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Iron Status and Resting Immune Function in Female Collegiate Swimmers

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Iron deficiency may lead to anemia and may result in compromised endurance exercise performance. Iron deficiency has also been reported to adversely affect the immune system and has been associated with attenuation of natural killer cell (NK) activity. This study was conducted to examine the relationship between iron status and NK activity in highly conditioned female athletes. Ten collegiate female swimmers (SWM) and 9 inactive females (SED) participated in this investigation. Resting blood samples were obtained and analyzed for serum iron and ferritin. NK activity (% lysis) was determined using a whole blood method (^{51}Cr release assay). No significant relationship was found between iron and NK activity ($r = 0.55$, $p = .09$), nor between serum ferritin and NK activity ($r = 0.33$, $p = .35$) for SWM. ANOVA revealed significantly greater NK activity for SWM (51.63 ± 15.79%) versus SED (30.34 ± 13.67%). Serum ferritin levels were not significantly different between SWM (20.38 ± 8.62 ηg · ml^{-1}) and SED (16.79 ± 10.53 ηg · ml^{-1}), nor were iron values different between groups (16.54 ± 2.17 μmol · L^{-1} SWM; 11.92 ± 2.61 μmol · L^{-1} SED). A significant relationship between iron status and resting immune function could not be established. Exercise training may affect NK activity; however, the influence of iron status on immune function requires further evaluation.

Key Words: natural killer cell, ferritin, cytotoxicity

Introduction

Iron deficiency has been identified as one of the world's most common nutritional deficiencies (6,13). The prevalence in the U.S. is also high, with up to 11% of adolescent and women of child bearing age reported to be iron deficient (15). A negative iron balance can develop when iron loss exceeds the rate of iron absorption (14). This may lead to one of three stages of iron deficiency: iron depletion, iron deficient erythropoiesis, and iron deficiency anemia, which is classified by iron

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store depletion and hemoglobin concentration below 12 g · dl^{-1} in women (14). Iron store depletion has been characterized by serum ferritin levels below 12 ηg · ml^{-1} (6).

Iron deficiency may occur with frequency in elite, endurance-trained athletes (8, 9), with female athletes being at an increased risk (8, 16, 28). Factors that may contribute to iron deficiency in endurance athletes include low dietary iron intake (13), impaired iron absorption (9), and increased iron loss via sweat (31), feces, urine, and menstruation (9). Intravascular hemolysis has also been reported to contribute to iron deficiency (11, 13).

Much attention has been given to the role of iron in oxygen transport and the potential endurance performance ramifications of iron deficiency and iron deficiency anemia (1, 5, 8, 9, 16, 17, 28, 33). The relevancy of iron status for endurance athletes has been well described (18, 29). Iron also plays an important role in proper functioning of the immune system. Iron has been reported to be a necessary element for optimal functioning of a number of immune system cells, including natural killer cells, neutrophils, and lymphocytes (5–7). Therefore, iron deficiency may not only compromise endurance performance and work capacity, but may also adversely affect the immune system. The iron deficient state has been associated with impaired cell-mediated immunity, including reductions in T lymphocyte number, compromised T lymphocyte response to a mitogenic challenge, and reductions in natural cell-mediated cytotoxicity (6, 30). Cell-mediated immunity is important for host defense against a variety of infection causing agents, including viruses.

It has been shown that an intense training session or prolonged exercise may transiently suppress the cytotoxic activity of natural killer cells (4, 23, 25). Pedersen (26) has hypothesized that this temporary suppression of immune function may place the host at an increased chance of infection. Further, periods of intense exercise training and competition have been associated with increased susceptibility to upper respiratory tract infections (19). Other investigators have identified a relationship between serum ferritin concentration and natural cell-mediated cytotoxicity (NK activity). Chandra et al. (6) reported that untrained subjects with severe iron deficiency as reflected by low serum ferritin concentrations (<12 ηg · ml^{-1}) were found to have impaired NK cell activity. Based on these findings, it is possible that highly trained female athletes who may be at risk of iron deficiency may, consequently, also be at a heightened risk of experiencing compromised immunity. Therefore, the purpose of this preliminary investigation was to determine if a relationship exists between measures of iron status and resting immune function in well-trained female, endurance athletes. Resting immune function was reflected by a 12-hour fast and no exercise in the preceding 15-hour period.

Methods

Ten members of the University of Toledo women's swim team (SWM) and 9 untrained, inactive females were used for comparison (SED). Medical history and informed consent were obtained from each subject prior to participation in the study. The study was approved by the Human Subjects Research Review Committee of the University of Toledo. SWM were tested in late season (February), prior to the conference championships. Subject characteristics are displayed in Table 1.

All subjects arrived at the laboratory between 0600 and 0800 hours following an overnight fast. After 15 min of seated rest, blood was drawn from an antecubital vein from each subject and analyzed for determination of serum ferritin, serum iron,

Table 1 Subject Characteristics for Female Collegiate Swimmers (SWM) and Sedentary Female Control Subjects (SED)

Subjects	Age (years)	Mass (kg)	Height (cm)
SWM	19.3 ± 0.95	62.2 ± 4.32	169.5 ± 4.33
SED	22.9 ± 4.62	60.6 ± 13.0	165.3 ± 2.97

Note. Values are mean ± *SD*. No significant differences ($p > 0.05$) present between groups.

mononuclear cell populations, and NK activity. A portion of the blood sample was centrifuged and the serum was separated and stored at –80 °C for later determination of serum iron and ferritin. Serum iron was determined using a colorimetric assay (Sigma Chemical, St. Louis, MO), while serum ferritin was determined using an immunoradiometric assay (Hybritech, San Diego, CA). Mononuclear cell populations were determined by direct immunofluorescence. Monoclonal antibodies conjugated with phycoerythrin (PE) and fluorescein isothiocyanate (FITC) and appropriate controls were added to whole blood aliquots prior to lysis. The absolute number and percentage of cells were determined for mononuclear cells bearing the cell surface markers CD3 (T cells), CD4 (CD3+CD4+, helper/inducer cells), CD8 (CD3+CD8+, suppressor/cytotoxic cells), CD56 (CD3–CD56+CD16+, NK cells). Leukocyte count and differential were determined on an electronic counter (Coulter S+4) and the differential determined by standard procedures.

NK cell activity (% lysis) was determined using a whole blood ^{51}Cr release assay, with human K562 human erythroleukemia cells (American Type Culture, Rockville, MD) serving as the target. The method used was a modification of that described by Baron et al.(3). NK activity was determined at each of four concentrations of cultured K562 cells (2×10^6, 1×10^6, $.5 \times 10^6$, $.25 \times 10^6$). K562 cells (1×10^7) were pre-incubated with 100 μCi of ^{51}Cr for 45 min at 37 °C and 5% CO_2. Following incubation, 150 μL of whole blood was pipetted into each of 12 microplate wells to which were added 50 μL of K562 cells in triplicate. The microplates were incubated for 4 hours at 37 °C and 5% CO_2. Following incubation, 100 μL of chilled medium were added to each well to terminate the assay. The plates were then centrifuged (10 min at 400 × g) and 100 μL of supernatant removed and the radioactivity counted for 5 min in a gamma counter (Gamma Trac 1191, Tm analytic). The spontaneous release of ^{51}Cr was determined for each concentration of target cells by incubation of control wells with 100 μL of plain medium. Maximum release was determined by incubating each of the target cell concentrations with 100 μL of detergent (10% Triton-X-100, Sigma Chemical, St. Louis, MO). NK activity was expressed as the mean percent lysis of the four target cell concentrations such that:

$$\%\ \text{lysis} = 100 \times [(\text{CPM}_{\text{test}} - \text{CPM}_{\text{spontaneous}}) / (\text{CPM}_{\text{maximum release}} - \text{CPM}_{\text{spontaneous}})].$$

Coefficient of variation for triplicate samples in our lab has been found to be < 3% for this assay.

Statistical Analyses

Analysis of variance (ANOVA) was used to analyze group data for NK cell number, NK cytotoxicity, hemoglobin, hematocrit, serum iron, and serum ferritin. Correlational analyses were conducted within groups for examination of possible relationships between serum ferritin and natural cell-mediated cytotoxicity, and between serum iron and natural cell-mediated cytotoxicity. Statistical significance was accepted at the $p < 0.05$ level.

Results

ANOVA revealed significantly higher NK activity (% cytotoxicity) in SWM (51.63 ± 4.99) than in SED (30.33 ± 4.56) (Figure 1). NK cell numbers were $297 \cdot 10^6$ cells $\cdot L^{-1} \pm 31.62$ (SWM) and $220 \cdot 10^6$ cells $\cdot L^{-1} \pm 32.45$ (SED), but were not significantly different (Figure 2). However, CD3–CD16+CD56+ cell percentages were significantly higher in SWM (Table 2). CD3+ and CD3+CD4+ cell numbers and percentages were not different between groups (Table 2). CD3+CD8+ cell concentration was higher ($p < .05$) and percentage tended to be higher in SED ($p = .08$; Table 2).

SWM was shown to have significantly higher hemoglobin (14.57 ± 0.15 g · dl^{-1} vs. 13.27 ± 0.17 g · dl^{-1}) and hematocrit (42.64 ± 0.51% vs. 39.78 ± 0.54%) than SED (Table 3). Serum ferritin levels were not significantly different between SWM (20.38 ± 2.73 ηg · ml^{-1}) and SED (16.79 ± 3.51 ηg · ml^{-1}), nor were serum total iron values different (16.54 ± 0.69 μmol · L^{-1} in SWM, and 11.92 ± 0.87 μmol · L^{-1} in SED; Table 3). A significant relationship was not observed between serum ferritin and NK ($r = 0.33$, $p = .35$) for SWM or for SED ($r = -0.02$, $p = .48$); however, a trend

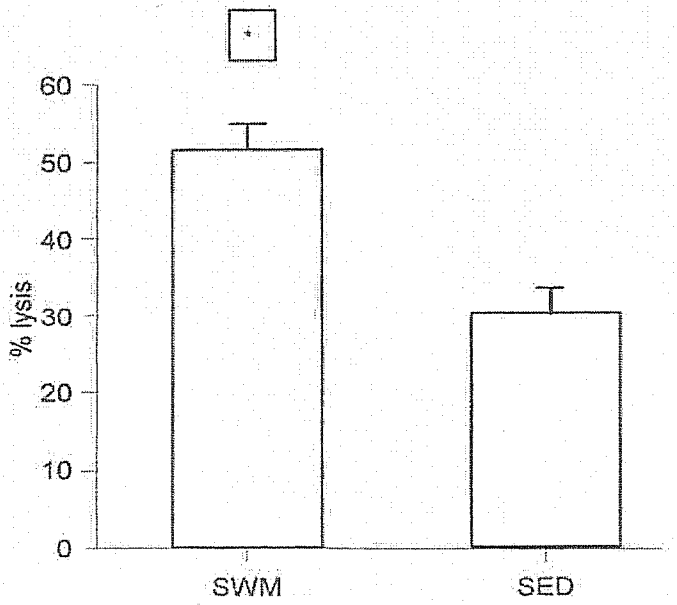

Figure 1 — Resting natural cell-mediated cytotoxicity (NK activity): collegiate female swimmers (SWM) versus female sedentary controls (SED). Values are the mean lysis of four target concentrations. *Significant difference between groups ($p < 0.05$).

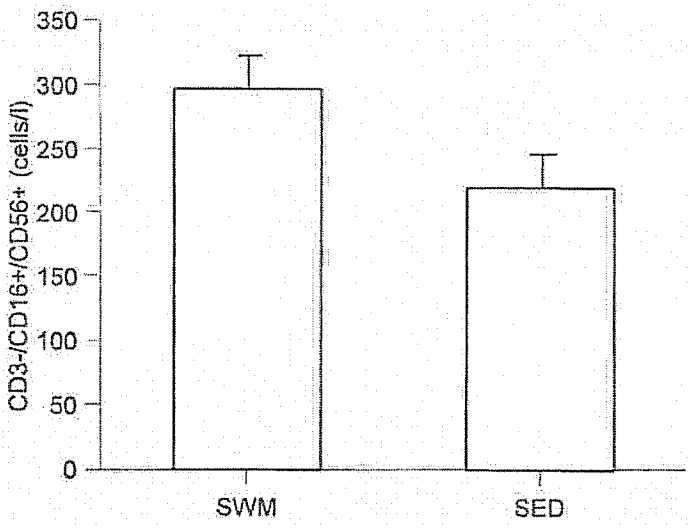

Figure 2 — Resting natural killer cell number (CD3–CD16+CD56+) for collegiate female swimmers (SWM) and female sedentary control subjects (SED). No group differences present.

Table 2 Mononuclear Cell Population Numbers and Percentages for Female Collegiate Swimmers (SWM) and Sedentary Female Control Subjects (SED)

	Cells/ml		Percent	
Marker	SWM	SED	SWM	SED
CD3+	1712 ± 157.65	1851 ± 142.74	74.6 ± 2.57	75.33 ± 1.44
CD3+CD4+	1105 ± 110.96	1084 ± 94.96	48.2 ± 2.82	43.89 ± 1.48
CD3+CD8+	579 ± 69.89	758 ± 69.17*	25.1 ± 1.90	31.22 ± 2.01*
CD3-CD16+/ CD56+	297 ± 31.62	220 ± 32.45	13.8 ± 1.72	9.44 ± 1.61*

*Significantly different from SWM ($p < 0.05$).

Table 3 Iron Status Measurements for Female Collegiate Swimmers (SWM) and Sedentary Female Control Subjects (SED)

Variable	SWM	SED
Total iron ($\mu mol \cdot L^{-1}$)	16.54 ± 0.69	11.92 ± 0.87
Ferritin ($ng \cdot ml^{-1}$)	20.38 ± 2.73	16.79 ± 3.51
Hemoglobin ($g \cdot dl^{-1}$)	14.57 ± 0.15	13.27 ± 0.17*
Hematocrit (%)	42.64 ± 0.51	39.78 ± 0.54

*Significantly different from SWM ($p < 0.05$).

was present for a significant relationship between serum iron and NK for SWM only ($r = 0.55$, $p = 0.09$). In addition, pooling of SWM and SED data failed to reveal any significant correlations between iron status and NK.

Discussion

There have been numerous reports of iron deficiency in highly trained athletes (8, 9, 12, 16). The frequency of iron deficiency in the athletic population has been attributed to low dietary iron intake (13), impaired absorption, and an increased rate of iron loss (9, 31). However, it should also be noted that some investigators have observed the incidence of iron deficiency in athletes to be similar to that of the general population (1, 32). Despite the conflicting reports, iron deficiency has been shown to have a frequent occurrence in elite, endurance-trained, female athletes (8, 16, 28). Aside from the potentially adverse effects of iron deficiency on endurance exercise performance, iron deficiency may also result in compromised immune system function. The susceptibility to viral infections has been proposed to be greater in those who train frequently at high intensities and high volumes (19). While it is likely that many factors would contribute to such a relationship, one factor to be considered is iron status. Iron deficiency (serum ferritin <12 ηg/ml^{-1}) has been reported to be associated with compromised NK cell activity (6).

Iron deficiency, the most commonly identified nutritional problem worldwide (5), may lead to impaired lymphocyte proliferative response to mitogen and may diminish the cytotoxic capability of natural killer cells. Therefore, it was proposed that a relationship between iron status and immune function might be established by determining resting immune function (NK activity) and markers of iron status in a group of highly trained female endurance athletes and a group of sedentary female control subjects.

Based on the data obtained in this investigation, cellular immune function as assessed by natural cell-mediated cytotoxic activity was not influenced by iron status in female collegiate swimmers. Despite significantly higher cytotoxic activity in the SWM group, the two groups did not differ on indices of iron status: serum total iron and serum ferritin levels. The SWM group did exhibit significantly greater hemoglobin concentrations, while percent hematocrit was not different between groups (Table 3). However, due to the homogeneity of the two groups with regard to iron status, it was not possible to attribute observed differences in natural cell-mediated cytotoxicity to these measurements. Therefore, in contrast to data from Chandra (6), no relationship was observed between natural cell mediated cytotoxicity and serum ferritin concentration. It is notable, however, that both groups had serum iron values that were considered to be within normal ranges (Table 3), while serum ferritin values (Table 3) were low but not indicative of iron store depletion (ferritin <12 ηg · ml^{-1}) for either group. In order to better assess the relationship between iron status and immune function, it is necessary to insure that a range is present for iron status. Therefore, pre-screening of participants is necessary. This is a key limitation to the present investigation.

Despite a failure to observe any differences in iron status between the two groups, the percentage of NK cells and NK cytotoxic activity were significantly higher in the SWM group. This is the first investigation that has noted significantly elevated resting NK cytotoxic activity in trained female swimmers in comparison to sedentary women. These results are similar to the findings of other investigators

who have examined differences in immune function between endurance trained subjects and sedentary controls (21, 22, 24). For example, Pedersen et al. (24) observed significantly greater NK cell activity in male racing cyclists than untrained males at rest. Nieman et al. (20) observed significantly elevated NK activity following 6 weeks of moderate endurance training in previously sedentary mildly obese women in comparison to a sedentary control group. The endurance training group was also found to have reduced upper respiratory infection symptoms (20). Nieman et al. (21) have also found high-fit elderly women to have significantly greater NK cell activity than sedentary elderly women. Finally, marathon runners have been shown to have significantly elevated resting NK cytotoxic activity when compared to sedentary control subjects (22).

Foot strike hemolysis has been identified as a contributing factor to iron deficiency in the running population (10). Additionally, repetitive musculoskeletal stress may also have an adverse effect on ferritin (2), further compromising iron storage capacity. However, the non-weight bearing nature of swimming may place swimmers at a lower risk of iron deficiency or anemia relative to runners. As a result, other populations of female athletes may be more susceptible to iron deficiency, and it is possible that stronger correlational evidence between iron status and immune function may be found by examining these groups.

In summary, in support of observations of other investigators, the endurance trained female swimmers were found to have significantly greater resting NK activity than a group of sedentary, female control subjects. However, no differences in iron status as determined by serum ferritin and serum iron concentrations were observed between sedentary and endurance trained females. As a result, a relationship between iron status and immune function could not be identified. Further research is warranted to better examine the influence of iron status on resting immune function. In addition, while it is possible that iron status may affect immune responses to acute exercise, the role that iron status plays under resting conditions must first be determined. Therefore, recommendations for further research include correlating training volume and nutrient intake with iron status and measures of immune function, conducting a comprehensive analysis of iron losses and the effect on iron storage and resting immune status, evaluating the influences of hormone treatments on iron status and immune function, and examining the influence of iron supplementation on resting immune status.

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