Volume : 3 | Issue : 8 | Aug 2014 • ISSN No 2277 - 8179

Research Paper

Dynamics of Serum Iron and Hepcidin in Japanese Rugby League Players

Medical Science

KEYWORDS : rugby; athlete; hepcidin; iron; ferritin.

Mitsugi Shimoda	Second Department of Surgery, Dokkyo Medical University, Tochigi, Japan
Akihito Abe	Second Department of Surgery, Dokkyo Medical University, Tochigi, Japan
Yasunori Chiba	Panasonic Rugby Team, Ohta, Japan.
Shigeyuki Mitsuhashi	Department of Orthopedic Surgery, Fujisawa City Hospital, Kanagawa, Japan.
Keiichi Kubota	Second Department of Surgery, Dokkyo Medical University, Tochigi, Japan

ABSTRACT

Background: There have been no studies reporting about hepcidin and its relation with stress due to training or competition in contact sports such as rugby. There are also no studies reporting about the relation of hepcidin with iron, ferritin, or hemoglobin.
Research question: The aim of this study was to investigate the dynamics of serum iron and hepcidin in rugby players.
Type of study: Observational parallel-group study.
Methods: Forty Eight male rugby players were accrued from the top domestic league for this study. Laboratory data were analyzed on an annual basis. The players were divided into two groups according to the duration of participation in top league games (total competition time 300min for group A and <300min for group B), and these two groups were compared.
Results: The levels of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), creatine phosphokinase (CPK), blood urea nitrogen, ferritin, and hepcidin were increased significantly in all of the players after training or competition. In contrast, the total protein, albumin, alkaline phosphatase, creatinine, red blood cell count, hemoglobin, haematocrit, and platelet count showed a significant decrease. Comparison of the two groups showed that the levels of GOT, GPT, LDH, CPK, creatinine, uric acid, hepcidin, and ferritin were higher in group A than in group B.
Conclusions: These findings suggest that the yearly variations of iron, hemoglobin, ferritin, and hepcidin levels were demonstrated to be associated with biochemical parameters. In addition, starting players have a higher work load than substitute players despite undergoing the same training.

Introduction

Maintaining adequate oxygen-carrying capacity is essential for elite athletes to achieve optimum performance. However, many athletes have low serum iron levels that negatively influence oxygen-carrying capacity. There have been reports that anemia in athletes is related to iron deficiency, but the cause of such iron deficiency remains unknown[1-3].

Hepcidin is a peptide synthesized by the liver that reduces iron utilisation[4] in humans. Increased expression of hepcidin lowers the blood level of iron by suppressing release of iron from liver cells and reticuloendothelial cells, as well as by suppressing intestinal uptake of iron and increasing ferritin level. In recent years, high hepcidin levels have been reported in patients with postoperative complications of gastrointestinal surgery or organ transplantation. These reports have described that hepcidin was induced by some cytokines, such as interleukin-6 (IL-6), therefore resulting in increased ferritin and decreased serum iron levels [5-8]. However, there have been no reports about hepcidin in athletes playing contact sports such as rugby or about its relation with stress due to training or competition, as well as the relations among iron, ferritin, hemoglobin, and hepcidin. Therefore we investigated in this study the relationship between iron related parameters and factors used in regular medical check, such as total protein (TP), albumin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase (ALP), creatine phosphokinase (CPK), uric acid (UA), blood urea nitrogen (BUN), blood glucose (BG), creatinine and blood counts etc.

In this study, we collected the blood samples from elite male rugby league players, and analyzed them to assess the relationships among hepcidin, iron, hemoglobin, ferritin, and the cytokine IL-6, as well as with regular medical check parameters.

Methods

A total of 48 male athletes from a single team in the Japan Top Rugby League were evaluated. Blood samples were collected a total of three times per year: 1) at the beginning of heavy training and stress period (May 2010), when physical stress from training was the highest (heavy training and stress period), 2) at the end of which focused on strategy, and thus caused the least physical stress (August 2010, adjustment phase before start of the rugby season: light training and unstress period), and 3) after the 7th league game when physical stress tended to be maximal during the season (October 2010: on season). All blood samples were collected immediately after practice or the game, as well as before food intake. The parameters measured were TP (g/L), albumin (g/L), GOT (U/L), GPT (U/L), ALP (U/L), gamma-glutamyl transpeptidase (γ-GTP, U/L), CPK (U/L), UA (μmol/L), BUN (mmol/L), BG (mmol/L), creatinine (μmol/L), electrolytes (Na, K, and Cl, mEq/dL), white blood cells (WBC, $x10^9$/μl), red blood cells (RBC, $x10^{12}$/μl), hemoglobin (g/L), haematocrit (%), platelets ($x10^9$/μl), iron (μmol/L), ferritin (pmol/Lng/mL), hepcidin (ng/mL), and IL-6 (pg/mL). Serum samples for measurement of hepcidin were obtained from each subject and stored at -80°C until analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS), as reported previously[9]. IL-6 levels were determined by using a Human IL-6 Quantikine ELISA kit (R&D System, Minneapolis, MN). Parameters and data were analyzed on yearly basis. The rugby players were divided into two groups according to the duration of participation in the top league games (total competition time[3]300min (group A) or <300min (group B) for further comparison. The mean playing time of all the subjects was 293 minutes, therefore 300 minutes was selected as the cut-off value. For this retrospective analysis, informed consent was obtained from each subject and all participants have accepted publication of the results. All blood samples undertaken by two authors (MS and AB), and transfer to the medi-

cal lab (Kotobiken Medical Laboratories Inc., Tsukuba, Japan, Model 7170, Hitachi, Inc., Tokyo, Japan). The protocol of this study was approved by the Ethical Committee on Clinical Investigation of Dokkyo Medical University Hospital, and also has confirmed that this study meets the ethical standards of the journal[10], and that the study procedures were in accordance with the ethical standards of the Committee and with the Declaration of Helsinki.

Results are expressed as the median and range. Comparisons between two groups were done with the Mann-Whitney U test (two-sided) and a probability (P) value<0.05 was considered to indicate statistical significance.

Results

The median age was 25.5 years (range: 18.6-34.8 years), median body weight was 92.0kg (range: 65.3-121kg), and median height was 181cm (range: 166.2-196.0cm). Results for all subjects are shown in Table 1. Compared with the values measured at the end of light training and unstress period (August 2010), the levels of GOT (P=0.00001), GPT (P=0.001), CPK (P=0.0005), UA (P=0.0004), BUN (P=0.0005), and ferritin (P=0.043) were all significantly higher during heavy training and stress period (May 2010). On the other hand, ALP (P=0.038), creatinine (P=0.0001), hemoglobin (P=0.004), platelets (P=0.009), and iron (P=0.045) were all significantly lower during heavy training and stress period than during light training and unstress period.

Compared with during the on season (October 2010), the levels of CPK (P=0.0036), BS (P=0.031), BUN (P=0.018), and hepcidin (P=0.032) were significantly higher during light training and unstress period (August 2010), while the levels of TP (P=0.0038), albumin (P=0.036), RBC (P=0.027), hemoglobin (P=0.007), haematocrit (P=0.0019), and platelets (P=0.013) were significantly lower. Comparison of groups A and B showed that group A had significantly higher GOT (P=0.006) and CPK (P=0.008) levels than group B during heavy training and stress period (Table 2). Both TP (P=0.009) and albumin (P=0.019) were lower in group A during light training and unstress period, whereas Cl (P=0.014) was higher in group A than group B. Group A also had significantly higher levels of GOT (P=0.006), GPT (P=0.041), CPK (P=0.021), creatinine (P=0.0001), WBC (P=0.001), hepcidin (P=0.008), and IL-6 (P=0.007) during the on season.

Discussion

In this study, we have analyzed the blood samples collected from Japanese male rugby players who participated in the top level of competition. We have chosen to accrue the necessary sample population from the top rugby team to select the best physically fit population. The team to which the players belonged has won the premiership of the Japan Top Rugby League Division once, and has also won the Japan Rugby championship 4 times in the past. Recently, there have been several reports about iron metabolism in female athletes, which have indicated that anemia in female athletes is due to lack of iron[8,11]. In male athletes, there is no menstrual cycle to deplete iron stores, so iron deficiency is generally thought to be due to insufficient oral intake (unless there is intestinal bleeding). However, it is unlikely for athletes to eat an unbalanced diet with insufficient iron these days, and none of the subjects of this study had a poor diet. Therefore we hypothesized that that there is another factor affecting iron metabolism in male athletes. Our previous research on invasive gastrointestinal surgery showed that an increase of IL-6 and some cytokines leads to a dramatic increase of hepcidin, resulting in elevation of ferritin and in reduction of serum iron as well as a decline of hemoglobin[4, 6]. In other words, this research showed that the levels of iron and hemoglobin are reduced after invasive surgery via a mechanism that is unrelated to intraoperative bleeding. For the current study, we hypothesized that in athletes playing aggressive contact sports such as rugby, variation of serum hepcidin could affect iron metabolism. Peeling, et al was the first to report on the dynamics of hepcidin in athletes[12], followed by McClung et al reporting on hepcidin in female athletes[13]. Auersperger et al studied hepcidin and iron metabolism in marathon runners[13], and concluded that iron decreases as hepcidin increases. We obtained similar findings during stressful heavy training and stress period and during the on season, where the increased levels of GOT, GPT, CPK, and UA led to elevation of hepcidin and a converse decrease of hemoglobin and iron. During less intense light training and unstress period, hepcidin decreased, and there was a restoration of the other parameters along with recovery of iron and hemoglobin levels. This suggests that physical stress associated to sports influences the levels of hepcidin, iron, and hemoglobin. The median hepcidin level was >40ng/mL throughout the year in our study population, which is lower than after pancreatoduodenectomy (PD) (74ng/mL)[7], but is clearly higher than the average adult male level (19.1ng/mL)[11].

Comparing the two groups based on total playing time, group A had significantly higher GOT and CPK levels during intensive training. This finding suggests that, even with the same training program, the level of physical stress differs among rugby players. As a player's playing time becomes longer, training methods and menu may differ from those players with a shorter playing time. Group A also had higher levels of GOT, GPT, CPK, hepcidin, IL-6, and WBC during the on season, suggesting the influence of heavy physical stress during competition. In addition, although there were no significant differences, levels of hemoglobin, iron, and ferritin were tend to be worse than that of group B, suggesting that the athletes with a longer playing time were subjected to more severe physical stress as well as having abnormal iron metabolism. The players were in a similar state to patient after PD[7], suggesting that iron supplementation would be prudent. Also, because rugby players show marked yearly variation of iron metabolism parameters such as hemoglobin, iron, and ferritin, their condition should not only be monitored by regular blood tests, but also through assessing special parameters such as hepcidin, CRP, and IL-6, and adequate guidance should be offered with regard to conditioning.

Conclusion

Blood samples were collected and analyzed to assess yearly trends, as well as the correlations among iron, hemoglobin, ferritin, and hepcidin. From the results of this study, the yearly variations of iron, hemoglobin, ferritin, and hepcidin levels were demonstrated to be associated with regular medical check biochemical parameters such as GOT, GPT, CPK, and UA. These findings also suggested the importance of collecting blood samples at relevant times instead of once per year to adequately understand and monitor each player's condition.

Table 1: Results for all players in the 2010 season

	Heavy training and stress period (May)	Light training and unstress period (August)	On season (October)	P value
TP (g/L)	77 (69-81)	78 (70-87)	**74 (68-84)	**0.0038
Albumin (g/L)	49 (44-53)	48 (42-51)	**46 (41-50)	**0.036

GOT (U/L)	*33 (19-79)	28 (15-54)	29 (14-63)	*0.00001
GPT (U/L)	*29 (19-86)	24 (9-61)	26 (11-65)	*0.001
ALP (U/L)	*256 (130-482)	277 (133-461)	253 (110-431)	*0.038
γ-GTP (U/L)	25 (13-106)	25 (13-109)	21 (12-98)	0.254
CPK (U/L)	*537 (106-3604)	163 (98-1726)	**304 (149-2610)	*0.0005 **0.0036
UA (μmol/L)	*416.4 (273.6-571.1)	368.8 (196.3-517.5)	380.7 (196.3-541.3)	*0.0004
BG (mmol/L)	4.72 (3.22-6.38)	4.61 (2.44-7.22)	5.00 (3.33-7.60)	0.076
BUN (mmol/L)	*6.14 (3.82-9.53)	5.75 (3.78-7.68)	**6.46 (4.03-8.25)	*0.0005 **0.018
Creatinine (μmol/L)	*74.7 (62.5-100.7)	83.9 (67.9-116.7)	78.5 (61.0-115.1)	*0.0001
Na (mmol/L)	140 (138-145)	141 (137-144)	141 (139-144)	0.990
K (mmol/L)	4.1 (3.3-4.7)	3.8 (3.4-4.4)	4.2 (3.5-4.9)	0.066
Cl (mmol/L)	102 (99-105)	102 (98-105)	102 (99-107)	0.210
WBC (X109/L)	8.9 (4.78-15.8)	8.7 (5.4-14.5)	8.5 (4.4-26.7)	0.239
RBC (X1012/L)	501 (405-572)	510 (431-572)	**497 (446-569)	0.027
Hemoglobin (g/L)	*153 (128-175)	157 (132-183)	**150 (139-143)	*0.004 **0.007
Haematocrit (%)	44.5 (38.8-53.2)	45.9 (38.9-53.0)	**43.5 (40.3-49.7)	**0.019
Platelets (X109/L)	*243 (159-408)	270 (167-361)	**233 (124-333)	*0.009 **0.013
Iron (μmol/L)	*16.8 (8.6-37.2)	19.2 (5.9-31.5)	16.5 (8.1-40.1)	*0.045
Ferritin (pmol/Lng/mL)	* 260.7 (53.9-750.5)	224.7 (65.2-770.7)	267.4 (76.4-878.6)	*0.043
Hepcidin (ng/mL)	43.9 (2.1-99.4)	40.6 (3.4-92.3)	**48.1 (7.0-96.3)	**0.032
IL-6 (pg/mL)	14.0 (5.5-24.7)	9.0 (5.1-11.7)	6.3 (3.2-62.0)	0.264

Median (range), *During heavy training and stress period (May) vs. after Light training and unstress period (August). **After Light training and unstress period (August) vs. during on season (October).

TP - total protein; GOT - glutamic oxaloacetic transaminase; GPT - glutamic pyruvic transaminase; ALP - alkaline phosphatase; γ-GTP - gamma-glutamyl transpeptidase; CPK - creatine phosphokinase; UA - uric acid; BG - blood glucose; BUN - blood urea nitrogen; WBC - white blood cell count; RBC - red blood cell count; IL-6 – interleukin-6

Table 2: Comparison of groups A and B in the 2010 season

	Group	Heavy training and stress period (May)	Light training and unstress period (August)	On season (October)	P value
TP (g/L)	A	76 (69-80)	#74 (70-81)	76 (68-82)	#0.009
	B	77 (71-81)	81 (70-87)	74 (68-84)	
Albumin (g/L)	A	48 (44-50)	#46 (43-51)	47 (43-50)	#0.019
	B	49 (45-53)	49 (42-51)	46 (41-50)	
GOT (U/L)	A	*43 (30-79)	23 (17-54)	31 (21-63)	*0.006 0.006
	B	31 (19-77)	22 (15-47)	26 (14-53)	
GPT (U/L)	A	29 (19-72)	28 (14-61)	31 (16-65)	*0.041
	B	29 (13-86)	21 (9-51)	24 (11-46)	
ALP (U/L)	A	241 (130-326)	271 (133-344)	251 (110-347)	*0.137
	B	257 (157-482)	294 (154-461)	262 (156-431)	
γ-GTP (U/L)	A	24 (15-106)	26 (13-109)	24 (12-98)	0.206
	B	13-102 (26)	14-70 (22)	12-98 (20)	
CPK (U/L)	A	*1119 (435-3604)	196 (129-1726)	360 (208-892)	*0.008 #0.021
	B	520 (106-3174)	151 (98-409)	263 (149-2610)	
UA (μmol/L)	A	404.5 (345.0-523.5)	368.8 (309.3-481.8)	404.5 (291.5-541.3)	0.784
	B	416.4 (273.6-571.1)	374.8 (196.3-517.5)	374.8 (196.3-487.8)	

BG (mmol/L)	A	4.6 (3.2-6.4)	4.3 (2.4-7.2)	5.3 (3.3-6.1)	0.112
	B	4.8 (3.7-6.4)	4.9 (4.1-6.2)	5.0 (4.2-7.6)	
BUN (mmol/L)	A	6.0 (5.4 -7.3)	5.9 (4.3-7.7)	6.9 (5.1-8.3)	0.120
	B	6.1 (3.8-9.5)	5.6 (3.8-7.6)	6.3 (4.0-8.1)	
Creatinine (µmol/L)	A	76.3 (62.5-89.2)	81.6 (67.9-116.7)	86.9 (70.2-115.1)	0.0001
	B	74.7 (63.3-100.7)	87.7 (69.4-116.7)	73.2 (61.0-105.2)	
Na (mmol/L)	A	140 (139-143)	141 (139-143)	141 (137-144)	0.806
	B	141 (138-143)	140 (138-145)	141 (138-144)	
K (mmol/L)	A	4.0 (3.3-4.5)	3.8 (3.4-4.3)	4.2 (3.6-4.7)	0.665
	B	4.1 (3.3-4.7)	3.8 (3.4-4.4)	4.2 (3.5-4.9)	
Cl (mmol/L)	A	102 (99-105)	#102 (100-105)	102 (99-107)	#0.014
	B	102 (99-105)	101 (98-104)	102 (99-107)	
WBC (X109/L)	A	7.7 (6.0-13.1)	7.9 (6.5-13.6)	13.7 (5.6-26.7)	0.001
	B	9.0 (4.8-15.8)	8.7 (5.4-14.5)	6.8 (4.4-18.3)	
RBC (X1012/L)	A	493 (448-543)	498 (453-564)	494 (446-569)	0.167
	B	512 (405-572)	520 (431-572)	500 (447-566)	
Hemoglobin (g/L)	A	150 (135-159)	153 (134-169)	147 (141-170)	0.088
	B	155 (128-175)	161 (132-183)	150 (139-173)	
Haematocrit (%)	A	44.1 (38.8-53.5)	44.2 (38.9-49)	43.5 (40.8-49.7)	0.168
	B	44.5 (38.8-51.4)	46.2 (38.9-53.0)	43.5 (40.3-49.7)	
Platelets (X109/L)	A	229 (159-274)	270 (167-312)	230 (124-290)	0.077
	B	247 (183-408)	270 (167-361)	233 (163-333)	
Iron (µmol/L)	A	16.7 (9.3-34.4)	17.4 (5.9-26.9)	15.9 (11.6-28.8)	0.274
	B	17.2 (8.6-37.2)	17.2 (5.9-31.5)	17.0 (8.2-40.1)	
Ferritin (pmol/Lng/mL)	A	213.5 (168.5-750.5)	233.7 (152.8-770.7)	305.6 (13.8-878.6)	0.184
	B	260.7 (53.9-665.1)	220.2 (65.2-575.2)	243.4 (76.4-698.8)	
Hepcidin (ng /mL)	A	42.6 (27.7-75.7)	44.7 (20-74.1)	60.0 (25.3-96.3)	0.008
	B	44.9 (2.1-99.4)	37.1 (3.4-92.3)	40.9 (7.0-85.4)	
IL-6 (pg/mL)	A	14.7 (6.5-22.7)	9.9 (6.8-10.4)	48.7 (32.8-62.0)	0.007
	B	13.3 (5.5-24.7)	8.6 (5.1-11.7)	15.7 (3.2-35.8)	

Median (range), # and : Group A vs. Group B

TP - total protein; GOT - glutamic oxaloacetic transaminase; GPT - glutamic pyruvic transaminase; ALP - alkaline phosphatase; γ-GTP - gamma-glutamyl transpeptidase; CPK - creatine phosphokinase; UA - uric acid; BG - blood glucose; BUN - blood urea nitrogen; WBC - white blood cell count; RBC - red blood cell count; IL-6 – interleukin-6.

REFERENCE

1. Corsetti R, Lombardi G, Lanteri P, et al. Haematological and iron metabolism parameters in professional cyclists during the Giro d'Italia 3-weeks stage race. Clin Chem Lab Med 2012; 50(5): 949-956. | 2. Korsten-Reck U, Seufert J, Dickhuth HH, et al. Hypogonadism and anemia in an athlete. Int J Sports Med 2012; 33(2): 154-156. | 3. Reinke S, Taylor WR, Duda GN, et al. Absolute and functional iron deficiency in professional athletes during training and recovery. Int J Cardiol 2012; 156(2): 186-191. | 4. Hentze MW, Muckenthaler MU, Galy B, et al. Two to tango: regulation of Mammalian iron metabolism. Cell 2010; 142(1): 24-38. | 5. Park KH, Sawada T, Kosuge T, et al. Surgical inflammation induces hepcidin production after abdominal surgery. World J Surg 2012; 36(4): 800-806. | 6. Bohne F, Martinez-Llordella M, Lozano JJ, et al. Intra-graft expression of genes involved in iron homeostasis predicts the development of operational tolerance in human liver transplantation. J Clin Invest 2012; 122(1): 368-382. | 7. Kosuge T, Sawada T, Shimoda M, et al. Increased hepcidin production impairs iron metabolism after pancreatoduodenectomy. World J Surg 2010; 34(10): 2452-2456. | 8. Garuti C, Tian Y, Montosi G, et al. Hepcidin expression does not rescue the iron-poor phenotype of Kupffer cells in Hfe-null mice after liver transplantation. Gastroenterology 2010; 139(1): 315-322 e1. | 9. Uehata T, Tomosugi N, Shoji T, et al. Serum hepcidin-25 levels and anemia in non-dialysis chronic kidney disease patients: a cross-sectional study. Nephrol Dial Transplant 2012; 27(3): 1076-1083. | 10. Harriss DJ, Atkinson G. Update--Ethical standards in sport and exercise science research. Int J Sports Med 2011; 32(11): 819-821. | 11. Kanda J, Mizumoto C, Kawabata H, et al. Clinical significance of serum hepcidin levels on early infectious complications in allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2009; 15(8): 956-962. | 12. Peeling P, Dawson B, Goodman C, et al. Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. Eur J Appl Physiol 2008; 103(4): 381-391. | 13. Auersperger I, Knap B, Jerin A, et al. The effects of 8 weeks of endurance running on hepcidin concentrations, inflammatory parameters, and iron status in female runners. Int J Sport Nutr Exerc Metab 2012; 22(1): 55-63. |