

## Original Article

# Effects of 4 weeks iron supplementation on haematological and immunological status in elite female soccer players

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The effects of 4 weeks iron supplementation on haematological and immunological status were studied in 25 elite female soccer players aged 20-28 years. The subjects were randomized and assigned to one of the following two groups; subjects given 40 mg/day iron supplementation (S group) or those given placebo (C group). The oral iron supplementation (40 mg elemental iron) was taken in 15 ml solution once a day by the S group, and the C group took a placebo for 4 weeks. Daily energy and protein intakes met the Korean Recommended Dietary Allowances. Blood haemoglobin concentration did not change in the S group, but decreased significantly ( $P<0.05$ ) in the C group over the 4-week experimental period. Haematocrit, mean cell volume, mean cell haemoglobin and total iron binding capacity decreased significantly, and mean cell haemoglobin concentration increased significantly ( $P<0.05$ ) in both the S and C groups. Plasma ferritin concentration increased significantly ( $P<0.05$ ) in the S group, but did not change in the C group. The change of plasma immunological parameters and erythrocyte anti-oxidative enzyme activities were almost the same between the S and C groups. These results suggest that 4 weeks of iron supplementation by elite female soccer players significantly increased body iron stores and inhibited decrease of haemoglobin concentration induced by soccer training.

**Key words:** iron supplementation, haematological parameter, immune function, elite soccer player, Korean

## Introduction

Iron deficiency is one of the leading nutritional problems in the world.<sup>1,2</sup> Its most common clinical manifestation is anaemia, and the work impairment caused by iron deficiency anaemia has been thoroughly documented.<sup>3-6</sup> Iron deficiency reduces physical performance<sup>4,6-8</sup> probably through combined effects on oxygen consumption<sup>8-10</sup> and muscle metabolism.<sup>10-12</sup> Many studies suggest that elite female athletes may be at increased risk of iron deficiency.<sup>13-16</sup> Clement and Asmundson<sup>13</sup> reported that 82% of female Canadian distance runners were iron deficient, as estimated by serum ferritin levels, which are believed to accurately reflect the size of the body iron stores.<sup>16</sup> Another report found that despite normal haemoglobin (Hb) and serum iron values, bone marrow showed either an absence or only traces of iron.<sup>14</sup> Several other investigators have confirmed this surprisingly high incidence of iron deficiency in active persons.<sup>9,15</sup>

As female athletes are already at increased risk due to the superimposed requirements related to menstruation, the possibility of increased iron demand associated with exercise is of particular concern to those engaged in physical activity. As a result, a variety of supplementation regimens are recommended to ensure adequate iron status.<sup>17</sup> These intervention programs are predicated on the assumption that nutritional iron deficiency does indeed lead to significant disability. Reduction of iron deficiency is also aimed at reducing the risk of developing anaemia and perhaps other performance-related problems.<sup>17</sup> Rowland *et al.*,<sup>18</sup>

noted that 4-week oral iron treatment improved serum ferritin levels (8.7 to 26.6  $\mu\text{g/L}$ ) in nonanaemic iron deficient runners. Schoene *et al.*,<sup>10</sup> studied the effect of 2 weeks of iron therapy on exercise performance in trained, mildly iron deficient female athletes. They reported that performance was unchanged after therapy.<sup>19</sup>

On the other hand, the immune system seems particularly sensitive to the availability of iron.<sup>19</sup> Iron is needed for DNA synthesis, and for the activity of the iron-dependent enzymes that are involved in the killing of microorganisms. An iron deficiency can thus cause an overall atrophy of immune tissues.<sup>20</sup> Alterations in immune responses can occur early in the course of reduction of iron stores.<sup>21</sup> Various immune responses can be suppressed by vigorous and intensive athletic training.<sup>22-24</sup> Thus, exercise-induced immunological impairment may be related to iron deficiency. However, evaluation of the relationship between iron status and immune function has not been adequately investigated in elite female athletes. Thus, the purpose of this investigation was to determine whether 4 weeks of iron treatment would improve haematological

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**Table 1.** Example of daily schedule for the subjects<sup>1</sup>

Time of day (h)	Items
06:00 - 07:30	Climbing and walking
08:00 - 10:00	Breakfast and rest
10:00 - 12:00	Soccer training
12:30 - 14:30	Lunch and rest
14:30 - 18:00	Soccer training
18:30 - 20:00	Dinner and rest
20:00 - 21:30	Weight training

<sup>1</sup>One month before Universiade Game.

parameters in elite female athletes with nonanaemic iron deficiency. In addition, we investigated the effects of iron supplementation on immunological status in female iron deficient athletes.

## Methods

### Subjects

Twenty-five elite female soccer players (20-28 years old) were recruited from the Korean national team for this study. The subjects severely trained for 7-9 hours every-day. An example of a daily training schedule is shown in Table 1. All procedures were approved in advance by the Ethics Committee of the Korean Sports Medical Nutrition Institute and were in accordance with the Helsinki Declaration of 1964, as revised in 2000. After a detailed explanation of this study, each subject gave her informed written consent. The subjects were determined to be free of disease by a medical examination before the study. No subjects were using illegal drugs or taking medications that affect body weight. The day of the menstrual cycle when they began and ended the study was noted because fluctuations in metabolic parameters can occur during the cycle.<sup>25</sup> Subjects started the iron supplementation with their training season immediately after biochemical pre-tests. Because the experimental period was 4 weeks, most of the women were at about the same point of their cycle (mid-follicular phase) when blood haematological and immunological parameters were remeasured at the end.

Subjects were randomized and assigned to one of the following two groups: (1) subjects given 40 mg/day iron supplementation (S group) or (2) those given placebo (C group). The characteristics of subjects belonging to the S and C groups are shown in Table 2.

### Supplementation

Iron supplement and placebo were purchased commercially (Daewoong Pharmaceutical Ltd., Seoul, Korea). The experimental treatment consisted of oral iron supplementation (40 mg elemental iron) taken in 15 ml solution once a day, as tolerated. The C group took a placebo, which appeared identical to the active agent and was taken in 15 ml solution once a day, as tolerated.

### Dietary intake

The daily food intake of the subjects was not controlled, but energy and protein intake met the Korean Recommended Dietary Allowances (RDA). A dietary assessment

**Table 2.** Characteristics of subjects

		S	C
		N = 11	N = 14
Age	years	22.6 ± 2.0	23.8 ± 2.8
Height	cm	165.2 ± 6.0	163.9 ± 5.7
Weight	kg	57.9 ± 4.5	57.0 ± 4.9
Body mass index	kg/m <sup>2</sup>	21.3 ± 1.7	21.2 ± 1.2
Percentage body fat	%	24.1 ± 3.1	23.7 ± 3.1
Fat mass	kg	14.0 ± 2.5	13.5 ± 2.1
Fat free mass	kg	43.9 ± 3.1	43.5 ± 3.9

Values are means ± SD. S, Iron supplement group; C, Control group.

was performed using a 24-h recall method. The subjects were asked to record their complete food intake during the 3 days of the study. The daily intake of nutrients was calculated from these records using a nutritional analysis program (CAN-pro, Korean Nutrition Society).

### Measurement procedures

Subjects underwent several measurements before starting the experiment and again after the 4 weeks while still in training. End measurements were conducted >24 h after the previous exercise. The procedures were performed in the following order: blood and plasma biochemical tests (haematological and iron-related measurements<sup>26</sup>, white blood cell counts<sup>27</sup>, leucocyte differential<sup>27</sup>, plasma immunoglobulin<sup>28</sup>, erythrocyte antioxidative enzyme activities<sup>29-31</sup>). Evaluations of biochemical parameters of blood and plasma were requested from Green Cross Reference Laboratory Co., (Seoul, Korea).

### Body composition

The subjects' height, weight and measurements were taken by conventional methods. Skinfold thickness was determined by caliper. Percentage of body fat, fat mass and FFM were calculated from skinfold thickness (subscapular and triceps) as described previously.<sup>32,33</sup> Percentage of body fat (%BF) is calculated with the following formula:

$$BS = W^{0.425} \times H^{0.725} \times 71.84 / 10000$$

*BS*, Body surface area (m<sup>2</sup>); *W*, body weight (kg); *H*, height (cm)

$$BD = 1.0923 - 0.000514 \times$$

$x = (SFt + SFs) \times BS / W \times 100$

*BD*, Body density (kg/m<sup>3</sup>); *SFt*, triceps skinfold thickness (mm); *SFs*, subscapular skinfold thickness (mm)

$$\%BF = (4.570 / BD - 4.142) \times 100$$

### Statistical analysis

The mean and standard deviation (SD) were reported for all measurements. Data were analysed using repeated measures ANOVA followed by Student's paired t-tests to show differences in variables from baseline to 4 weeks and using Student's unpaired t-tests to show differences in variables between the S and C groups. A value of  $P < 0.05$  was considered to be significant.

## Results

### Dietary intake

Daily nutrient intakes during the experiment and percentages of RDA are shown in Table 2. Daily intake of energy and nutrients were not different between the S and C groups. Energy intake was about 100% of RDA and protein intake was over 100% of RDA for both the S and C groups (Table 3). Calcium, iron, and vitamin A were insufficient compared to RDA (Table 3). Percentages of energy as protein, fat and carbohydrate were 14.8, 28.2 and 57% for the S group and, 15.1, 28.9, and 56% for the C group. The sources of the iron from daily meals included a combination of meats, fish, eggs, beans, grains, and vegetables. Mean percentages of iron sources were 32.4% for animals, 67.6% for plants in S group and 29.4% for animals, 70.6% for plants in C group.

### Haematological parameters

All pre- and post-experiment blood and plasma haematological test results were within the standard values for adult Korean women. Blood haemoglobin concentration did not change in the S group, but decreased significantly ( $P < 0.05$ ) in the C group over the 4-week experimental period (Table 4). Red blood cells and plasma iron concentration did not change in either the S or C group (Table 4). Haematocrit, mean cell volume (MCV), mean cell haemoglobin (MCH) and total iron binding capacity (TIBC) decreased significantly, and mean cell haemoglobin concentration (MCHC) increased significantly ( $P < 0.05$ ) in both the S and C groups (Table 4). The change in MCH was significantly ( $P < 0.05$ ) greater in the C group than in the S group (Table 4). Plasma ferritin concentration increased significantly ( $P < 0.05$ ) in the S group, but did not change in the C group (Table 3).

### Immunological parameters

White blood cells and plasma immunoglobulin were not different between pre- and post-experiment results in either the S or C group (Table 5). Percentages of neutrophils and basophils did not change in either the S or C group over the 4-week experimental period (Table 5). The percentage of lymphocytes decreased, and percentages of monocytes and eosinophils increased in both the

S and C groups, but the change of eosinophils in the C group was not significant (Table 5).

### Antioxidative enzyme activities

Erythrocyte glutathione peroxidase (GPx) and Catalase activities were not different between pre- and post-experiment results in either the S or C group (Table 6). Superoxide dismutase (SOD) activity increased significantly ( $P < 0.05$ ) in both the S and C groups over the 4-week experimental period (Table 6).

### Discussion

The results show that 4 weeks iron supplementation (a daily dose of 40 mg elemental iron) to elite female soccer players significantly increased plasma ferritin concentration and inhibited decrease of Hb concentration induced by soccer training. The change in Hb values pre- to post- was not different in the S group. This is in agreement with Pate *et al.*,<sup>34</sup> who found that oral iron supplementations, when administered to nonanaemic female athletes, have no statistically significant effects on Hb levels. However, they did note a modest improvement from 14.4 to 15.0 g/dl over their treatment period (5-9 weeks with 50 mg elemental iron per day). There was thus some question prior to the present study as to whether changing Hb levels would influence our results. Newhouse *et al.*,<sup>35</sup> reported that 8-week iron supplementation (100 mg elemental iron per day) did not influence Hb concentration (13.4 to 13.5 g/dl) in prelatent or latent iron deficient females. Rowland *et al.*,<sup>18</sup> demonstrated that 4-week iron supplementation (975mg ferrous sulfate per day) has no effect on Hb concentration (13.1 to 12.9 g/dl) in female endurance runners. These results suggest that oral iron supplementation did not increase blood Hb level in female athletes. However, the results of the present study may indicate that iron supplementation inhibits the decrease in Hb level induced by heavy training. In this study, most of the haematological parameters (Hb, Ht, MCV, MCH, MCHC, and TIBC) decreased over the 4-week experimental period (training season) in both the S and C groups. Newhouse and Clement<sup>36</sup> suggested that iron deficiency induced by

**Table 3.** Dietary intake

		S N = 11	C N = 14
Energy	kcal	2154 ± 377 (107)*	2083 ± 343 (103)
Protein	g	78 ± 10 (133)	66 ± 11 (129)
Fat	g	68 ± 11	66 ± 13
Carbohydrate	g	312 ± 69	292 ± 61
Fibre	g	4.5 ± 0.6	4.1 ± 1.2
Calcium	mg	538 ± 121 (75)	571 ± 154 (82)
Phosphorus	mg	1103 ± 214 (156)	1118 ± 141 (160)
Iron	mg	13.4 ± 2.8 (84)	13.3 ± 2.1 (82)
Sodium	g	4.1 ± 0.9	3.9 ± 1.0
Potassium	g	2.7 ± 0.5	2.5 ± 0.5
Vitamin A	mgRE	676 ± 141 (97)	614 ± 146 (88)
Vitamin B1	mg	1.2 ± 0.2 (118)	1.1 ± 0.2 (112)
Vitamin B2	mg	1.3 ± 0.3 (105)	1.2 ± 0.2 (103)
Niacin	mgNE	15.1 ± 2.4 (117)	14.7 ± 2.1 (114)
Vitamin C	mg	152 ± 79 (277)	105 ± 36 (192)
Cholesterol	mg	521 ± 119	490 ± 112

Values are means ± SD. S, Iron supplement group; C, Control group. RE, equivalent retinol weight; NE, equivalent niacin weight. \*Percentage of recommended dietary allowance.

**Table 4.** Pre- and post-experiment haematological measurements

		S N=11			C N=14		
		Before	After	Change	Before	After	Change
Haemoglobin	g/dl	12.8 ± 1.4	12.2 ± 0.9	-0.6 ± 0.9	13.2 ± 1.3	12.3 ± 1.2*	-0.9 ± 0.6
Red blood cells	x10 <sup>6</sup> /mm <sup>3</sup>	4.24 ± 0.17	4.19 ± 0.27	0.03 ± 0.25	4.31 ± 0.32	4.20 ± 0.30	-0.04 ± 0.27
Haematocrit	%	40.6 ± 3.2	37.8 ± 2.4*	-2.8 ± 3.1	41.9 ± 3.3	38.3 ± 3.1*	-3.6 ± 1.7
MCV	fl	95.8 ± 6.4	90.3 ± 5.6*	-5.5 ± 1.8	97.4 ± 2.2	91.1 ± 3.0*	-6.3 ± 2.0
MCH	pg	30.1 ± 2.8	29.5 ± 2.5*	-0.6 ± 0.5	30.6 ± 1.2	29.3 ± 1.4*	-1.4 ± 0.7#
MCHC	g/l	31.3 ± 1.4	32.3 ± 1.3*	1.0 ± 0.8	31.6 ± 0.8	32.1 ± 0.7*	0.6 ± 0.6
Plasma iron	µg/dl	77 ± 34	78 ± 31	1 ± 26	71 ± 18	85 ± 46	14 ± 45
TIBC	µg/dl	479 ± 67	388 ± 75*	-91 ± 36	456 ± 39	392 ± 50*	-64 ± 11
Plasma ferritin	µg/l	21.5 ± 28.3	33.3 ± 33.4*	11.2 ± 8.3	16.6 ± 9.4	24.1 ± 15.8	7.6 ± 15.3

Values are means ±SD. S, Iron supplement group; C, Control group. MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; TIBC, total iron binding capacity.  $P < 0.05$  vs. pre-experiment values (repeated measures ANOVA and Student's paired *t*-test). #  $P < 0.05$  vs. S group (Student's *t*-test).

**Table 5.** Pre- and post-experiment immunological measurements

		S N=11			C N=14		
		Before	After	Change	Before	After	Change
White blood cell	x10 <sup>3</sup> /mm <sup>3</sup>	3.88 ± 1.67	4.52 ± 1.00	0.64 ± 0.91	4.10 ± 1.60	4.60 ± 0.22	0.50 ± 0.88
Neutrophil	%	51.1 ± 4.8	49.6 ± 10.6	-1.5 ± 4.2	49.6 ± 5.9	54.7 ± 9.8	5.1 ± 4.9
Lymphocyte	%	42.4 ± 4.2	34.6 ± 8.2*	-7.8 ± 3.1	43.6 ± 7.1	34.6 ± 8.2*	-9.0 ± 4.7
Monocyte	%	4.0 ± 1.3	7.2 ± 1.8*	3.2 ± 1.8	4.4 ± 1.9	7.1 ± 2.4*	2.7 ± 2.0
Eosinophil	%	2.1 ± 1.5	4.0 ± 3.3*	1.9 ± 0.5	2.1 ± 1.9	2.9 ± 1.5	0.8 ± 0.9
Basophil	%	0.5 ± 0.5	0.6 ± 0.5	0.1 ± 0.5	0.5 ± 0.5	0.6 ± 0.5	0.1 ± 0.5
IgG	mg/dl	1207 ± 171	1258 ± 225	51 ± 26	1211 ± 197	1210 ± 188	-1 ± 45
IgA	mg/dl	170 ± 68	172 ± 67	2 ± 30	213 ± 84	214 ± 74	1 ± 51
IgM	mg/dl	134 ± 46	156 ± 53	22 ± 39	135 ± 30	146 ± 29	11 ± 22

Values are means ±SD. S, Iron supplement group; C, Control group. \* $P < 0.05$  vs. pre-experiment values (Repeated measures ANOVA and Student's paired *t*-test).

sports training is not a true anaemia in that iron is not limiting red blood cell production. An increase in plasma volume is presumed to account for most of the initial drop in Hb, although red blood cell destruction also contributes to the decrease.<sup>37,38</sup> Evidence in favor of the latter concomitant change includes: (1) the degree of change in Hb concentration is greater than that accountable to increased plasma volume<sup>31</sup> (2) there is an increase in mean red blood cell size<sup>38,39</sup> (3) red blood cell osmotic fragility decreases and (4) serum haptoglobin decreases.<sup>38,40</sup> Short-term haematological change induced by sports training is thus an early adaptation to endurance exercise.<sup>38</sup>

Inadequate dietary iron intake appears to be a major contributing factor to the prevalence of iron deficiency. In this study, iron intakes of the subjects who completed the 4-week experimental period averaged 13.3 mg/day for the C group. It should be noted that no condition except iron deficiency has been reported to produce a low serum ferritin concentration.<sup>41</sup> Many female athletes had intakes below the Korean recommended intake of 16 mg/day, which reinforced the common finding that it is difficult for the menstruating female to meet her iron demands when consuming the typical Western diet.<sup>42</sup>

The difference between the S and C groups was the change in plasma ferritin levels. The S group's mean level of plasma ferritin rose 54.9%. Although statistically significant, this rise in plasma ferritin is still modest when one considers that the normal range extends to 160 µg/l, and that the mean level of a large screening (n=1104) of U.S. female nonathletes was 69.6 µg/l.<sup>9</sup> Schoene *et al.*,<sup>10</sup> supplemented with a similar dosage (300 mg/day), but for only two weeks, and found an increase in ferritin levels from 10.0 to 22.1 µg/l. In the present study, it was hoped that 4 weeks of supplementation would be sufficient to bring the mean ferritin levels to greater than 60 µg/l. Ferritin levels below 64 µg/l may still indicate an iron-deficient state.<sup>43</sup> Heinrich *et al.*,<sup>43</sup> correlated iron absorption with serum ferritin concentration. Diagnostic <sup>59</sup>Fe<sup>2+</sup> absorption appeared to be a more sensitive indicator of depleted iron stores. It was concluded that serum ferritin values up to 64 µg/l could still be representing prelatent iron deficiency. Exhausted iron stores cannot be definitely excluded as a possibility until serum ferritin concentration rises above this level. Newhouse *et al.*,<sup>35</sup> suggested that supplementation of prelatent/latent iron deficient female athletes should ideally be continued for perhaps 16 weeks to ensure that mean levels reach the 60-70 µg/l range.

**Table 6.** Pre- and post-experiment antioxidative enzyme activities

		S N=11		C N=14	
		Before	After	Before	After
GPx	U/g Hb	0.90 ± 0.59	0.70 ± 0.68	0.73 ± 0.38	0.84 ± 0.47
SOD	U/g Hb	5854 ± 1666	10020 ± 2431*	6756 ± 1600	9736 ± 2534*
Catalase	U/g Hb	768 ± 850	566 ± 939	668 ± 710	534 ± 428

Values are means±SD. S, Iron supplement group; C, Control group. GPx, glutathione peroxidase; SOD, superoxide dismutase; Hb, haemoglobin.

\*P<0.05 vs. pre-experiment values (Repeated measures ANOVA and Student's paired t-test).

The immune system itself appears to be particularly sensitive to the availability of iron.<sup>19</sup> Iron deficiency depresses various aspects of immune function, including the lymphocyte proliferative response to mitogen stimulation,<sup>44</sup> macrophage interleukin-1 production,<sup>45</sup> and natural killer cell cytotoxic activity<sup>46</sup>; the latter possibly owing to the reduced production of interferon associated with iron deficiency. Phagocyte function is impaired by low iron availability, as evidenced by decreased bactericide, lowered myeloperoxidase activity and a decrease in the oxidative burst.<sup>47</sup> In contrast, high concentrations of ferric ions inhibit phagocytosis of human neutrophils *in vitro*.<sup>48</sup> In this study, the change in immunological parameters and antioxidative enzyme activities were nearly the same for both the S and C groups. The percentage of lymphocytes and monocytes, and SOD activity changed during the 4-week experimental period in both the S and C groups. These results may show an adaptation to heavy training.

In conclusion, the results demonstrate that 4 weeks of iron supplementation (a daily dose of 40 mg elemental iron) given to elite female soccer players significantly increased body iron stores and inhibited decrease of Hb concentration induced by soccer training, but did not influence immune functions and antioxidative enzyme activities. Iron supplementation would appear to be necessary for elite female athletes, but a more detailed study is required to clarify the effects of iron supplementation.

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