemoglobin, myoglobin, and some essential enzymes fundamental to energy production. On the other hand, exercise can affect iron status of athletes. At the end of last century, it was reported that strenuous physical exercise could increase red cell destruction. In 1960s, the possibility that this increased red cell destruction might produce 'sport anemia' was suggested [1]. Since then, considerable research effort has been devoted to investigating the effects of exercise on body iron status. However, in spite of numerous investigations, there is still no general agreement as to whether exercise can induce a true iron deficiency or 'sport anemia' [2–8].

Most studies in the past have addressed this issue by measuring plasma indices of iron status and tissue iron levels. Almost no study has been conducted to approach the issue from a cellular viewpoint. Little is known about the effect of strenuous exercise on cellular iron accumulation. It is generally believed that transferrin (Tf) and transferrin receptor (TfR) mediated iron uptake is the major route for cellular iron accumulation. The number of TfR on the cellular membrane is an important factor in determining the amount of iron acquired by a cell *in vivo* [9–11]. In a previous study, therefore, we first investigated the effect of strenuous exercise (3 month of swimming) on TfR expression and transferrin-bound iron (Tf-Fe) uptake in bone marrow erythroblasts in rats [12] to address further the issue of exercise and iron status at cellular level. It was found that the TfR number on the cell surface was significantly increased in the strenuously exercised rats. The rate of Tf internalization in erythroblast of the exercised rat increased proportionally to the increase of cellular surface TfR. The parallel and significant increased rate of Tf-Fe uptake by

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erythroblast of the exercised rats was also observed. This increased TfR expression, induced by strenuous exercise, implied that exercise could lead to a decreased intracellular iron level that, in turn, led an increase in cellular TfR number. This result, plus the low plasma iron concentration and transferrin saturation, and the marked decreased non-heme iron concentrations in the liver and spleen, probably represented the early signs of iron deficiency. However, our findings did not favor the viewpoint that strenuous exercise could produce "sport anemia" because no significant difference was found in hemoglobin concentrations between the strenuously exercised and the control rats.

It has been well documented that exercise increases erythropoiesis, erythrocyte and hence iron turnover [13,14]. The magnitude of the increase in iron turnover is determined by many factors of which the length and the intensity of exercise are two important ones [15]. Our previous study [12] demonstrated that 3-month of strenuous exercise could not lead to a true iron deficiency or "sport anemia". However, it does not mean that it could not be developed after a longer period (i.e. 6 month or more) and/or a greater intensity of strenuous exercise. If iron turnover increased above the available iron provided from the limited body iron stores and/or the normal dietary intake, by a longer period and/or greater intensity of exercise, probably a true iron deficiency or 'sport anemia' might occur in strenuously exercised rats. In order to test this possibility, we investigated the changes in TfR expression and Tf-Fe uptake as well as hematological indices of iron status and tissue non-heme iron concentrations after different periods (3, 6, and 12 months) of strenuous exercise in the present study.

2. Materials and methods

2.1. Animals and exercise protocol

The use of animals for this study was approved by the Department of Health of Hong Kong Government and the Animal Ethics Committee of the Hong Kong Polytechnic University. Female SD (Sprague-Dawley) rats, aged 2 month, 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-hr periods of light and dark. After being kept under the standard laboratory conditions for one week, the animals were randomly assigned to one of the following six groups: 3 (n = 10), 6 (n = 11) and 12 month (n = 8) of strenuous exercise groups and their corresponding control groups (n = 9, 8 and 6 respectively). Laboratory rodent diet for rats (PMI Nutrition International, 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 [16] and Prasad and Pratt [17]. The rats in exercised groups swam in groups of three in a glass swimming basin (45 cm width × 80 cm length × 80 height) filled with tap water to a depth of 50 cm so that the rats could not rest by supporting their body with tails. 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 60-min in the second week. The 2-week swimming period was considered to be a training period [16] so that the increased exercise could be tolerated later. After the training period, 120-min of exercise per day (9:00 –11:00 AM) were given, lasting for different periods (3, 6 or 12 month). After swimming, rats were placed in a dry tank and warmed with overhead lamps. The rats in the corresponding control groups remained sedentary in their cages and received approximately the same amount of handling as the exercised animals (except swimming) throughout the entire experiment.

2.2. Bone marrow mononuclear cells

A method described previously [12] was used for cell preparation. Briefly, animals were fasted for twenty-four hours following the last exercise regimen at the end of the 3, 6, or 12 months of experiment. After blood sample was collected, the bone marrow cells were isolated and suspended in ice-cold saline buffered with 20 mM Hepes (pH 7.4) at 4°C. The crude cell suspension was passed through two layers of nylon mesh [18]. The mononuclear cells were obtained by Histopaque (Sigma Chemical Co., USA) density gradient sedimentation. The cells were then washed three times with ice-cold Hanks' Balanced Salt Solution (HBSS) buffered with 20 mM Hepes, pH 7.4, containing 2% bovine serum albumin. Subsequently the cells were suspended in the same buffer to a final concentration of 1×10^8 cells/ml. The cell suspension was kept at 4°C for no longer than 3 h before use. To determine the percentage of erythroblast in cell suspensions, cell counts were carried out on dried cell smears stained with May-Grunwald-Giemsa stains

2.3. Hematological indices of iron status and tissue non-heme iron measurement

At the end of different experimental periods, fasting blood samples were collected for the measurement of hematological iron indices. The liver, spleen, heart, kidney and brain were removed, weighted and stored in a freezing chamber below –70°C for subsequent determination of tissue non-heme iron concentrations. Hemoglobin (Hb) concentration was determined by cyanmethemoglobin method [19]. Hematocrit (Hct) was measured by the microcapillary hematocrit assessment. Plasma iron (PI) and total iron-binding capacity (TIBC) were determined using commercial kits (Sigma Co. St Louis, MO, U.S.A.). Transferrin saturation (TS = PI/TIBC %) was calculated. Tissue non-heme iron concentrations were measured according to the method described by Kaldor [20].

Table 1
Hematological indices of iron status in the stremuously exercised and the control rats at the end of the different experimental periods

	3 month		6 month		12 month	
	Exercise (n = 10)	Sedentary (n = 9)	Exercise (n = 11)	Sedentary (n = 8)	Exercise (n = 8)	Sedentary (n = 6)
PI (g/dl)	136.7** ± 14.0	213.1 ± 18.6	111.6*** ± 10.5	184.0 ± 27.1	112.4* ± 11.2	193.8 ± 25.5
Hb (μg/dl)	14.8 ± 0.7	15.9 ± 1.2	14.1 ± 0.7	15.2 ± 0.8	13.9 ± 2.1	14.5 ± 1.1
Hct (%)	39.6 ± 0.5	40.7 ± 0.9	39.7 ± 0.9	40.4 ± 1.1	39.7 ± 0.9	42.1 ± 0.7
TIBC (μg/dl)	434.5 ± 27.1	423.4 ± 15.0	419.5 ± 15.5	418.5 ± 36.0	394.2 ± 25.1	398.3 ± 19.6
TS (%)	31.3*** ± 22.4	49.7 ± 5.4	26.3** ± 1.9	43.8 ± 4.9	28.4*** ± 1.6	47.7 ± 4.4

The rats in the strenuous exercise groups swam in a group of three in glass swimming basin filled with tap water (35 ± 1°C) of 50 cm depth, 2 h/d and 5 d/week for 3, 6, or 12 months. The animals in the control groups received approximately the same amount of handling as the strenuously exercised rats. At the end of the different experimental periods, the blood was collected from rats in the strenuous exercise and the control groups for the measurement of hematological indices of iron status. The results were Mean ± SE. Hb: Hemoglobin; Hct: Hematocrit; PI: Plasma iron; TIBC: Total Iron-Binding Capacity; and TS: Transferrin Saturation (%). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

2.4. Transferrin binding assay and transferrin receptor-mediated iron uptake

Rat apotransferrin was obtained from Sigma Chemical Co., St Louis, USA. Iron^{59} ($FeCl_3$, 5 μCi/μg) and ^{125}I (NaI, carrier free) were purchased from Radiochemical Center, Amersham, England. Transferrin was labeled with ^{125}I and/or ^{59}Fe as previously described [21]. Binding assay was performed as described by Muta et al. [22] In brief, 2×10^5 mononuclear cells were incubated with various amounts of labeled Tf ranged from 0.12 to 70 nM in a total incubation volume of 0.1ml HBSS buffered with Hepes (pH 7.4) containing 2 mg/ml of bovine serum albumin (BSA) at 4°C for 60 min. For nonspecific binding, 7 μM of unlabeled ferric Tf was added. All assays were done in triplicate. At the end of the incubation period, the cell samples were placed on top of 120 μl dibutylphthalate : 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 γ-counter. The transferrin binding data were transformed to Scatchard plot. The number of transferrin receptor per cell and the apparent dissociation constants were calculated from Scatchard plot analysis.

Tf and Tf-Fe uptake was determined according to the method described previously [21,23]. Briefly, the cell suspension was pre-warmed in a shaking water bath for 10 min at 37°C. [^{125}I][^{59}Fe] transferrin was then added at a final concentration of 0.5 μM in a total volume of 3.2 ml of HBSS, containing BSA 2 mg/ml. After the desired incubation periods, 100 μl incubation suspension containing 2×10^6 mononuclear cells was removed. After washing with 2 ml of cold PBS three times, the cells were incubated with 200 μl of Pronase (1 mg/ml) in ice cold phosphate buffered saline for 30 min at 4°C. This treatment leads to the separation of membrane bound and intracellular Tf and iron. For the measurement of transferrin and iron in the cytosolic and stromal fractions, the method described previously was used [21,23].

2.5. Analytical methods

Cell counts were made using an hemocytometer. Radioactivity was measured in a three-channel γ-counter (Packard 5003 COBRA Q). Fractal analysis was applied to describe the time course of Tf internalization according to the method described previously [24]. The data were expressed as means ± standard error (S.E.). The statistical calculation was performed using the student's *t*-test.

3. Results

3.1. Effect of length of exercise on hematological iron indices and tissue non-heme iron concentrations

The results of hematological indices of iron status were illustrated in Table 1. Blood Hb concentration and Hct in three strenuous exercise groups had no significant changes as compared to their corresponding control groups. All values of Hb and Hct measured at the end of different periods of exercise were within normal range. In addition, no significant difference was found in total iron-binding capacity between all exercise and their corresponding control groups as well as among the three exercised groups, indicating a lack of significant effect of strenuous exercise and its length on the concentration of plasma Tf. However it was found that PI and TS were significantly lower in the three exercised groups than those in the corresponding control groups. The data on tissue mean non-heme iron concentrations (Table 2) showed that liver, spleen, kidney and heart had significant lower iron stores in the exercise rats than the control animals. The decreased PI, TS and tissue iron levels indicated that strenuous exercise might lead to a low iron status. However, the results also demonstrated that the strenuous exercise-induced lower iron status did not change progressively with prolonged periods of exercise as no difference in changes of PI, TS and tissue iron levels was found among the three exercise groups.

Table 2
The measurements of tissue non-heme iron in the strenuously exercised and the control rats at the end of the different experimental periods

Tissue	3 month		6 month		12 month	
	Exercise (n = 10)	Sedentary (n = 9)	Exercise (n = 11)	Sedentary (n = 8)	Exercise (n = 8)	Sedentary (n = 6)
Liver	0.166* ± 0.007	0.215 ± 0.016	0.174** ± 0.015	0.234 ± 0.017	0.174* ± 0.016	0.235 ± 0.16
Spleen	1.193* ± 0.080	1.728 ± 0.209	1.265** ± 0.177	1.875 ± 0.111	1.379*** ± 0.083	1.937 ± 0.071
Kidney	0.055* ± 0.003	0.072 ± 0.005	0.074* ± 0.007	0.090 ± 0.007	0.064* ± 0.006	0.084 ± 0.005
Heart	0.019* ± 0.001	0.022 ± 0.001	0.021** ± 0.002	0.027 ± 0.002	0.020* ± 0.001	0.027 ± 0.003

At the end of the different experimental periods, the liver, spleen and kidney were removed and stored in freezing chamber below −70°C. Subsequently, tissue non-heme iron concentrations (μg/mg protein) were determined according to Kaldor. The results were Mean ± SE (mg/g). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. the sedentary group.

3.2. Binding of [^{125}I] transferrin to erythroblast

The bone marrow mononuclear cells were incubated with radioactive iodinated Tf at 4°C, a temperature at which only receptor binding takes place on the cell surface [25,26]. Scatchard analysis of the Tf binding data was performed to determine the number of TfR per cell and the apparent dissociation constant. The average number of Tf binding sites was 603,189 ± 107,562, 890,150 ± 164,849 and 384,695 ± 46,275 molecules/erythroblast in the three control groups respectively (Figure 1). The exercised rat erythroblasts had significantly higher levels of specific surface Tf binding sites as compared with the corresponding controls. The average number of Tf binding sites was 1,374,137 ± 243,676, 2,175,360 ± 462,737 and 1,012,759 ± 249,423 molecules/erythroblast in 3, 6, and 12 months of exercise groups respectively (Figure 1), being about 2.28, 2.44 and 2.63 folds higher than those of the corresponding controls. The collected data demonstrated that strenuous exercise could significantly increase TfR expression on the surface of bone marrow erythroblast, however, the expression did not increase progressively with the increase in the duration of strenuous exercise after 3 month of exercise. No significant difference was found in the ratios of the increased TfR expression induced by different periods (3, 6 and 12 months) of strenuous exercise. The apparent dissociation constants were 12.2 ± 1.7, 9.3 ± 1.9 and 8.9 ± 2.0 nM in the 3, 6 and 12 months of exercised rats and 12.5 ± 0.9, 12.7 ± 2.2 and 9.4 ± 2.6 nM in the corresponding sedentary animals respectively, no significant differences being found between all exercise and their corresponding control groups ($P > 0.05$). The results indicated that exercise and its length had no effect on the binding affinity of TfR with Tf. Figure 2 showed specific [I^{125}] Tf binding to the surface of erythroblasts of 3, 6 and 12 months of the strenuously exercised and the sedentary animals (Figure 2. **A**) and Scatchard plot analysis (Figure 2. **B**).

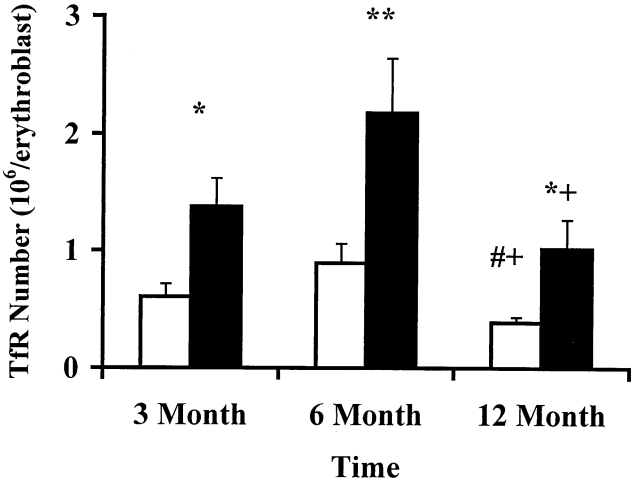

Fig. 1. Effects of the different periods of strenuous exercise on the transferrin receptor expression in rat bone marrow erythroblasts. Binding assay was performed as described in the text. Specific transferrin binding sites were determined from Scatchard analysis of binding curves (Open bars-the sedentary rats, Black bars-the exercised animals). The results were Mean ± SE. * $p < 0.05$; **$p < 0.01$ vs. control, # $p < 0.05$ vs. 3 month sedentary group and + $p < 0.05$ vs. corresponding 6 month groups.

3.3. Transferrin internalization and receptor-mediated iron uptake

The bone marrow erythroblasts were incubated with 0.5 μM of [^{59}Fe][^{125}I] transferrin for the various periods at 37°C. The approaches employed by Karin and Mintz [25] were used to release cell surface bound ligand with Pronase digestion and to determine the Tf internalization. Tf internalization increased with the increase in the incubation time in a linear manner during initial incubation period, and then reached a steady state (Figure 3). After 30 min of incubation, the intracellular Tf approached to levels of 8.28 ± 1.94, 10.73 ± 3.30 and 6.60 ± 0.93 fmole/10^6 cells in the erythroblasts of the 3 (Figure 3. **A**), 6 (Figure 3. **B**), and 12 months (Figure 3. **C**) of the exercised rat. These values were about 2.68, 2.13 and 2.63 folds more of their corresponding control values (3.09 ± 0.36, 5.03 ± 1.26, and 2.51 ± 0.88 fmole/10^6 cells) respectively. There were significant differences between all three exercised and the corresponding control groups ($P < 0.05$). At most of the other time points observed (i.e. 5, 10, 20 min), it was observed that the rate of Tf endocytosis in the exercised rat erythroblasts were significantly higher than those in

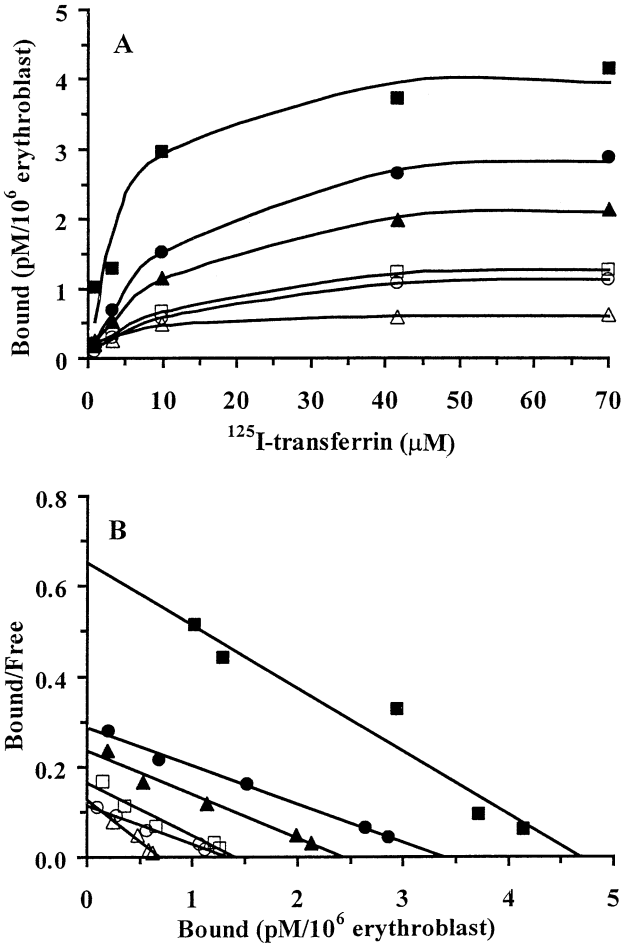

Fig. 2. A: Specific [^{125}I] transferrin binding to bone marrow erythroblasts of the 3 (●), 6 (■), and 12 (▼) months of the strenuously exercised rats and their corresponding controls (○, □, ▽). The saturation concentration of the binding is about 35 μM of transferrin, For assay condition, see Materials and Methods. **B**: Scatchard plots of the saturation results showed in A.

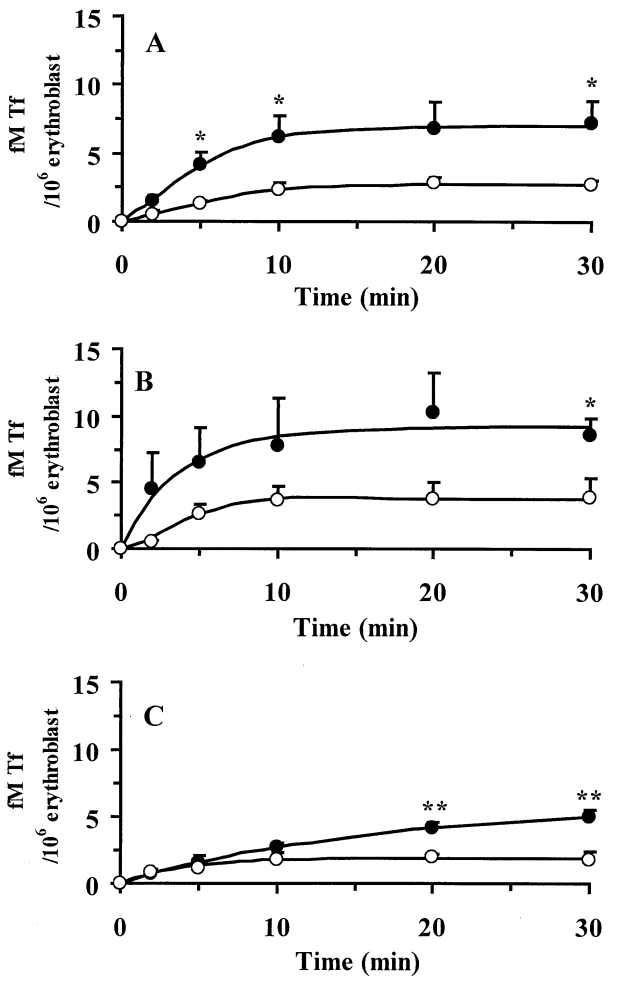

Fig. 3. Time course of transferrin internalization in erythroblasts of 3 (**A**), 6 (**B**), and 12 (**C**) months of the strenuously exercised (●) and their corresponding control (○) rats. The cells were incubated with 0.5 μM of [^{59}Fe][^{125}I] transferrin in Hank's balanced salt solution at 37°C. At the indicated time points, the cells were washed and treated as described in the text. Data were expressed as Mean ± SE. ** $P < 0.01$; * $P < 0.05$ vs. control.

the corresponding controls as well (Figure 3). This implied that the rate of Tf internalization in the exercised rats increased proportionally to the increase in TfR number on the membrane of the cells. The mean cycle time of Tf, calculated according to our previous description [12] was about 2–3 min (Table 3). No significant differences in all groups were found.

The intracellular radioactive iron accumulation increased in a linear fashion with the increase in the incubation time. Figure 4 showed the profiles of intracellular iron accumulation in different periods of exercised and their corresponding control rats. After 30 min of incubation, the total radioactive iron accumulation in erythroblasts was about 2.43, 2.28, and 2.94 folds higher in the 3, 6, 12 months of exercised rat than in their corresponding control rats. Comparison of the slope of regression lines indicated that the rates of iron accumulation in the exercise and the control groups were significantly different ($P < 0.05$). In the exercised rat erythroblasts, the rates of intracellular iron accumulation were 7.07 (3 month), 8.79 (6 month) and 5.96 (12 month) fmole/10^6 cells/min. While in the control rat, corresponding rates were 2.91, 3.85, and 2.03 fmole/10^6 cells/min respectively.

Table 3
The mean cycle time of transferrin in the exercised and the control rats

Month	Exercise (min)	Sedentary (min)	P value
3	2.91 ± 0.58 (n = 10)	2.60 ± 0.71 (n = 9)	NS*
6	2.90 ± 0.36 (n = 11)	2.97 ± 0.11 (n = 8)	NS
12	2.87 ± 0.48 (n = 8)	2.71 ± 0.58 (n = 6)	NS

The mean cycle time of transferrin was calculated from the following relationship: Mean cycle time = Transferrin uptake (fmol/10^6 cells) / Rate of transferrin-bound iron uptake (fmol/10^6 cells/min). The results were Mean ± SE. * $P > 0.05$.

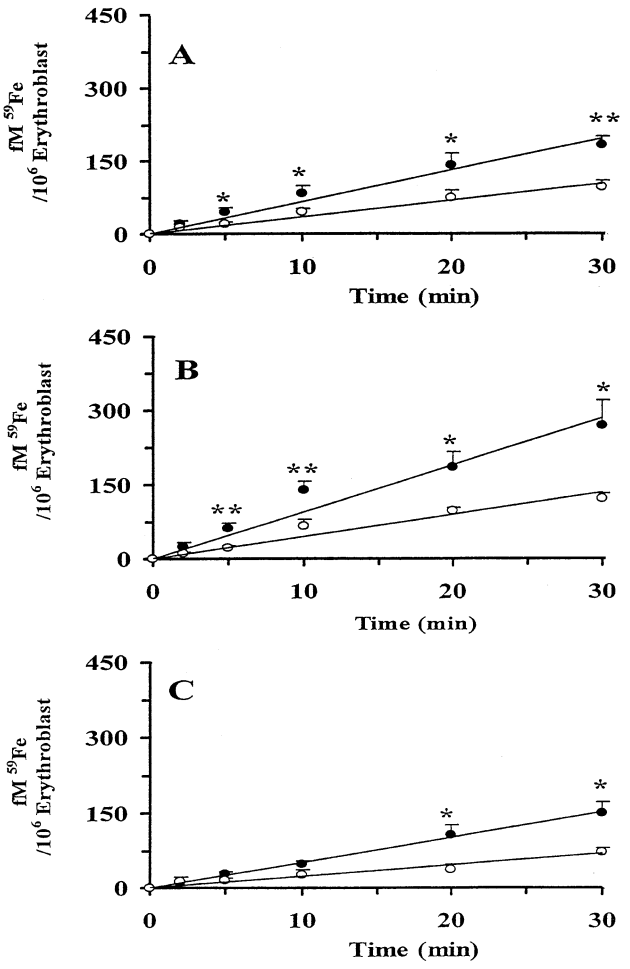

Fig. 4. Transferrin-bound iron uptake by erythroblasts of 3 (**A**), 6 (**B**) and 12 (**C**) month of the strenuously exercised and the corresponding control rats. The results were Mean ± SE. The equations of regression lines: **A,** $y = 6.57x + 12.19$, $R^2 = 0.971$ (UL: upper line, exercise); $y = 3.45x + 15.61$, $R^2 = 0.963$ (LL: lower line, control); **B;** $y = 9.47x + 43.07$, $R^2 = 0.955$ (UL); $y = 4.47x + 29.90$, $R^2 = 0.941$ (LL); and **C,** $y = 5.16x + 24.18$, $R^2 = 0.998$ (UL); $y = 2.28x + 24.18$, $R^2 = 0.948$ (LL). * $p < 0.05$, ** $p < 0.01$ vs. corresponding control.

4. Discussion

The findings obtained from the present study confirmed our earlier observation [12] that three month of strenuous exercise could induce a significant increase in TfR expression, Tf endocytosis and consequently Tf-Fe uptake in bone marrow erythroblasts of female rats. Also, results showed that longer periods of strenuous exercise (6 or 12 month of swimming) had a similar effect. However, contrary to our expectation, longer periods (6, 12 month) of exercise did not lead to more significant changes in TfR expression, Tf internalization and Tf-Fe uptake in the cells as compared to 3 month of strenuous exercise. No significant difference was observed in the ratios of increased TfR expression, Tf endocytosis and Tf-Fe uptake in erythroblasts induced by different periods (3, 6 and 12 months) of strenuous exercise. It suggested therefore that the length of exercise had no significant effect on the magnitude of the increase in these measurements. In addition, a notable decline of plasma iron concentration and Tf saturation, non-heme iron levels in the liver and spleen, found in 3 month of exercised rats, was also observed in 6 and 12 months of exercised animals. However there was no significant difference in the magnitudes of the reduction among these groups. Furthermore, longer period (6 or 12 month) of strenuous exercise did not induce a significant decline in blood hemoglobin and hematocrit as found in 3 month of exercise. These results pointed out that the increase in the length of the strenuous exercise could not produce a true iron deficiency or 'sport anemia' under our experimental conditions, although the findings suggested the existence of the exercise-induced low iron status.

Data obtained from the three control groups showed that age had a significant effect on TfR expression, and hence Tf-Fe uptake and Tf internalization in bone marrow erythroblasts of female SD rats. Compared to young growing rats (5 month age, 2 month-start age plus 3 month-experimental period), mature adult rats (8 month age, 2 month plus 6 month) had higher TfR number, Tf-Fe uptake and Tf internalization rates, being about 147.6, 132.3 and 162.8% of young growing animals respectively. Old age rats (14 month age, 2 month plus 12 month) had the lowest TfR number, Tf-Fe and Tf uptake rates, being about 63.8, 69.7, and 81.2% of young animals and 43.2, 52.7 and 49.9% of mature adult rats respectively. Although strenuous exercise induced a significant increase in Tf expression and Tf-Fe and Tf uptake in the cell in all three age groups, however, the increased magnitudes of these measurements in the three age groups were not significantly different, all being about 2–3 folds of the controls. It indicated that under circumstances where dietary iron was not limiting, normal rats in different ages had the ability to respond to strenuous exercise to keep normal plasma hemoglobin level and the effective oxygen transport. Probably it was due to the increase in TfR expression, stimulated by a relatively low cellular iron status induced by strenuous exercise, that iron uptake and hemoglobin synthesis were increased in the bone marrow erythroblasts.

It was reported that exercise could lead to an expansion of blood volume. The expansion of blood volume with exercise implies that total RBC mass may be increased despite the trend toward lower hemoglobin concentration with exercise [6,27–30]. Because of the existence of an expended blood volume induced by exercise, it was reasonable to believe that the normal hemoglobin concentration in the exercised rats observed in this study reflected that there was more total blood hemoglobin in the strenuously exercised rats than in the control animals. In other words, the rate of hemoglobin synthesis and the amount of iron uptake by the cells were increased in the strenuously exercised rats and thus blood oxygen transport ability was elevated to meet the increased metabolic demand of body tissues and cells. This increased iron acquired by bone marrow cells and used for the increased hemoglobin synthesis probably

shifted from iron storage sites such as the liver and spleen, as iron levels in these tissues were found to decrease. Ruckman and Sherman [16] reported that there was an overall trend toward iron depletion in the liver and spleen of the exercised rats and that the trend of decreased iron levels in organs could be related to the increased hemoglobin. Strause et al [31] also demonstrated that exercised rats had less total iron in the liver and spleen than sedentary rats. Similarly, lower iron stores in the liver and spleen of the exercised compared with the sedentary rats were observed by other researchers [17]. These findings supported the view that exercise could lead to a shift of iron from storage sites to bone marrow cells for the increased hemoglobin synthesis. However, it should be pointed out that although bone marrow cells acquired more iron in the exercised than in the sedentary rats, probably intracellular iron level in exercised rats was actually less than that in the control animals. Gagne et al [32] recently reported that stainable bone marrow iron was lowered significantly by exercise. It was most likely due to the increase in iron turnover as well as the speed of hemoglobin synthesis and release from cells induced by exercise. It was this increase in the speed of iron (or Hb) transport across the cell membrane (uptake and efflux) that led to the relatively low intracellular iron, the latter might in turn stimulate the expression of TfR to result in a parallel increase in surface and intracellular TfR [33].

In conclusion, our findings indicated that strenuous exercise was able to bring about a remarkable decline in plasma iron and Tf saturation, non-heme iron concentrations in the liver and spleen and a significant increase in TfR expression, Tf endocytosis and TfR-mediated iron uptake in bone marrow erythroblasts of rats. It suggested the existence of low or sub-optimal iron status in exercised animals. However, these changes were not progressively increased or decreased with the increase in the duration of strenuous exercise. In addition, plasma hemoglobin and hematocrit levels, same as those found in 3 month of swimming, were not significantly changed after 6 and 12 months of strenuous exercise. It was hence concluded that longer period of strenuous exercise could not produce true iron deficiency or ‘sport anemia’ in normal SD female rats fed on adequate diets under our experimental conditions. Further investigation will be required to elucidate the effect of the intensity of strenuous exercise on cell iron metabolism as well as hematological indices of iron status and tissue non-heme iron concentrations in SD female rats.

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