Nutritional Sciences 7(1), February, 2004, pp. 35-40

Effect of Iron Supplementation on Iron-Deficiency-Related Indices, Oxidative Stress and Antioxidative Enzyme Activity in Female Marathoners*

Hye-Young P. Kim§, Jee-Young Choi and Hyung-Sook Kang

Dept. Foods and Nutrition, Yongin University, Yongin-Si, Gyeonggi-Do 449-714, Korea

This study was performed to evaluate the effect of iron supplementation on iron-deficiency-related indices, oxidative stress and antioxidative enzyme activity in female marathoners. Fourteen teenage female marathoners participated in the study. Subjects were divided into two groups: mild anemic and control, depending on their hemoglobin (Hb) level. The mild anemic group had significantly lower RBC count and hematocrit (Hct) and Hb levels compared to the control group. The mild anemic group (≤12.5g Hb/dl, n=7) was given iron supplements (60mg Fe/day) for four weeks during the summer training period. RBC count, Hct and Hb levels showed an increasing tendency through iron supplementation, and significant differences in these variables between the anemic and control groups disappeared in the post-period. There was no difference in plasma malondialdehyde (MDA) between the anemic and control groups. However, catalase (CAT) and glutathione peroxidase (GPx) activity were significantly higher in the anemic group. The significant difference in enzyme activity between the groups disappeared in the post-period. In addition, superoxide dismutase activity significantly decreased after iron supplementation. In conclusion, antioxidative enzyme activity was up-regulated in an anemic condition and mild iron supplementation decreased the antioxidant enzyme activity of female marathoners while improving their anemic condition.

Key words : Anemia, Iron supplementation, Oxidative stress, Antioxidant enzyme, Female marathoners

INTRODUCTION

Iron deficiency is one of the most common nutritional problems in the world.[1] Iron deficiency is often expressed as a progressive condition that begins with normal body iron status and becomes subnormal or depleted because of low dietary iron intake, inadequate intestinal iron absorption or increased iron loss.[2] As this process continues, synthesis of iron-containing proteins, such as hemoglobin (Hb), becomes compromised. When Hb concentration falls below a specific cut-off value (12g/dl), the iron deficiency progresses to iron deficiency anemia.[3] The symptoms of anemia include impaired physical work performance, fatigue, anorexia, abnormal cognitive development, reduced immunocompetence, and growth abnormalities.

Iron is necessary not only for the formation of Hb and myoglobin, but also for the function of many metabolic enzymes and cytochromes.[4] Iron plays a major role in oxygen transport and oxygen utilization, both of which are important for the performance of marathoners.[5-8] Many studies have demonstrated significant decreases in red blood cell count and in the levels of Hb and ferritin in athletes.[9-14] Female runners were the most affected group in many cases.[15] Several investigators have proposed mechanisms by which iron balance could be affected by marathoners. Increased gastrointestinal blood loss after running and hemoglobinuria as a result of erythrocyte rupture within the foot during running has been reported.[16-18]

Iron can work as a potent prooxidant.[19] The superoxide radical is capable of releasing stored iron from ferritin and this iron can initiate the peroxidation of lipids.[20] In an animal model, iron overload has been reported to induce enhanced lipid peroxidation.[21] However, a recent study with humans has reported that iron supplementation does not affect oxidative stress in women with low iron status.[22] There are also other reports in animal studies that iron deficiency alters several enzyme activities including enhanced antioxidant enzyme activity.[23,24] Few studies have been performed to investigate the relationship between iron status and antioxidative enzyme activity in humans. Strenuous exercise has been shown to increase indicators of oxidative stress.[25] Therefore, it is important to clarify the effect of iron supplementation on oxidative stress in marathoners.

Oral iron therapy is frequently used to restore normal Hb value.[26] Some studies show no changes in the iron status of women athletes through iron supplementation over the course of their athletic season.[27,28] Conversely, other studies have reported improved Hb and ferritin concentrations through supplementation.[29-31] In this study, we evaluated the

* This work was supported by the Korea Research Foundation Grant (KRF-2002-041-G00112).
Accepted : February 11, 2004
§ To whom correspondence should be adressed.

overall effects of iron supplementation on iron-deficiency-related indices, oxidative stress and antioxidative enzyme activity in iron-deficient female marathoners.

METHODS

1. Subjects

This study was conducted on fourteen female teenage marathoners. After pre-period blood samples were drawn, female marathoners were divided into two groups depending on their Hb level (mildly anemic, ≤12.5g Hb/dl vs. control, >12.5g Hb/dl). Marathoners with mild anemia (≤12.5g Hb/dl, n=7) were given iron supplements (100mg Fe/day, ferrummate solution, Choong Whae Pharmacy) for four weeks during the summer training period. The iron-treated group took 60% of their prescribed iron dose during the treatment period. The average iron supplement dose was 60mg Fe/day.

2. Biochemical Analysis

Blood samples were taken in the early morning from an antecubital vein after a 12-h overnight fast. EDTA-added whole blood was used to analyze mean cell volume, hematocrit (Hct) and Hb using a Coulter counter (ADVIA120, Bayer, USA). Non-EDTA treated blood was centrifuged and serum was obtained. Serum iron concentration and total iron binding capacity (TIBC) were measured using a ferrozine colorimetric method (Sigma diagnostics, No.565, St. Louis, USA). Serum ferritin level was measured using a chemiluminescence immunoassay kit (Bayer Diagnostics, USA). Percent transferrin saturation was determined by computing the ratio of serum iron to TIBC.

Lipid peroxidation in the plasma was determined using a lipid peroxidation assay kit (Calbiochem., No. 437634, USA). Red blood cell glutathione peroxidase activity was measured using a coupled assay of Paglia and Valentine.[32] One unit of glutathione peroxidase (GPx) activity was defined as 1 μmole NADPH oxidized per min. The activity of RBC superoxide dismutase (SOD) was determined by the inhibition of xanthine- and xanthine-oxidase catalyzed reduction of ferricytochrome C.[33] One unit of SOD activity was defined as a 50% inhibition of reduction of ferricytochrome C. Catalase activity was determined following H_2O_2 reduction at 240 nm.[34]

3. Statistics

Data analysis, estimation of mean and standard deviation for each group was carried out using the SPSS package (SPSS 10.0, SPSS Institute, USA). A comparison between the anemic and control groups in the same period was performed using an independent student's t-test. A comparison between the pre- and post-period in the same group was performed using a paired t-test. Pearson's correlation coefficients were calculated among iron-deficiency- related blood indices, oxidative stress and antioxidant enzyme variables. A probability value of $p<0.05$ was chosen as the level of statistical significance.

RESULTS AND DISCUSSION

1. General Characteristics of the Subjects

General characteristics of the subjects are presented in Table 1. The mean age of the female marathoners was 16.9 ± 0.7 years. The subjects became runners at 12.3 ±2.0 years old and were exercising regularly for 4.7 ± 0.7 hrs/day. The mean height of the subjects was 163.4 ± 4.1 cm and the mean weight was 48.7± 4.5 kg. The mean BMI of the female marathoners was lower than normal (20-25) and was 18.18± 1.20.

Table 1. General characteristics of the subjects (n=14)

Variables	Mean ± S.D.
Age (yr)	16.9 ± 0.7
Carrier starting age(yr)	12.3 ± 2.0
Training (hr/day)	4.7 ± 0.7
Height (cm)	163.4 ± 4.1
Weight (kg)	48.7 ± 4.5
BMI (kg/m^2)	18.18 ± 1.20

2. Iron-deficiency-related Blood Parameters and Oxidative Stress of Subjects

Blood iron status of the female marathoners is presented in Table 2. The mean values in most of blood iron indices except total iron binding capacity (TIBC) were at the low end of the normal range. TIBC was at the high end of the normal range, which relates to iron deficiency. When the athletes were divided into two groups depending on their Hb level, the mild anemic group (≤12.5 gHb/dl) had significantly lower RBC count and Hct and Hb levels compared to the control group.

Table 2. Iron-deficiency-related blood parameters of female marathoners

Variables	Normal Range	Total	Anemic Group	Control Group
RBC ($10^6/mm^3$)	4.2 ~ 6.3	4.42 ± 0.60[1)]	4.00 ± 0.39*	4.83 ± 0.46
Hct (%)	38 ~ 54	39.50 ± 4.45	36.20 ± 3.42**	42.80 ± 2.39
Hb (g/dl)	13 ~ 17	12.63 ± 1.44	11.62 ± 1.10*	13.64 ± 0.95
sFe (μg/dl)	60 ~ 200	60.56 ± 21.77	61.75 ± 22.46	59.60 ± 23.82
TIBC (μg/dl)	250 ~ 450	457.2 ± 40.5	452.5 ± 48.7	461.0 ± 38.2
TS (%)	13 ~ 45	13.46 ± 5.37	13.86 ± 8.37	13.58 ± 6.60
Ferritin (μg/l)	10 ~ 449	16.55 ± 6.38	18.24 ± 7.80	14.86 ± 4.85

Mean ± S.D.
Hct: hematocrit, Hb: hemoglobin, sFe: serum ferritin, TIBC: total iron binding capacity, TS: transferrin saturation
* $p<0.05$, ** $p<0.01$ between the anemic and control groups

In comparison to non-athletic subjects in other studies,[35,36] marathoners had lower mean Hct, Hb, serum iron, and ferritin concentrations. Rowland et al.[31] also reported that female high school cross-country runners had low iron stores, as identified by depressed serum ferritin levels during a competitive season. Several other studies have indicated that endurance training reduces body iron stores and the effect was more pronounced in female athletes.[26,37-38]

The Hct, Hb, and ferritin levels of female marathoners were similar to those of aerobics students or soccer players in other studies.[39,40] Serum iron and transferrin saturation were similar to those of soccer players, but lower than those of aerobics students.

The values of plasma lipid peroxidation and RBC antioxidative enzyme activity of marathoners are shown in Table 3. There was no difference in plasma MDA, index of lipid peroxidation, between the anemic and control groups. However, catalase and GPx activity were significantly higher in the anemic group than in the control group.

Table 3. Oxidative stress and antioxidative enzyme activity of female marathoners

Variables	Total	Anemic Group	Control Group
MDA (μmol/l)	3.40± 1.63[1)]	3.41± 1.72	3.40± 1.74
Cat (U/mg Hb)	135.2 ±17.9	149.4 ±10.5**	121.0 ±10.4
GPx (U/g Hb)	44.0 ±17.4	54.5 ±10.1*	41.1 ± 3.2
SOD (U/mg Hb)	16.5 ± 4.1	17.4 ± 2.9	15.5 ± 5.3

1) Mean ± S.D.
MDA: malondialdehyde, Cat: catalase, GPx:glutathione peroxidase, SOD: superoxide dismutase
* p<0.05, ** p<0.01 between the anemic and control groups

Physical exercise has been implicated in the enhanced production of lipid peroxides.[25] Antioxidant enzymes are known to protect the cells from oxidative-radical induced reactions and acute bouts of exercise increase the activity of these antioxidant enzymes.[41-42] The reason for higher catalase and GPx activity in the anemic group is not clear. However, it is possible that antioxidant enzyme activity increases because anemic subjects are more prone to oxidative-radical induced reactions.

Correlation coefficients between iron-deficiency-related blood indices and oxidative stress are listed in Table 4. Catalase activity had a significant negative correlation with RBC count in the marathoners. Red blood cell count positively correlated with Hct and Hb. Ferritin level positively correlated with transferrin saturation and negatively correlated with TIBC.

3. Changes in Blood Iron Status after Iron Supplementation

Changes in blood iron status after iron supplementation are presented in Table 5. After an anemic group was given iron supplements during the summer training period, RBC count and Hct and Hb levels showed increasing tendencies and significant differences in these variables between the anemic and control groups disappeared in the post-period. The control group showed a significant decrease in red blood cell count in the post-period. Serum iron, ferritin and transferrin saturation were not significantly affected by iron supplementation and stayed at the low end of the normal range. Total iron binding capacity increased in both groups in the post-period. Therefore, the amount of iron taken by the subjects of this study was helpful, but not enough to increase many of the iron-deficiency related indices.

Studies on the use of iron supplements with athletes have sparked controversies. Pate et al.[28] noted a non-significant increase in blood Hb concentration in an iron-supplemented group (5-9 week supplementation) and a slight decrease in a placebo group as in our study. Fogelholm[43] also found no change in blood Hb in the eight-week iron-supplemented group, but blood Hb decreased in a placebo group. In general, those studies that used small amounts of iron (50mg Fe or less per day) as a supplement found little improvement in Hb and iron status,[27,28,40] while those that used larger amounts of iron in the supplement (100 mg Fe or more per day) reported beneficial effects.[8,44,45] There is no question that iron supplementation is beneficial in restoring Hb concentration and performance in anemic individuals. However, the dose and period of supplementation necessary to change iron status may vary depending on the conditions of the subjects. The optimum dose to improve the iron status of female marathoners should be further investigated.

Table 4. Correlation coefficients between iron-deficiency-related blood indices and oxidative stress and antioxidant enzyme activity in female marathoners

	MDA	Cat	GPx	SOD	RBC	Hct	Hb	sFe	TIBC	ferritin
Cat	0.027									
GPx	-0.170	0.620								
SOD	-0.053	-0.079	0.623							
RBC	0.432	-0.654*	-0.514	-0.069						
Hct	0.235	-0.569	-0.560	-0.132	0.910**					
Hb	0.283	-0.543	-0.509	0.008	0.909**	0.984**				
sFe	0.269	-0.090	-0.227	-0.052	0.349	0.284	0.290			
TIBC	0.228	-0.418	-0.037	0.450	0.210	-0.166	-0.091	-0.341		
ferritin	-0.295	0.217	0.223	-0.124	-0.479	-0.280	-0.333	0.504	-0.772*	
TS	0.200	0.004	-0.207	-0.150	0.283	0.296	0.280	0.981**	-0.508	0.607

MDA: malondialdehyde, Cat: catalase, GPx:glutathione peroxidase, SOD: superoxide dismutase, Hct: hematocrit, Hb: hemoglobin, sFe: serum ferritin, TIBC: total iron binding capacity, TS: transferrin saturation
* p<0.05, ** p<0.001

Table 5. Changes in blood iron-deficiency related indices after iron supplementation[1)]

Variables	Anemic group		Control group	
	Pre	Post	Pre	Post
RBC(106/mm^3)	4.00 ± 0.39*	4.31 ± 0.15	4.83 ± 0.46	4.42 ± 0.59***
Hct (%)	36.20 ± 3.42**	39.60 ± 1.82	42.80 ± 2.39	39.20 ± 3.90
Hb (g/dl)	11.62 ± 1.10*	12.70 ± 0.56	13.64 ± 0.95	12.28 ± 1.33
sFe (μg/dl)	61.75 ± 22.46	69.75 ± 46.29	59.60 ± 23.82	67.20 ± 30.72
TIBC (μg/dl)	452.5 ± 48.7	495.5 ± 39.5***	461.0 ± 38.2	506.4 ± 55.9***
TS (%)	13.86 ± 8.37	13.91 ± 5.71	13.58 ± 6.60	13.10 ± 5.73
Ferritin(μg/l)	18.24 ± 7.80	17.84 ± 9.89	14.86 ± 4.85	13.22 ± 7.28

1) Iron solution was supplemented for 4 weeks only to the anemic group.
Hct: hematocrit, Hb: hemoglobin, sFe: serum ferritin, TIBC: total iron binding capacity, TS: transferrin saturation
* p<0.05, ** p<0.01 between the anemic and control groups at the same period.
*** p<0.05 between the pre- and post- period in the same group.

Table 6. Changes in oxidative stress and antioxidative enzyme activity after iron supplementation[1)]

Variables	Anemic Group		Control Group	
	Pre	Post	Pre	Post
MDA (μmol/l)	3.41 ± 1.72	2.13 ± 1.75	3.40 ± 1.74	2.36 ± 1.54
Cat (U/mg Hb)	149.4 ± 10.5**	139.5 ± 14.0	121.0 ± 10.4	133.2 ± 18.0
GPx (U/g Hb)	54.5 ± 10.1*	46.5 ± 10.3	41.1 ± 3.2	38.3 ± 9.0
SOD (U/mg Hb)	17.4 ± 2.9	14.0 ± 3.1***	15.5 ± 5.3	16.9 ± 5.7

1) Iron solution was supplemented for 4 weeks only to the anemic group.
MDA: malondialdehyde, Cat: catalase, GPx:glutathione peroxidase, SOD: superoxide dismutase
* p<0.05, ** p<0.01 between the anemic and control groups at the same period.
*** p<0.05 between the pre- and post- period in the same group.

4. Changes in Lipid Peroxidation and Antioxidative Enzyme Activity after Iron Supplementation

Changes in blood oxidative stress and red blood cell antioxidative enzyme activity after iron supplementation are presented in Table 6. MDA, an index of lipid peroxidation, had a decreasing tendency in both groups in the post-period. Catalase and GPx activity showed decreasing tendencies after iron supplementation and no differences between the anemic and control groups were found in the post-period. In addition, SOD activity significantly decreased after iron supplementation in the anemic group ($p<0.05$). These results indicate that RBC antioxidative enzyme activity is up-regulated in the anemic condition and that iron supplementation normalizes RBC antioxidant enzyme activity in female marathoners along with promoting an improvement in anemic conditions.

In animal studies, Kim and Chung[23)] found that RBC superoxide dismutase activity increased when there was an iron deficiency. Willis et al.[24)] also reported that cytochrome C, tricarboxylic acid cycle enzymes, and manganese SOD activity in the skeletal muscle of iron-deficient rats increased during training. They speculated that heme and nonheme iron responses to training were altered by iron deficiency. The Hb concentration to supply oxygen to the whole body is lower in an anemic condition. Therefore, it is likely that the red blood cell antioxidant enzyme is more active to protect small but essential Hb from oxidative stress.

CONCLUSION

Regular exercise results in a depletion of body iron stores, placing a significant number of young women at risk of iron deficiency. The prevalence of iron-deficiency anemia is likely to be higher in athletic groups, especially younger female marathoners, partly due to increased loss via hemolysis caused by repeated foot contact with the ground. In this study, mildly anemic female marathoners (≤12.5 g Hb/dl) were given iron supplements (60 mg/day for 4 weeks) and the effect of iron supplementation on blood iron status, oxidative stress and antioxidative enzyme activity was analyzed. The base-line values of RBC count, Hct and Hb concentration were significantly lower in the mild anemic group compared to the control group. When iron supplements were given to female mild anemic marathoners, levels of RBC count, Hct and Hb had increasing tendencies and significant differences in these variables between the anemic and control groups disappeared in the post-period.

There was no difference in the oxidative stress of the blood between the groups, but anemic female marathoners had higher catalase and GPx activity than the control group. After iron supplementation to the anemic group, the differences in Catalase and GPx activity disappeared. Superoxide dismutase activity also decreased after iron supplementation. In conclusion, supplementation of iron, at the dose we used, to mild anemic female marathoners proved beneficial in terms of improving blood iron status and normalizing

antioxidant enzyme activity.

Literature Cited

1) DeMaeyer E, Adiels-Tegman M. The prevalence of anemia in the world, *World Health Stat Q38* : 302-316, 1985
2) Bothwell TH, Charlton RW, Cook JB, Finch CA. Iron metabolism in man, Blackwell Scientific, Oxford, United Kingdom, 1979
3) Johnson MA. Nutrition monitoring and nutrition status assessment. *J Nutr* 120 : 1468-1491, 1990
4) Lyle RM, Weaver CM, Sedlock DA, Rajaram S, Martin B, Melby CL. Iron status in exercising women: the effect of oral iron therapy vs increased consumption of muscle foods. *Am J Clin Nutr* 56 : 1049-1055, 1992
5) Schena F, Pattini A, Mantovanelli S. Iron status in athletes involved in endurance and in prevalently anaerobic sports. In: Kies CV, Driskell JA. Sports Nutrition. CRC Press Inc., New York, 1995
6) Karamizrak SO, Islegen C, Varol SR, Taskiran Y, Yaman C, Mutaf I, Akgun N. Evaluation of iron metabolism indices and their relation with physical work capacity in athletes. *Br J Sports Med* 30 : 15-19, 1996
7) Lamanca JJ, Haymes EM. Effects of low ferritin concentration on endurance performance. *Int J Sport Nutr* 2 : 376-385, 1992
8) Schoene RB, Escourrou P, Robertson HT, Nilson KL, Parsons JR, and Smith NJ. Iron repletion decreases maximal exercise lactate concentration in female athletes with minimal iron-deficiency anemia. *J Lab Clin Med* 102(2) : 306-312, 1983
9) Weaver CM, Rajaram S. exercise and iron status. *J Nutr* 122 : 782-787, 1992
10) Szygula Z., Erythrocytic system under the influence of physical exercise and training. *Sports Medicine* 10(3) : 181-197, 1990
11) Rowland TW. Iron deficiency in the young athlete. Pediatric Clic North Am 37(5) : 1153-1163, 1990
12) Balaban EP. Sports anemia. Clin Sports Med 11(2) : 313-325, 1992
13) Newhouse I, Clement D. Iron status in athletes: An update. Sports Med 5 : 337-352, 1988
14) Hass JD, Brownlie T. Iron deficiency and reduced work capacity: A critical review of the research to determine a causal relationship. *J Nutr* 131 : 676s-690s, 2001
15) Beard J, Tobin B. Iron status and exercise. *Am J Clin Nutr* 72 : 594s-597s, 2000
16) Stewart JG, Ahlquist DA, McGill DB, Ilstup DM, Schwartz S, Owen RA. Gastrointestinal blood loss and anemia in runners. Ann Intern Med 100 : 843-845. 1984
17) Siegel AJ, Hennekens CH, Solomon HS, Van Boeckel BV. Exercise-related hematuria: findings in a group of marathon runners. *J Am Med Asso* 241 : 391-392, 1979
18) Brune M, Magnusson B, Persson H, Hallberg L. Iron losses in sweat. *Am J Clin Nutr* 43 : 438-443, 1986
19) Premkumar K, Bowlus CL. Ascorbic acid does not increase the oxidative stress induced by dietary iron in C3H mice. *J Nutr* 134 : 435-438, 2004
20) Blemond P, van Eijk HG, Swaak AJ, Koster JF. Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes. Possible mechanism in inflammation diseases. *J Clin Invest* 73 : 1576-1579, 1984
21) Britton RS, O'Neill R, Bacon BR. Hepatic mitochondrial malondialdehyde metabolism in rats with chronic iron overload. Hepatology 11 : 93-97, 1990
22) Binkoski AE, Kris-Etherton PM, Beard JL. Iron supplementation does not affect the susceptibility of LDL to oxidative modification in women with low iron status. *J Nutr* 134 : 99-103, 2004
23) Kim HYP, Chung HS. Effect of dietary iron and coffee intake on oxidative stress and antioxidative enzyme activities of rats. *Korean J Nutr* 35(9) : 919-925, 2002
24) Willis WG, Brooks S, Henderson S, Dallman P. Effects of iron deficiency and training on mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 62 : 2442-2446, 1987
25) Ji LL. Oxidative stress during exercise: Implication of antioxidant nutrients. Free Rad Biol Med 18(6) : 1079-1086, 1995
26) Powell PD, Tucker A. Iron supplementation and running performance in female cross-country runners. *Int J Sports Med* 12 : 462-267, 1991
27) Haymes EM, Phul JL, Temples TE. Training for cross country skiing and iron status. Med Sci Sports Exerc 18 : 162-167, 1986
28) Pate RR, Maguire M, Van Wyk J. Dietary iron supplementation in women athletes. Phys Sports Med 7 : 81-86, 1979
29) Hercberg S, Galan P, Soustre Y, Dop MC, Devanlay M, Dupin H. Effects of iron suplementation on serum ferritin and other hematological indices of iron status in menstruating women. Ann Nutr Metab 29 : 232-238, 1985
30) Risser WL, Lee EJ, Poindexter HBW, West MS, Pivarnik JM, Risser JMH, Hickson JF. Iron deficiency in female athletes: its prevalence and impact on performance. Med Sci Sports Exer 20 : 116-121, 1988
31) Rowland TW, Deisroth MB, Green GM, Kelleher JF. The effect of iron therapy on the exercise capacity of nonanemic iron-deficient adolescent runners. *Am J Dis Child* 142 : 165-169, 1988
32) Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70 : 158-169, 1967
33) Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. *J Lab Clin Med* 35 : 337-341, 1975
34) Johansson LH, Hankan Borg LA. Spectrophotometric method of determination of catalase activity in small tissue sample. *Anal Biochem* 174 : 331-336, 1988
35) Kim SH, Kim HYP, Kim WK, Park OJ. Nutritional status, iron-deficiency-related indices, and immunity of female athletes. *Nutrition* 18: 86-90, 2002
36) Kim WK, Kim HYP. Effects of dietary iron intake on immune status in male college students. *Nutri Sci* 1(1), 51-55, 1998

37) Martin DE, Vroon DH, May DF, Pilbeam S. Physiological changes in elite male distance runners training for Olympic competition. Phys Sports Med 14 : 152-171, 1986
38) Wishnitzer R, Vorst E, Berrebi A. Bone marrow iron depression in competitive distance runner. *Int J Sports Med* 4 : 27-30, 1983
39) Kim HYP, Jang YA, Lee PY. Iron status of female athletes involved in aerobic sports. *Nutri Sci* 1(1) : 29-33, 1998
40) Kang HS, Kim HYP, Choue RW. Effect of iron supplementation on iron status and immunity status of elite female soccer players. *Korean J Nutr* 36(7) : 729-735, 2003
41) Ji LL. Antioxidant enzyme responses to exercise and aging. Med Sci Sports Exer 25(2) : 225-231, 1993
42) Clarkson PM. Antioxidants and physical performance. Crit Rev Food Sci Nutr 35 : 131-141, 1995
43) Fogelholm M, Jaakkola L, Lampisjarvi T. Effects of iron supplementation in female athletes with low serum ferritin concentration. *Int J Sports Med* 13 : 158-162, 1992
44) Plowman SA, McSwegin PC. The effects of iron supplementation on female cross-country runners. *J Sports Med* 21 : 407-416, 1981
45) Hinton PS, Giordano C, Brownlie T, Haas JD. Iron supplementation improves endurance after training in iron-depleted, non-anemic women. *J Appl Physiol* 88 : 1103-1111, 2000