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Relationship Among Reduced Level of Stored Iron and Dietary Iron in Trained Women

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Summary

The aim of the study was to assess the relationship between dietary iron intake (both haeme- and non-haeme-iron) and its status in trained female subjects. Forty female athletes and forty non-trained women of the same age participated in this study. Blood samples were taken to assess haematological (red blood cell count – Er, haemoglobin concentration – Hb, packed cell volume – PCV) and iron related parameters (serum ferritin concentration – SF, serum iron concentration – SI, total iron binding capacity – TIBC). A self questionnaire was used to record food intake for seven days and diets were analyzed on the basis of mean daily nutrient intake, energy values, iron intake and sources of dietary iron. According to established clinical criteria for iron deficiency some athletes and control subjects shown iron depletion (20 % and 10 %, respectively), iron deficiency erythropoiesis (10 % and 7.5 %, respectively) and iron-deficient anaemia (10 % and 7.5 %, respectively). There was no difference in the mean total dietary iron intake between the two female groups, while the mean intake of haeme-iron was significantly lower in the control group. The findings in the present study are: (1) the significant relation exists between serum ferritin level and quantity of haeme-iron intake (but not with total iron intake), and (2) 10 % of female athletes have iron deficiency.

Key words
Athletes – Anaemia – Ferritin – Iron intake

Introduction

It would seem somewhat paradoxical that trained athletes, who presumably consume more kilojoules than sedentary individuals, should become deficient in iron considering the correlation between the total energy and the content of this mineral in their diet (Burskirk 1977). A high percentage of female athletes, especially those involved in endurance exercise, have been found to have some degree of iron deficiency (Clement and Sawchuk 1984, Lampe *et al.* 1986). On the other hand, iron deficiency is one of the most prevalent nutritional problems in the world (DeMeager and Adiels-Tegman 1985). The iron status of well-trained individuals involved in strenuous physical activity has been extensively studied (Newhouse and Clement 1988). However, most of these studies did not evaluate the role of dietary iron in the physiology of exercise and iron status. It is possible that some athletes do not follow dietary regimes that are nutritionally adequate. The purpose of this study was to assess the relationship between dietary iron intake and its stores in female endurance trained athletes and a non-trained control group.

Materials and Methods

Subjects

Forty healthy non-smoking females aged 18–24 years were recruited from various collegiate athletic teams to participate in this study. All had been engaged for at least 2 years in a regular training program. The subjects participated in one of the following forms of exercise: swimming, gymnastics or tennis held at a campus recreational facility. The participants were asked to exercise at a target heart rate of 60–75 % of their heart rate reserve (maximum minus resting heart rate) for 30–60 min (not including warm-up and cool-down) at least three times a week. Compliance with the exercise schedule was assessed by

having the subjects document the type, intensity, duration and frequency of activity in training protocols which they submitted to the investigators on a weekly basis. The control sample of forty females was recruited among university students who had not participated in any form of regular exercise. Information concerning their medical history, oral contraceptive use and regularity of menstrual cycles was obtained *via* pre-experimental health questionnaire from all subjects. Neither the athletes nor controls were taking any medication or drugs of any kind and were in good health. All were free of symptoms of infections. Subjects were familiarized with the scope of the study. None of the subjects were amenorrhoeic and haematological and dietary data were collected at all phases of the menstrual cycle.

Haematological measurements

Blood samples were taken following a 12 h fast from the antecubital vein between 08.00 and 10.00 h. Two various blood samples were collected into a vacutainer tube containing no anticoagulants and the other into an EDTA vacutainer. Full blood counts were obtained from Cell Coulter (Coulter S-plus) and provided information on the number of red blood cells (Er), concentration of haemoglobin (Hb) and haematocrit (PCV). Quality control (daily analysis of a standard blood preparation) was within acceptable limits in all cases. Iron-related analyses were performed by the following methods: serum ferritin concentration (SF) – microplate enzyme immunoassay (Ferrizime, Abbott IMX); serum iron concentration (SI) and total iron binding capacity (TIBC) – a colorimetric method using ferrozine as chromogen adopted for semi-automated analysis on an Abbott apparatus (ABA 100 Analyzer). Transferrin saturation (TSat) was calculated as the ratio of serum iron to TIBC and expressed as a percentage. The intra- and interassay coefficients of variation for SF were 7 % and 10 %, respectively.

Dietary record

A self-administrated questionnaire was used to record food intake during seven successive days. A nutritionist gave instructions for the written protocol and a standardized apparatus consisting of a balance, measuring cup and spoon was used to measure the mass of all solid and semisolid foodstuffs or the volume of all liquids drunk during this 7-day period. The recorded diets were analyzed by a dietitian on the basis of food composition tables compiled by the Polish Institute for Nutrition (Nadobna *et al.* 1994). The proportion of haeme- and non-haeme-iron in the diet was calculated according to the guidelines of Monson *et al.* (1978).

Statistical analysis

Statistical analysis was carried out with the X^2 test, t-test, analysis of variance and Pearson's correlation coefficient using the SPSS/PC statistical package.

Results

The physical characteristics of the subjects are presented in Table 1. No significant differences in age, height or body mass were found.

Table 1
Physical characteristics of the subjects

	Controls	Athletes
Age (yr)	21±2	20±2
Height (cm)	164±4	168±6
Mass (kg)	58.7±2.8	56.8±1.9

Data are means ± S.D.

The mean values of haematological and iron-related parameters are presented in Table 2. The only significant difference between athletes and the controls was found for serum ferritin levels. The mean ferritin concentration in athletes was lower than in the controls.

Table 2
Parameters of iron status in the studied groups

	Controls	Athletes
Haemoglobin (reference range 7.4–9.3 mmol/l)	8.7±0.5	8.5±0.3
PCV (reference range 38–46 %)	41.6±1.8	41.3±2.2
Serum iron (reference range 61.0–178.7 μg/l)	100.5±21.2	108.9±17.8
TIBC (reference range 43–81 μmol/l)	67±7	61±7
Transferrin saturation (reference range 18–50 %)	23.0±4.3	23.2±5.1
Serum ferritin (reference range 20–110 μg/l)	46.2±8.7	39.9±10.6*

*Data are means ± S.D., *p<0.05*

The mean values of many haematological and iron status parameters of female athletes approached the lower border of the reference ranges. On the individual basis, many subjects displayed a low iron status. Eight athletes had serum iron concentration either below or equal to the lower limit of the reference range (20 μg/l), while eight athletes were below 30 μg/l. Four athletes had haemoglobin concentrations below 7.4 mmol/l. Four female athletes also had low percentage transferrin saturation, i.e. below 18 %. Three stages of iron deficiency anaemia were designated when combinations of three established clinical criteria were met. Iron depletion (SF<20 μg/l; T-Sat>18 %; Hb>1.86 mmol/l) was indicated in six female athletes. Iron-deficient erythropoiesis (SF<20 μg/l; T-Sat ≤18 %; Hb>1.86 mmol/l) was found in four athletes. Iron-deficient anaemia (SF<12 μg/l; T-Sat<16 %; Hb<1.86 mmol/l) was present in three athletes.

Similar analysis of blood parameters was conducted in the control group. The mean value of all parameters was within the appropriate reference range. However, seven control subjects had serum iron concentrations below 30 μg/l, seven ferritin concentrations below or equal to 20 μg/l and two haemoglobin concentrations below 1.86 mmol/l. When established clinical criteria for iron deficiency were designated as described above, the control group exhibited iron depletion (four subjects), iron-deficient erythropoiesis (three subjects) and iron-deficient anaemia (three subjects).

Table 3
Energy and nutrient intakes in the studied groups

	Controls	Athletes
Energy (kcal)	2449±421	2752±573
Total proteins (g)	80.1±28	93.3±24
Animal	52.3±16	59.4±21
Vegetable	28.1±19	33.7±20
Lipids (g)	92.8±8	101.3±12
Carbohydrates (g)	269.3±80	312.7±99
Vitamin C (mg)	68.6±20	80.3±29
Folic acid (μg)	162.0±39	189.9±33
Iron (mg)	10.8±3	12.2±5
% of energy derived from		
Proteins	15.0	14.4
Lipids	36.4	38.2
Carbohydrates	48.6	47.4

Data are means ± S.D.

Dietary iron intakes

The mean daily nutrient intakes and energy values are presented in Table 3. The mean daily iron intake and sources of dietary iron are given in Table 4. As for the mean energy intake, each group failed to meet the allowances recommended in Poland (RDA), and although the protein intake was adequate, the diets were suboptimal with regard to iron and folate. The iron content in the diet was 4.4 and 4.5 mg/1000 kcal for controls and athletes, respectively. The mean of total dietary iron intake was not different between the two female groups but the haeme-iron proportion was significantly different. The mean haeme-iron intake was significantly lower in the control group ($p<0.05$, Table 4).

Table 4
Daily iron intake and sources of dietary iron

	Controls	Athletes
Total (mg/l)	10.8±3	12.2±5
Haeme (mg/l)	0.7±0.6	1.1±0.6*
Non-haeme (mg/l)	10.1±1.4	11.3±1.1
Source of iron (%)		
Meat and fish	34.2	36.3
Cereals	35.3	38.3
Vegetables and fruits	16.3	15.8
Sugar sweeteners and other	4.2	3.6
Dairy products	10.0	6.0

*Data are means ± S.D., * $p<0.05$ controls versus athletes*

Table 5
Correlation coefficients between serum ferritin and dietary intake

	Controls	Athletes
Animal proteins	0.32**	0.34**
Meat and fish	0.39**	0.42**
Vegetables	NS	NS
Dairy products	0.22*	0.19*
Cereals	NS	NS

*NS – not significant, * $p<0.05$, ** $p<0.01$*

No significant correlation was observed between two biochemical iron status indicators and iron intake. However, a relationship does exist between serum ferritin and the amount of haeme iron. Table 5

summarizes the correlations between serum ferritin and dietary iron intake in the study groups. There was a statistically significant relationship between serum ferritin level and meat-fish and animal protein intake.

Discussion

There is strong association between participation in endurance exercise and low iron status. The athletes have been found to have lower indices of iron stores than sedentary control subjects, and in some cases their low haematological parameters suggest a condition termed "sports anaemia" (Eichner 1988).

In this study, the mean values of many of the iron status parameters tended towards the lower limit of the reference ranges, while 10 % of the investigated female athletes and 7.5 % of the control female sedentary subjects have exhibited anaemia.

Bothwell *et al.* (1979) described three stages of iron deficiency anaemia. Stage one, called iron depletion, is characterized by decreased iron stores mainly in the bone marrow, liver and spleen. This occurs when iron loss exceeds iron absorption over a period of time. Iron depletion is characterized by serum ferritin concentration lower than 12 μg/l. If the iron supply to the marrow necessary for red blood cell production is further reduced, the synthesis of transferrin is increased, resulting in a decreased transferrin saturation. A transferrin saturation less than 16 % and a serum ferritin lower than 12 μg/l is indicative of stage two called iron deficient erythropoiesis. In the final stage of iron deficiency anaemia, there is a decrease in total body iron and subsequently a decrease in circulating haemoglobin levels. This phase is characterized by serum ferritin less than 12 μg/l, transferrin saturation smaller than 16 % and haemoglobin lower than 1.86 mmol/l.

In this study we investigated the effects of moderate endurance training on iron-related parameters in women at rest. The results are in agreement with the findings of other authors (Frederickson *et al.* 1983, Parr *et al.* 1984, Weight *et al.* 1992). Although numerous reports have addressed this topic, their interpretation is hampered by the wide variation in laboratory criteria of iron deficiency. The most reliable studies are those based on serum ferritin measurements. However, even with this parameter, the cut-off level used to define iron deficiency ranged from 12 μg/l used in most clinical reports to 40 μg/l, a criterion that would classify most women in the reproductive age as iron-deficient (Matter *et al.* 1987). Factors found to influence serum ferritin levels include age, sex, blood donation, diet (especially the intake of meat), alcohol consumption, a history of disease (especially liver and inflammatory diseases), smoking and body composition (Legget *et al.* 1990). On the other hand, iron should be available in a soluble form for optimal absorption in the intraluminal part of the gastrointestinal tract. Generally, the oxidation potential in the gastrointestinal tract determines which valence state of iron will be predominantly formed and how much of iron will be absorbed. Various dietary factors critically influence iron availability but it should be kept in mind that many interactions of iron with other food components actually take place at the same time. In the present study the amount of ascorbic acid, calcium, dietary fibre, tea and coffee ingested daily were also noted but were found to bear no relationship to the iron status. In fact, the only study to report significant correlation between dietary iron and ferritin levels was that of Deuster *et al.* (1986). On the other hand, there are several mechanisms by which physical activity has been suggested to change body iron in athletes. These include: 1. gastrointestinal bleeding due to intestinal ischaemia or trauma or stress-induced gastritis; 2. intravascular haemolysis, followed by urinary excretion of haemoglobin due to: a) compression of erythrocytes by the feet striking the ground or the contraction of large muscles, b) exercise acidosis, c) an increase in the circulatory rate, d) an increase in body temperature, e) a catecholamine-induced increase in the osmotic and mechanical fragility of erythrocytes, or f) the release of haemolyzing factor from the spleen, 3. haematuria due to renal vasoconstriction or renal or bladder traumas and 4. sweating. However, any discussion about the pathogenesis of anaemia in athletes is dominated by the issue of iron consumption, absorption, trafficking, utilization or loss.

Several investigators have suggested that dietary factors contribute to the development of iron deficiency with endurance training and that the type of dietary iron ingested may be important (Weight *et al.* 1992). On the other hand, haeme-iron is absorbed by an independent route and is not inhibited by other determinants of iron status (Hallberg 1981).

In our study, most of female athletes and control subjects consumed nutritionally inadequate diets. These data are comparable with other studies. Snyder *et al.* (1989) studied female runners who were carefully matched for age, their aerobic capacity and miles run per week, and observed a significantly lower mean serum ferritin levels of 7 μg/l in those who consumed a vegetarian-type diet as compared with 20 μg/l in those who consumed ample quantities of red meat. In another report, iron-deficient female runners (SF<15 μg/l) had significantly lower haeme-iron intake than iron-replete athletes (SF>64 μg/l) (Matter *et al.* 1987).

This study also indicates that inadequate iron consumption is a significant risk factor for iron deficiency in athletes much more than for sedentary subjects. The percentage (5 % for athletes and 7.5 % for the controls) of subjects who did not reach the recommended total iron intake is low, but the quality of iron present in the diet is the determining factor in

meeting iron requirements. Haeme-iron is present only in meat and fish and, singly, represents 5–10 % of total iron sources. Absorption studies show that its bioavailability is nearly 25 % and is not influenced by other dietary factors (Hallberg 1981). Conversely, non-haeme-iron represents 90–95 % of total dietary intake which has a low bioavailability (often below 5 %) and can be severely affected by other nutritional factors (Hallberg 1981). Haeme-iron content in our study was low for both athletes and sedentary subjects (4.5 and 4.4 % of total iron intake, respectively).

In conclusion, an important finding emerging from the present study is that a significant relation exists between serum ferritin levels and the quantity of haeme-iron intake (but not with that of total iron intake). This confirms the importance of considering the detailed analysis of the diet and could reinforce the necessity for analyzing the diet particularly in relation to the bioavailability of the iron ingested, i.e. the quantity of haeme-iron and non-haeme-iron together with the dietary activators and inhibitors of its absorption. The second finding is that a high percentage of female athletes are in various stages of iron deficiency. Future research directed towards analyzing the relationship between iron depletion and the protective effect of adequate dietary iron is needed. On the other hand, more precise methods should be used for identification of milder forms of iron deficiency (e. g. the soluble truncated form of human transferrin receptor).

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