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*Article*

# The Impact of Differences in Athletic Performance on Blood Indices in University Soccer Players

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**Abstract:** [Background] Athletes need a diet that suits them to maintain and improve their performance. In recent years, dietary supplements have become more accessible and widely used by athletes, but there is concern that they may lead to reduced energy expenditure. In this study, physical characteristics of football players, blood composition and dietary surveys were conducted to investigate the effects of different athletic performance of athletes on iron nutritional status. [Methods] Dietary surveys, physical activity surveys, and measurements of physical characteristics were performed on 83 Japanese university soccer players. [Results] Body weight, muscle weight, reticulocytes, erythrocytes, hemoglobin, hematocrit and hepcidin were significantly higher in team A than in team B. Body fat mass and body fat percentage were significantly lower in team A than in team B. In terms of dietary content, potassium, iodine and niacin were significantly higher in team A than in team B. [Conclusion] There were no significant differences in dietary content. Although hepcidin levels were higher in team A than in team B, hemoglobin and ferritin levels were not reduced. Further studies are needed.

**Keywords:** athlete; nutrition; diet; iron; hepcidin

## 【Introduction】

In 2010, the International Olympic Committee (IOC) stated that "a diet providing sufficient energy from a wide range of commonly available foods can meet the carbohydrate, protein, fat and micronutrient requirements for training and competition" [1]. Therefore, in our study "Dietary Self-Management in University Soccer Players: Are There Differences by Athletic Performance?" (Takahashi 2023), we reported that athletes with higher athletic performance had better dietary self-management skills [2]. Athletes with higher athletic ability consumed more iron than athletes with general athletic ability, and ferritin levels in blood indicators were higher in athletes with higher athletic ability. However, there was no difference in hemoglobin concentration.

Iron is a micronutrient found in very small amounts in the body, but it plays an important role in processes such as oxygen transport and energy metabolism. Iron is also an essential component of hemoglobin, which is required for oxygen transport in red blood cells. Therefore, if an athlete's body is deficient in iron, the oxygen-carrying capacity of active muscles is reduced, leading to a decrease in athletic performance. In other words, iron is also involved in oxygen transport to the muscles during exercise and plays an important role in energy production during exercise [3]. Iron deficiency therefore leads to reduced athletic performance in endurance sports [4]. In addition, iron metabolism disorders such as iron recycling from the spleen and macrophages and iron absorption in the duodenum via hepcidin are also associated with iron deficiency [3]. Exercise is known to increase the inflammatory cytokine interleukin-6 (IL-6), which is secreted 3 hours later and inhibits iron absorption. In other words, it has been suggested that athletes with high athletic ability who are highly active will secrete high levels of hepcidin and ferritin levels should be lower.

Therefore, focusing on hepcidin, this study investigated the effects of different sports on blood conditions.

## 【Methods】

### 1. Subjects

The subjects of this study were 83 people (ages: 19-22 years) who belong to a university soccer club. The purpose and content of the study were fully explained to the subjects, and they voluntarily agreed to participate in the study. In order to evaluate self-management ability based on athletic ability, the university athletes were divided into two groups: a high athletic ability group (Group A, n = 31), consisting of students whose members include many who will become professional soccer players from 2025, and a general athletic ability group (Group B, n = 52).

This study was approved by the ethics committee of the Kyoto Prefectural University (No. 309).

### 2. Measurement Items

#### (1) Body measurements

Height was measured with a height meter, and body weight, body fat percentage, and skeletal muscle weight were measured using the impedance method with an InBody 770 body composition measuring device (InBody Japan Inc.).

#### (2) Blood Analysis

Blood samples were drawn from the antecubital vein after each subject had been seated quietly for at least 15 min. The samples were analyzed in a local commercial laboratory (Kyoto Microbiology Research Institute, Kyoto, Japan). Red blood cells (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), ferritin, hepcidin, unsaturated iron binding capacity (UIBC), ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, platelets, C-reactive protein (CRP) and white blood cells (WBC) and haptoglobin were measured. Anemia was defined as an Hb level of <13 g/dL. Iron depletion was defined as a ferritin level of <20 µg/L. Hemolysis was determined by the indirect bilirubin concentration, which is the total bilirubin minus the direct bilirubin concentration.

#### (3) Dietary survey

Participants were instructed to document their meals on two non-game days within the study period (July 2024) using the approximate quantity method and capture images with a smartphone. During photography, the participants were asked to include their student ID alongside the meal to aid in portion size determination. Nutrients and other intakes were analyzed from the collected records and photographs using specialized nutrition calculation software (Eiyō Plus ver. 1.0; Kenpakusha, Tokyo). Daily intakes and energy density-adjusted values were calculated for 22 items: energy; protein; fat; carbohydrate; potassium; calcium; magnesium; phosphorus; iron; zinc; copper; retinol activity equivalent; vitamin D, K, B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, and C; alpha-tocopherol; folic acid; and salt equivalent. Additionally, data on the frequency and quantity of protein supplement consumption were gathered and incorporated into nutrient and other intake analyses. However, the nutrient content of supplements other than proteins, such as multivitamins, was not included in the analysis.

#### (4) Physical activity survey

The physical activity questionnaire was administered over two days on the same day as the dietary questionnaire. Behavioural data were collected at approximately 5-minute intervals from awakening to bedtime using recording forms. Physical activity intensity was assessed using the Physical Activity Recording Standards for Health Promotion 2013,14 and the revised METs table for physical activity<sup>15</sup>. The intensity and duration of training at their club was determined by analysing videos of training sessions. The physical activity survey was completed over two days on the same day as the dietary survey. Behavioural data were collected at approximately 5-minute intervals from

awakening to bedtime using recording forms. In this study, we therefore investigated the effects of differences in the athletes' sports on blood conditions, focusing on hepcidin.

Daily energy expenditure (kcal/day) was calculated using the formula  $\text{daily energy expenditure} = \text{body weight} \times 1.05 \times \Sigma(\text{exercise intensity} \times \text{time})$ . The participants participated in club activities six days a week, with some undertaking independent training.

### 3. Statistical Analysis

Descriptive statistics were reported as mean (SD). Differences in means between the two groups were analysed using the t-test. The unpaired t-test was used to compare normally distributed data, whereas the Mann-Whitney U test was used to compare non-normally distributed data between groups. Pearson's correlation coefficient was used to assess correlation for normally distributed data, whereas Spearman's rank sum correlation coefficient was used for non-normally distributed data. Statistical analysis was performed using JMP Pro 18 (SAS Institute, Cary, NC, USA). P values <0.05 were considered to indicate statistical significance.

## 【Results】

The participants' physical characteristics are listed in Table 1. The height, weight, and muscle mass were significantly higher in Team A than in Team B. The body fat percentage was significantly lower in Team A than in Team B. There were no significant differences in the height, BMI, or body fat mass between the groups.

Table 2 shows the blood test results of the two groups. There were no anemic subjects in either group, and reticulocytes, RBCs, plasma iron, hemoglobin, hematocrit, and hepcidin were significantly higher in Team A than in Team B. Values for plasma iron, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, unsaturated iron binding capacity, ferritin, aspartate aminotransferase, alanine aminotransferase, creatinine, platelets, C-reactive protein, WBCs, and haptoglobin did not differ significantly between groups. Table 3 shows a comparison of the nutritional intake of both groups. Group A had significantly higher levels of potassium, iodine, and niacin than Group B. The energy, protein fat, carbohydrate, calcium, magnesium, phosphorescence, iron, zinc, copper, iodine, vitamin A, D, E, K, B1, B2, B6, B12, folic acid, C, dietary fiber, and salt equivalent intake of the two groups did not differ significantly.

#### 1. Screening for the presence of hepcidin and related substances

Regarding the correlation between hepcidin levels and body composition, blood indices, and dietary questionnaires, Pearson's correlation results showed significant positive correlations between reticulocytes, WBCs, creatinine, and ferritin in group A ( $p < 0.05$ ,  $p < 0.04$ ,  $p < 0.01$ , and  $p < 0.01$ , respectively) (Table 4) whereas in group B, serum iron and ferritin levels showed a significant positive correlation ( $p < 0.01$ ,  $p < 0.01$ , respectively) (Table 5).

Spearman's analysis showed positive correlations for reticulocytes, WBCs, and ferritin in Team A ( $p < 0.01$ ,  $p < 0.01$ , and  $p < 0.01$ , respectively); positive correlations for serum iron and ferritin in Team B ( $p < 0.05$ ,  $p < 0.01$ , respectively); and positive correlations for serum iron and ferritin in Team B ( $p < 0.05$ ,  $p < 0.01$ , respectively). In the combined analysis of the two groups, body fat mass and percentage, WBCs, CRP level, and ferritin level were positively correlated with hepcidin levels (Table 6).

## 【Discussion】

This study investigated the effects of iron nutritional status on different levels of competition in university soccer players. The results showed that erythrocyte and hemoglobin concentrations were significantly higher in team A than in team B. However, hepcidin was also significantly higher in team A than in team B.

Hemoglobin concentration was significantly higher in team A than in team B. This may be because Team A consumed more iron than Team B, but there was no significant difference compared



to the dietary survey. In addition, the intake of vitamin C, a nutrient that enhances iron absorption, tended to be higher in team A than in team B ( $p < 0.07$ ). These results suggest that iron absorption was increased, leading to the prevention of anemia. However, it should be noted that hemoglobin levels were within the normal range in both teams. In general, the recommended iron intake for athletes is 15 mg/day [5]. Both groups had adequate hemoglobin concentrations, although they did not meet the 15 mg/day limit. Ferritin concentrations were found in Team A and Team B. Mielgo Ayuso et al reported that 30-99 ng/ml is considered functional iron deficiency for screening for iron deficiency and a serum ferritin level of at least 100 ng/ml is required [6]. Both groups exceeded 100 ng/mL and are unlikely to have iron deficiency anemia.

On the other hand, hepcidin was significantly higher in Team A than in Team B. It has been reported that muscle glycogen decreases and hepcidin increases when energy intake remains inadequate [7]. When comparing the energy intake and activity of the athletes, the activity of both teams was higher than the energy intake: -700 kcal for team A and -500 kcal for team B. It is possible that the increased hepcidin in team A, which failed to meet its energy intake requirements, could be due to insufficient energy intake. A correlation between hepcidin and WBC and CRP was also observed in the A team correlation; WBC and CRP reflect an inflammatory response. It is possible that Team A athletes have a chronic inflammatory state, especially as this was not a post-exercise blood sample.

The main sources of energy during exercise are carbohydrate and lipids. Carbohydrate in particular is known to be an important source of energy for athletes [8] and it has been reported that muscle glycogen is depleted at the point where exercise cannot be continued [9]. Williams & Rollo have highlighted the importance of having sufficient muscle glycogen stores for soccer player [10] and improved performance has been reported in players consuming high carbohydrate diets with dietary carbohydrate levels of ~8 g/kg BW [11,12]. The latest nutritional guidelines for soccer recommend a daily carbohydrate intake of 5-7 g/kg body weight on days of moderate intensity training and 7-12 g/kg body weight on days of high intensity training and before matches [13]. The results of this study showed that Team A (5.7 g/kg body weight) and Team B (6.0 g/kg body weight). It has been reported that Asians rely more on carbohydrates for energy [14]. Based on the above, it is possible that increased carbohydrate intake may help both Team A and Team B to build a body that can withstand higher intensity and longer training sessions.

While the possibility of chronic inflammation was suggested in Team A, the hemoglobin concentration was significantly higher in Team A than in Team B, although the blood indices were in the normal range in both teams. Energy intake was also inadequate in both groups. It has been suggested that chronic low energy availability (LEA) may also affect biomarkers involved in erythrocyte turnover [15–19]. A study by Hennigar et al. in active men reported that a 55% reduction in EA over 28 days resulted in a decrease in hemoglobin concentration [20]. This reduction in hemoglobin (anemia), which does not respond to iron administration, suggests that it may be due to inhibition of red blood cell production by LEA [20]. When erythropoiesis is reduced as a result of energy deprivation, the number of erythroid progenitor cells in the bone marrow decreases, maintaining iron stores for vital functions. This response has been reported to underlie the development of iron-limiting anemias, including iron deficiency anemia and anemia due to chronic disease and inflammation [21]. Testosterone is also a potent stimulator of erythropoiesis [22–25]. However, the effects and mechanisms by which testosterone affects human erythropoiesis during severe energy deprivation remain unclear. Injection of testosterone into energy-restricted mice increased the incorporation of  $^{59}\text{Fe}$  into newly formed erythrocytes, an indirect measure of erythropoiesis, compared to energy restriction alone [26]. Testosterone replacement has been reported to suppress hepcidin in healthy adults, thereby increasing iron utilisation and maintaining red blood cell production during energy deficiency [27]. It has also been suggested that testosterone administration may be an effective treatment for maintaining erythropoiesis during energy deficiency and may be particularly relevant for certain types of anemia associated with chronic disease [28,29]. Exercise is known to increase testosterone secretion [30]. Although testosterone was

not measured in this study, it is possible that the significantly higher hemoglobin concentration in team A, despite higher hepcidin concentration than in team B, was related to higher testosterone secretion due to higher activity levels.

It is known that obesity increases hepcidin levels and has been reported to be due to inflammation [31–34]. In the present study, hepcidin levels were significantly higher in Team A than in Team B, even though Team A had less body fat than Team B. Nirengi et al. reported that hepcidin levels were higher in athletes with less body fat than in those with more body fat [35]. Body fat is usually positively correlated with hepcidin concentration [31–35]. However, there are few reports on the relationship between hepcidin and body fat in athletes. More research is needed on the relationship between hepcidin and body fat in athletes.

## V. Conclusion

The present study investigated the effects of different levels of competition on blood indices, mainly iron nutritional status, in university soccer players. The results showed that erythrocyte and hemoglobin concentrations were significantly higher in team A than in team B, while hepcidin concentrations were significantly higher in team A than in team B.

Team A showed a positive correlation between hepcidin and reticulocyte, WBC and ferritin; Team B showed a positive correlation between hepcidin and plasma iron and ferritin.

There are few reports on indicators of exercise capacity differences in relation to hepcidin. More research is needed on the effects of differences in exercise capacity on hepcidin.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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