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Effects of Dietary Iron Intake on Immune Status in Male College Students*

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ABSTRACT

This study was performed to investigate the effect of dietary iron intake on the immune status of male college students. Twenty healthy male university students participated in the study. The mean age of the subjects was 22.6 years old, mean height was 173.3 cm and mean body weight was 68.4 kg. The mean daily iron intake of the subjects was 19.9 mg, 158.1% of the Korean recommended dietary allowances (RDA). The blood iron status and immune responses of the subjects were analyzed and compared between the high dietary iron group consuming more than 100% of the RDA of iron (Hi-Fe) and the low dietary iron group consuming less than 100% of the RDA of iron (Low-Fe). The serum iron concentration and percent saturation of transferrin were within the normal range in both groups. However, the Hi-Fe group had higher serum iron and percent saturation of transferrin than the Low-Fe group ($p<0.05$). When differential white blood cell counts were compared, the Low-Fe group had a lower percentage of neutrophils than the Hi-Fe group ($p<0.1$). The plasma IL-2 concentration, immunoglobulin levels and lymphocyte subsets were not affected significantly by the differences in iron intake as shown in this study. Serum iron had a positive correlation with monocyte percentage but had a negative correlation with IgM concentration. The results of this study suggest that slightly-low dietary iron intake without anemia has no effects on the cell-mediated and humoral immunities of healthy male university students. However, natural defenses, such as neutrophils and monocytes, seem to be more sensitively affected by changes in dietary iron intake.

KEY WORDS : blood iron status · immune status · iron intake.

INTRODUCTION

Nutrition is an important determinant of immune response. Epidemiologic and clinical data suggest that nutritional deficiencies alter the immune system.[1] It is well known that protein-energy malnutrition is associated with significant impairment of many immune systems.[2] Some trace elements, such as iron and zinc, also have important roles in immune functions of the body.[3][4]

Our immune system can be divided into natural and acquired immune responses.[5] The natural, nonspecific defenses include the neutrophils, monocytes, complements and so on. When microbes invade the body, blood neutrophils are attracted to the injury site and begin phagocytosis. In more extensive injuries, blood monocytes migrate to the site, transform into macrophages, and help neutrophils eliminate microbes. These processes are naturally present and are not influenced by prior contacts with antigenic agents. If nonspecific natural defense reactions are not sufficient to eliminate the infection, the toxin intrusion in the blood activates acquired immune responses. The acquired, antigen-specific defenses include the B-cell system of humoral immunity and the T-cell system of cell-mediated immunity. These immune systems are acquired in that they are specific reactions induced by prior exposure to antigenic determinants.

Several studies have reported that iron deficiency affects immune cell functions.[3][6] Especially, natural immunity and cell-mediated immunity were found to be markedly impaired in subjects with iron deficiency.[7-9] However, humoral immunity was not found to be changed much by iron deficiency. There are reports that alterations in immune responses can occur early in the course of reduction of iron intake.[10] Therefore, in this study, we aimed to investigate the effect of dietary iron intake on immune status. The blood iron status and immune responses of the subjects were analyzed and compared between the group consuming more than 100% of the RDA of iron (Hi-Fe)and the group consuming less than 100% of the RDA of iron (Low-Fe).

MATERIALS AND METHODS

This study was conducted on twenty healthy male university students residing in Seoul. A dietary assessment was

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performed using a 24-hour recall method. Food intake data was analyzed for nutrient content using a nutritional analysis program (Ewha program, Korea).[11] The dietary nutritional intake values were compared with the Korean Recommended Dietary Allowances.[12]

Resting blood samples were taken from an antecubital vein following a 12-hr overnight fast. A portion of the whole blood samples with ethylene diamine tetra-acetic acid (EDTA) preservation was used for flow cytometric staining and hematologic analysis. the remaining portions with or without EDTA preservation were centrifuged to obtain plasma or serum and were stored frozen at $-70^{\circ}C$ until further analysis.

Hematologic analyses were performed with whole blood, including blood counts and differential white cell counts. Monoclonal antibodies were used for flow cytometric analysis. The antibody specificities used were CD3+ (total T-cell marker), CD4+ (helper T-cell marker), CD8+ (suppressor T-cell marker), CD19+ (B-cell marker), and CD56+ (natural killer cell marker). Twenty microliters of monoclonal antibodies were added to 100 μl of whole blood and the mixture was incubated at room temperature for 10 minutes. Red blood cells were then lysed and removed by centrifugation. The cells were then washed twice in phosphate-buffered saline solution, and the number of cells bound with the specific antibody per 10,000 cells were counted by flow cytometer. (FACScan, Becton-Dickinson, Mountain View, USA)

Plasma samples were analyzed for IgG, IgA, IgM, and IL-2 concentrations. Plasma IgG, IgA and IgM levels were analyzed by Nephelo's method. IL-2 concentration was measured by an enzyme-linked immunoabsorbent assay (Endogen, Boston, USA).

Iron-status indices of the subjects included hemoglobin concentration, mean cell volume, serum trans-ferrin saturation and serum ferritin concentration. The hemoglobin concentration and mean cell volume of the whole blood were measured with a Coulter counter. Serum iron concentration and total iron binding capacity (TIBC) were measured utilizing a ferrozine colorimetric method (Sigma diagnostics, No 565, USA). Percent transferrin saturation was calculated as follows : (serum iron/total iron binding capacity) $\times$ 100. Serum ferritin levels were measured using a ^{125}I-radioimmunoassay kit. (Amersham International, UK)

The comparison between the low dietary-iron consuming group (Low-Fe) and the high dietary-iron consuming group (Hi-Fe) was performed using an unpaired Student t-test.[11] A probability value of $p<0.05$ was chosen as the level of statistical significance. Pearson's correlation coefficients were calculated among blood iron and immunologic variables.

RESULTS AND DISCUSSION

1. General characteristics and daily nutrient intakes of the subjects

Physical characteristics of the subjects are presented in Table 1. The mean age of the subjects was 22.6 years old, mean height was 173.3cm, and mean body weight was 68.4kg. The height and body weight of the subjects were similar to Korean reference data, which is 172 cm and 66 kg between the agses of 20-29 years. Mean body mass index of the subjects was 22.8, and was considered normal. Of the 20 subjects, 20% (n=4) were underweight (BMI<20), 60% (n=12) were normal (20≤BMI<25), 15% (n=3) were over-weight (25≤BMI<30) and only one subject was considered obese (BMI≥30).

Daily nutrient intake and a comparison with the Korean RDA are shown in Table 2. Most of the nutrient intakes of the subjects were above the Korean RDA, and ranged from 94.0 % to 184.6 % of the RDA. Mean daily iron intake of the subjects was 19.9 mg, 158.1% of Korean RDA. Kim and Kim[14] reported that male subjects living in Taegu had a daily iron intake of 26.1 mg (217.5%), while Oh and Hwang[15] reported that male subjects living in Cheju had a dailyiron intake of 14.9 mg (124.2%). The differences in iron intake among the studies are probably due to multiple factors, such as differences in season, lo-

Table 1. Physical characteristics of subjects

	Mean±SE	Range
Age (year)	22.6± 2.5	19-26
Height (Cm)	173.3± 6.3	164-185
Weight (Kg)	68.4±10.7	55-84
BMI (kg/m^2)	22.8± 3.5	17.9-31.0

Data is mean±standard error of mean (SEM)

Table 2. Nutrient intake and % RDA of subjects

Nutrient	Intake	% RDA
Energy (Kcal)	2898.9±306.2[1)]	115.7± 12.3
Carbohydrate (g)	391.8± 44.5	
Protein (g)	93.7± 11.8	120.8± 15.5
Fat (g)	85.7± 10.0	
Vitamin A(RE)	823.0±982.0	117.5±140.3
Vitamin B_1 (mg)	1.7± 0.3	133.1± 93.1
Vitamin B_2 (mg)	2.0± 0.5	123.5±113.0
Niacin (mg)	16.4± 2.3	96.2± 51.6
Vitamin C (mg)	82.0± 15.2	149.0±106.7
Calcium (mg)	668.0±129.3	94.0± 71.9
Phosphate (mg)	1163.3±165.6	164.1± 23.9
Iron (mg)	19.9± 6.2	158.1±200.2
Fiber (g)	8.1± 3.2	

1) Mean±SE

cations, subjects, and survey methods. In this study, nine subjects were consuming less than 100% of the iron RDA (12mg) per day and the remaining eleven subjects were consuming more than 100% of the iron RDA per day.

2. Blood iron status of the subjects

Red blood cell counts and blood iron status of the subjects are presented in Table 3. Mean values for all blood iron parameters were within normal ranges and none of the subjects had iron-deficient anemia (Hb<13.0 g/dl and ferritin<10 μg/l). The mean hemoglobin and hematocrit values in this study were similar to those values in Oh and Hwang's report[15], but higher than the values in Kim and Kim's report.[14] The subjects in this study had higher mean serum iron, total iron binding capacity (TIBC), transferrin saturation and ferritin concentrations than the subjects in Kim and Kim's report.[14] When blood iron indices were compared between the high and low iron consuming groups, the Hi-Fe group had higher serum iron concentration and percent saturation of transferrin than the Low-Fe group ($p<0.05$). The Hi-Fe group also had slightly higher ferritin concentration in the blood than the Low-Fe group, but statistically significant difference was not found. It might be due to small sample size of the study.

Correlations of the various blood iron indices are shown in Table 4. Hematocrit and hemoglobin had significantly positive correlations with red blood cell counts. Very strong positive correlations were found between hematocrit and hemoglobin. In addition, serum iron had significant positive correlations with TIBC and percent saturation of transferrin. Kim and Kim[14] reported similar correlations among blood iron indices as shown in this study. In addition, they found that serum ferritin had positive correlations with hemoglobin and hematocrit. However, those correlations with ferritin were not found in this study. It is probably due to higher mean ferritin stores found in subjects of this study.

Table 3. Hematological indices according to dietary iron intake

	Hi-Fe[1]	Low-Fe	Normal range
RBC (10^6/mm^3)	5.14± 0.11[2]	5.25± 0.19	4.2 - 6.3
Hemoglobin (g/dl)	15.75± 0.39	16.12± 0.25	13.0 - 17.0
Hematocrit (%)	46.74± 1.21	47.62± 0.41	38 - 54
MCV (u^3)	91.00± 1.76	90.83± 1.56	79 - 100
MCH (μg)	30.88± 0.61	30.83± 0.40	26 - 34
MCHC (%)	33.88± 0.13	33.83± 0.31	32 - 36
Serum iron (μg/dl)	211.38±19.77	145.11±11.96*	60 - 200
TIBC (μg/dl)	401.13±14.48	363.17±11.07	250 - 450
Saturation percent (%)	52.23± 4.18	39.98± 3.19*	13 - 45
Ferritin (ng/ml)	296.43±27.81	271.17± 9.72	9.0 - 449

1) Hi-Fe : the group consuming more than 100% RDA of iron, Low-Fe : the group consuming less than 100% RDA of iron
2) Mean±SE $p<0.05$

Table 4. Correlation coefficients among blood iron indices

	Hb	Hct	S-Iron	TIBC	% Sat	Ferritin
RBC	0.6324*	0.6166*	-0.0068	-0.0523	0.0154	-0.0917
Hb		0.9521*	0.3624	0.2884	0.3271	0.3591
Hct			-0.0194	0.0186	0.2221	-0.0145
S-Iron				0.7117*	0.9601**	0.2306
TIBC					0.4944	-0.0730
Saturation						0.2815

*$p<0.05$ **$p<0.01$

3. Immunologic status of the subjects

White blood cells and percent of differential white blood cell counts are presented in Table 5. Mean values for white blood cells of the subjects were within normal ranges. However, when differential white blood cell counts were compared in according to dietary iron intake levels, the Low-Fe group had lower percentage of neutrophils than the Hi-Fe group ($p<0.1$).

Several investigators have reported that phagocytosis, or ingestion of bacteria, is normal in the presence of iron deficiency, but that bactericidal activity is profoundly impaired.[16-18] In this study, even though the functionality of neutrophils was not investigated, the decrease in neutrophil proportions may lead to the poor function of neutrophils.

Percentages of T- and B-cell subsets are shown in Table 6. T lymphocytes (CD3+) accounted for 64.4% of the total lymphocytes, in which the CD4+ cell (T helper cell) amounted to 49.6% and the CD8+ cell amounted to 48.8%. The CD19+ cell (B-cell marker) accounted for 12.8%

Table 5. Differential white blood cell counts(%) according to dietary iron intake

	Hi-Fe[1]	Low-Fe	Normal range
WBC (10^3/mm^3)	6.24±0.37[2]	6.05±0.31	4.2 - 11.0
Lymphocyte (%)	36.88±2.18	40.17±2.90	28 - 42
Neutrophil (%)	52.63±2.64	44.33±3.50*	45 - 60
Basophil (%)	0.38±0.18	0.17±0.17	0 - 1
Eosinophil (%)	2.88±0.67	3.50±1.77	1 - 5
Monocyte (%)	7.25±0.73	6.83±0.70	4 - 8

1) Hi-Fe : the group consuming more than 100% RDA of iron, Low-Fe : the group consuming less than 100% RDA of iron
2) Mean±SE $p<0.1$

Table 6. Lymphocyte subsets (%) according to dietary iron intake

	Hi-Fe[1]	Low-Fe	Total
CD 3^+	64.77±4.14[2]	63.78±3.77	64.35±2.76
CD 4^+	32.64±2.70	30.84±3.68	31.89±2.11
CD 8^+	29.80±2.96	32.52±5.61	30.93±2.78
CD 4^+/CD8^+	1.15±0.13	0.95±0.28	1.07±0.13
CD19^+	12.04±1.56	13.86±1.64	12.80±1.12
CD56^+	9.04±1.68	9.86±2.49	9.38±1.36

1) Hi-Fe : the group consuming more than 100% RDA of iron, Low-Fe : the group consuming less than 100% RDA of iron
2) Mean±SE

of total lymphocytes and the CD56+ cell (NK cell marker) accounted for 9.4% of total lymphocytes. However, differences in dietary iron intake shown in this study had no effects on lymphocyte subsets of the subjects.

Santos and Falcao[19] have reported that iron-deficient adults have significantly reduced peripheral blood helper T-(CD4+) cell counts. *In vitro* evidence also indicates that CD8+ and CD4+ cells are the lymphocytes most sensitive to limited iron availability.[20] In this study, no statistical differences in CD4+ and CD8+ cells were found by different iron intake level, but the Low-Fe group had a slightly lower CD4+ cell percentage and CD4+/CD8+ratio than the Hi-Fe group.

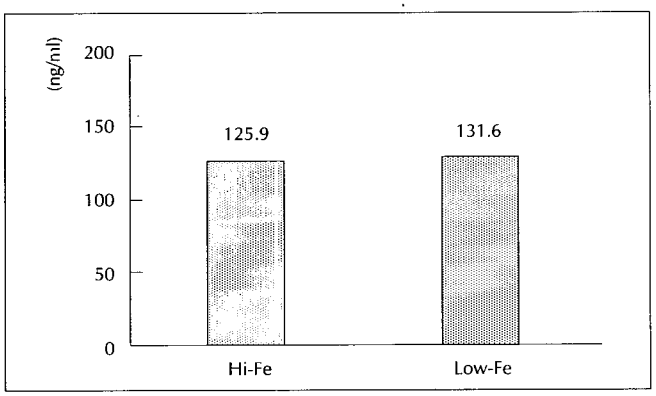

Fig. 1. IL-2 concentration of the subjects.

Table 7. Immunoglobulin levels according to dietary iron intake (mg/dl)

	Hi-Fe[1)]	Low-Fe	Normal range
Ig A	176.43±14.58[2)]	169.67±12.82	100 - 490
Ig G	1365.71±67.11	1411.67±65.24	800 - 1700
Ig M	167.21±27.89	213.00±24.20	50 - 320

1)Hi-Fe : the group consuming more than 100% RDA of iron, Low-Fe : the group consuming less than 100% RDA of iron
2)Mean±SE

It has been proposed that iron deficiency impairs T-lymphocyte functions, probably influencing IL-2 production, which enhances the expression of transferrin receptors.[21-24] However, in this study, slightly low dietary iron intake had no effects on plasma IL-2 concentration of the subjects (Fig. 1).

Plasma immunoglobulin levels of the subjects are shown in Table 7. Mean immunoglobulin levels of the subjects were within normal ranges. Differences in dietary iron intake shown in this study had no effects on humoral immunity of the subjects. Feng *et al*[25] reported that severe iron deficiency resulted in IgG subclass deficiencies, but other studies[7-9] showed no changes in humoral immunity even with severe iron deficiency.

Correlations between blood iron indices and immunological indices are shown in Table 8. Serum iron and total iron binding capacity had positive correlations with the proportion of monocytes. Meanwhile, serum iron and percent saturation of transferrin had negative correlations with IgM. Zimmer *et al*[26] also found a linear positive correlation between markers of immune status and blood iron status.

In conclusion, the differences in dietary iron intake without anemia has no effects on cell-mediated and humoral immunities of the healthy male universiy students. However, natural immunity, such as neutrophils and monocytes, seems to be more sensitively effected by changes in dietary iron intke.

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Table 8. Correlation coefficients between hematological and immunological indices

	Hb	Hct	S-Iron	TIBC	% Sat	Ferritin
WBC	0.2796	0.2855	-0.2541	0.0940	-0.0940	0.3083
Lymphocyte	-0.1299	-0.0725	0.2081	0.2588	0.0013	-0.0914
Neutrophil	0.1643	0.1134	-0.0194	0.0186	0.2221	-0.0145
Basophil	-0.4074	-0.2947	-0.4678*	-0.2348	-0.4339	0.1418
Eosinophil	-0.0284	0.0193	0.0604	0.0197	0.1412	-0.3840
Monocyte	-0.3094	-0.2651	0.5377*	0.4984*	0.4469	0.1873
$CD3^+$	0.0184	0.0222	-0.1549	0.1986	-0.0754	0.4334
$CD4^+$	-0.1951	-0.1463	-0.3009	0.1946	-0.2038	-0.2960
$CD8^+$	-0.1042	-0.1039	-0.2054	-0.1720	-0.2033	0.4397
$CD4^+/CD8^+$	-0.0469	0.0437	0.0422	0.4245	-0.0780	-0.5439
$CD19^+$	0.3112	0.1432	-0.1302	0.1930	-0.2007	-0.4328
$CD56^+$	-0.0042	0.1485	0.1481	-0.2129	0.1798	-0.0677
IgA	0.1555	0.0959	-0.2900	0.0947	0.5240	0.1619
IgG	0.0638	0.0789	-0.2914	-0.1085	0.3367	-0.1887
IgM	0.0982	0.0288	-0.5918*	-0.4646	-0.7198*	-0.2641
IL-2	0.3550	0.3443	0.2955	0.3031	0.2657	-0.0857

* $p<0.05$

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