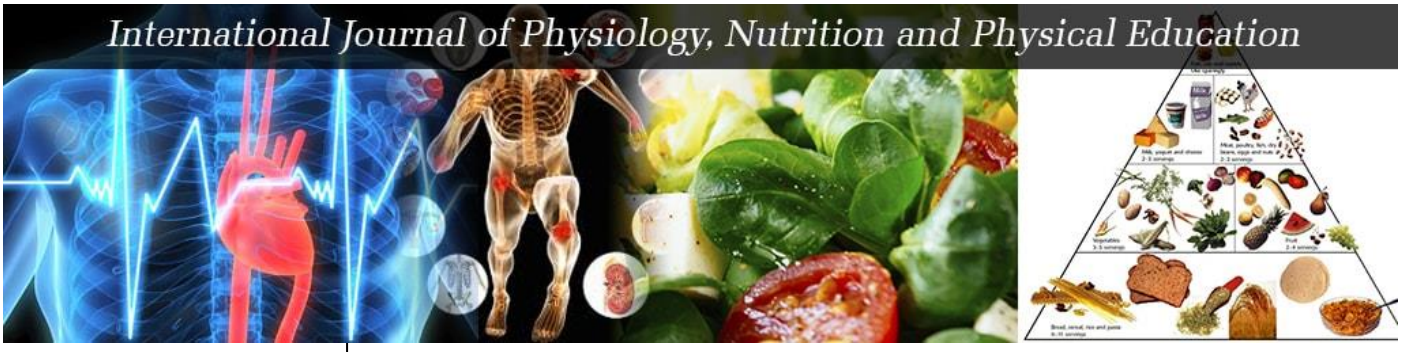

ISSN: 2456-0057
IJPNPE 2021; 6(1): 276-279

www.journalofsports.com
Received: 20-11-2020
Accepted: 23-12-2020

Usha Sri Kaniganti
Scientific Officer, Sports Authority of India, NSSC, Bangalore Karnataka, India

Corresponding Author:
Usha Sri Kaniganti
Scientific Officer, Sports Authority of India, NSSC, Bangalore, Karnataka, India

Diagnostic importance of hepcidin, soluble transferrin receptor (sTfR) and TfR-F index in monitoring the iron status of female hockey players

Usha Sri Kaniganti

DOI: https://doi.org/10.22271/journalofsport.2021.v6.i1e.2232

Abstract
Latent iron deficiency is highly prevalent among female athletes compared to the sedentary population. The present study is designed to assess whether the subnormal ferritin levels observed in female hockey players were due to true iron deficiency or due to exercise-induced metabolic adaptations. Twenty-six female field hockey players were selected for this study, after assessing the iron profile parameters they were divided into two groups namely the low ferritin group (LFG) and normal ferritin group (NFG) based on their ferritin levels (cut-off value -12ng/ml). Hepcidin, soluble transferrin receptor (sTfR), soluble transferrin receptor-ferritin index (TfR-F Index), levels were compared between groups. Though there was no significant difference in TfR levels, significant differences were observed in hepcidin, TfR-F Index, serum iron, TIBC, and TS levels between both the groups, indicating the incidence of depletion of stored iron in female players during the training cycle.

Keywords: soluble transferrin receptor, Hepcidin, Soluble transferrin receptor-ferritin index, Latent iron deficiency

1. Introduction
Latent Iron deficiency is very common among athletes. Despite having optimum levels of hemoglobin and serum iron levels, most of the female athletes exhibit low ferritin levels as an indication of depletion of storage iron. Low ferritin levels are common in athletes undergoing intensive physical training [1], Moreover, ferritin is not a reliable marker to diagnose the iron deficiency associated with inflammation as its levels augment and that can be misinterpreted as normal/optimum iron status. Iron deficiency anemia (IDA) is highly prevalent among field hockey players IDA might affect the aerobic performance and work capacity of the athletes [2].
Ferritin is widely used as a diagnostic marker to detect iron deficiency along with other parameters such as serum iron, total iron-binding capacity (TIBC), and transferrin saturation (TS). Reference ranges for serum ferritin vary across laboratories, but levels of 30 to 300 ng/ml and 10–200 ng/ml are considered normal for men and women respectively and the ferritin levels lesser than 12 ng/ml is often considered as an indicator of depletion of iron stores [3]. However, as ferritin is an acute phase reactant this may not be a reliable marker to diagnose iron deficiency associated with inflammation [4]. So besides the regular iron biomarkers, hepcidin, soluble transferrin receptor (sTfR), and sTfR/log ferritin index are considered more reliable markers to diagnose iron deficiency.
Hepcidin is a protein hormone responsible for the maintenance of iron homeostasis. Though the liver is the main source of systemic hepcidin, it is also produced locally by different organs. It maintains systemic iron availability by controlling the absorption of dietary iron and the distribution of iron among organs and tissues in the body. High hepcidin levels decrease the serum iron levels by decreasing the iron absorption from the intestine and by blocking the export of iron from hepatocytes, macrophages, and enterocytes, this can lead to decreased erythropoiesis and anemia. On the other hand, low hepcidin levels favour iron supply to bone marrow and increase the hemoglobin synthesis and erythropoiesis. Some of the recent studies have reported a significant surge in hepcidin levels after 3, 6, and 24 hours of post-exercise

and then declined to reach the baseline after 72 hours of post-exercise [5, 6]. Studies have also revealed that there is increased expression of hepatic hepcidin mRNA with the intensive exercise in both rats and humans and associated occurrence of anemia [7, 8]. An increase in hepcidin expression after exercise may result in the degradation of iron transporters such as divalent metal transporter 1(DMT1) on the duodenum and ferroportin (FPN1) on hepatocytes, macrophages, and enterocytes leading to reduced iron absorption from the small intestine and the trapping of iron in hepatocytes and macrophages [9–14].

The soluble transferrin receptor (sTfR) is a cleaved glycoprotein portion of membrane transferrin receptor (TfR) that is released into the serum after proteolysis. When transferrin iron complexes bind to transferrin receptors found on the surface of cells the iron is transported into the cells. During this process the transferrin receptors are cleaved from the surface of cells, enter the bloodstream, and become soluble transferrin receptors (sTfR). The number of transferrin receptors found on the surface of cells correlates with the level of iron within cells. When the intracellular iron level drops, the cells produce more transferrin receptors. As sTfR level varies with the change in expression of the transferrin receptor, it is a useful clinical parameter for assessing functional iron deficiency and erythropoiesis.

sTfR/log ferritin index (TfR-F index) is a calculated ratio of sTfR/log ferritin (TfR-F) is also used as an accurate indicator of true iron deficiency in patients with inflammation. TfR-F index was calculated as sTfR÷log ferritin, in which log refers to "base-10 log." As serum ferritin reflects the storage iron compartment and sTfR reflects the functional iron compartment the TfR-F index is reciprocally regulated and is widely used in the differential diagnosis of iron deficiency anemia (IDA). Low TfR-F index <1 indicates anemia of inflammation without iron deficiency, whereas a TfR-F index of >2 reflects true iron deficiency.

The present study aims to assess whether the subnormal ferritin levels observed in female athletes were due to true iron deficiency or due to the exercise-induced metabolic adaptations that take place in the athletic population.

2. Material and Methods

1. **Participants:** 26 female hockey players aged 16.7±1.3 years with more than 4 years of hockey-specific training experience were recruited for this study from the Sports Authority of India, Bengaluru. The details regarding the health status, hospitalization, medication, and supplementary usage were obtained through the questionnaires. Written informed consent was obtained from all the participants after explaining the procedure and purpose of the study.
2. **Specimen collection and assay:** Blood samples were collected from the participants in a seated position from the antecubital vein into plain evacuated tubes. All the participants were refrained from training 24 hours before the sample collection (Pre-exercise) to avoid acute exercise-induced shifts in plasma volume. After the collection, an aliquot of each sample was immediately mixed with EDTA solution to prevent clotting for hematology. The rest of the sample was collected in a plain tube with a clot activator. Following clotting, the sample is centrifuged at 1000 g for 10 minutes to separate the serum for the estimation of serum iron, ferritin, hepcidin, and soluble transferrin receptor.
3. **Estimation of Iron Profile related parameters:** Serum iron is estimated by ferrozine method, using Erba Fe125 test kit on EM360 fully automated biochemistry analyzer and the values are expressed as µg.dl^{-1}. Serum hepcidin was estimated by using, Hepcidin 25 (bioactive) test kit from DRG on an ELISA analyzer, and the values are expressed as ng.ml^{-1}. Ferritin and soluble transferrin receptor levels are measured by enzyme-linked immunosorbent assay (ELISA) test kits from Cal biotech and Bio Vendor respectively and the values were expressed as ng.ml^{-1}and µg.ml^{-1} respectively. Unsaturated Iron Binding Capacity (UIBC) was estimated calorimetrically by Ferrozine Chromozen method, using UIBC 125 test kit from Erba diagnostics and the values are expressed as µg.dl^{-1}. The sum of the UIBC and initial serum iron yields the total iron-binding capacity (TIBC) and the values are expressed as µg.dl^{-1}. Transferrin saturation is calculated from serum iron and TIBC values by using the standard equation.

$$\text{Transferrin Saturation \%} = \frac{\text{Serum Iron}}{\text{TIBC}} \times 100$$

Hemoglobin estimation: Anticoagulated whole blood samples collected in EDTA tubes were analyzed in Sysmex – PocH100i^{TM} automated hematology analyzer (Japan) to measure the hemoglobin.

4. **Grouping of participants:** Based on the ferritin test results participants are divided into two groups namely the low ferritin group (LFG with ferritin levels <12 ng/ml) and the normal ferritin group (NFG with ferritin levels >12 ng/ml) and other parameters are compared between the groups.
5. **Statistical analysis**: All the data were tested for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Variables that are normally distributed are presented in means and standard deviations, while those with non-normal distribution are expressed as medians and interquartile ranges (IQR). Between groups, the statistical analysis was performed using independent samples t-test for normally distributed data and by using the Mann -Whitney U test for non-normally distributed data. Statistical significance was set at $P < 0.05$. Effect size (Hedges' g for variables with normal distribution and r for variables with non-normal distribution) was calculated between the groups. All statistical analyses were performed using IBM SPSS statistical software (version 23).

3. Results

The mean and SD or median and IOR values of hemoglobin, serum iron, TIBC, TS, Hepcidin, sTfR, and TfR-F index, in both the groups were shown in Table-1. No significant differences were observed between the levels of hemoglobin and sTfR in both groups. However significant differences were observed in serum iron, TIBC, TS, hepcidin, and TfR-F index between both the groups.

Figure 1 depict the results of the TfR-F index, Hepcidin, and sTfR values in LFG and NFG. Figure 2 depicts the TfR-F Index distribution in both groups. Figure-3 depicts the hepcidin distribution in the LFG and NFG and Figure-4 depicts the Ferritin levels Vs TfR-F Index in all participants.

Table 1: Hemoglobin and Iron profile related parameter levels in both the groups

Parameter	LFG (n=15) (Mean ± SD/ Median &IQR)	NFG (n=11) (Mean ± SD/ Median &IQR)	Significance (2- tailed) p-value	Effect size (Hedges' g/r)
Hemoglobin ($g.dl^{-1}$)	12.54 ± 1.28	12.7 ± 1.30	0.758[¥]	0.124[H]
Serum iron ($\mu g.dl^{-1}$)	84.5 (39.9)	119.7 (45.1)	0.002[§**]	0.615[ɤ]
TIBC ($\mu g.dl^{-1}$)	464.36 ± 30.26	417.1 ± 60.21	0.032[¥*]	1.045 [H]
TS (%)	19.594 ± 8.295	32.09 ± 7.55	0.001[¥**]	1.563 [H]
Hepcidin ($ng.ml^{-1}$)	3.5 (2.0)	5.5 (2.0)	0.012[§*]	0.490 [ɤ]
sTfR ($\mu g.ml^{-1}$)	1.05 (0.51)	0.96 (0.26)	0.096[§]	0.325 [ɤ]
TfR-F index	2.2063 (1.93)	0.6559 (0.29)	0.000[§**]	0.748 [ɤ]

n=26,SD- standard deviation, IQR-Inter quartile range, [§-] Mann Whitney U test, [¥-] Independent t-test, [H] =Hedges'g effect size, [ɤ=]r effect size, **=significant ($p<0.05$), **=highly Significant ($p<0.01$)

4. Discussion and Conclusion

Though the hemoglobin and sTfR levels not significantly different between low ferritin and normal ferritin groups, a significant difference had been observed in levels of serum iron, TIBC, TS, hepcidin, and TfR-F index between low ferritin and Normal ferritin groups.

Though the mean & SD of serum iron and TS levels of LFG were within the reference range [15]. Serum iron and TS levels are significantly low in LFG compared to NFG and TIBC levels are significantly high in LFG in comparison with NFG.

Significantly low Hepcidin levels (3.5(2.0) $ng.ml^{-1}$) are observed in LFG in comparison with the NFG (5.5(2.0) $ng.ml^{-1}$) this might be the transient adaptation to restore the optimum ferritin by increasing the absorption of iron in LFG group.

The median of TfR-F index in LFG (2.2063) was higher than the reference range, i.e <1.6 [16] and was almost comparable with the value observed by Pauli Suominen et.al in stage I (depletion of storage of iron) subjects where STfR concentration remains stable but serum ferritin levels decrease gradually [17] and it is lesser than the values observed in iron-deficient erythropoiesis (IDE)/ Stage II and iron deficiency anemia (IDA)/ stage III subjects (depletion of functional iron components).

Hence it is concluded that the subnormal ferritin levels observed in female hockey players were due to the depletion of storage iron and if ignored that might lead to the development of iron deficiency anemia and might cause a decline in their performance. The periodic estimation of Hepcidin, Soluble transferrin receptor, and TfR-F index along with the regular iron profile parameters not only help to diagnose the iron-deficiency anemia but also to understand the severity/ stage of the iron deficiency in female hockey players, who are more liable to develop iron deficiency during their training cycle.

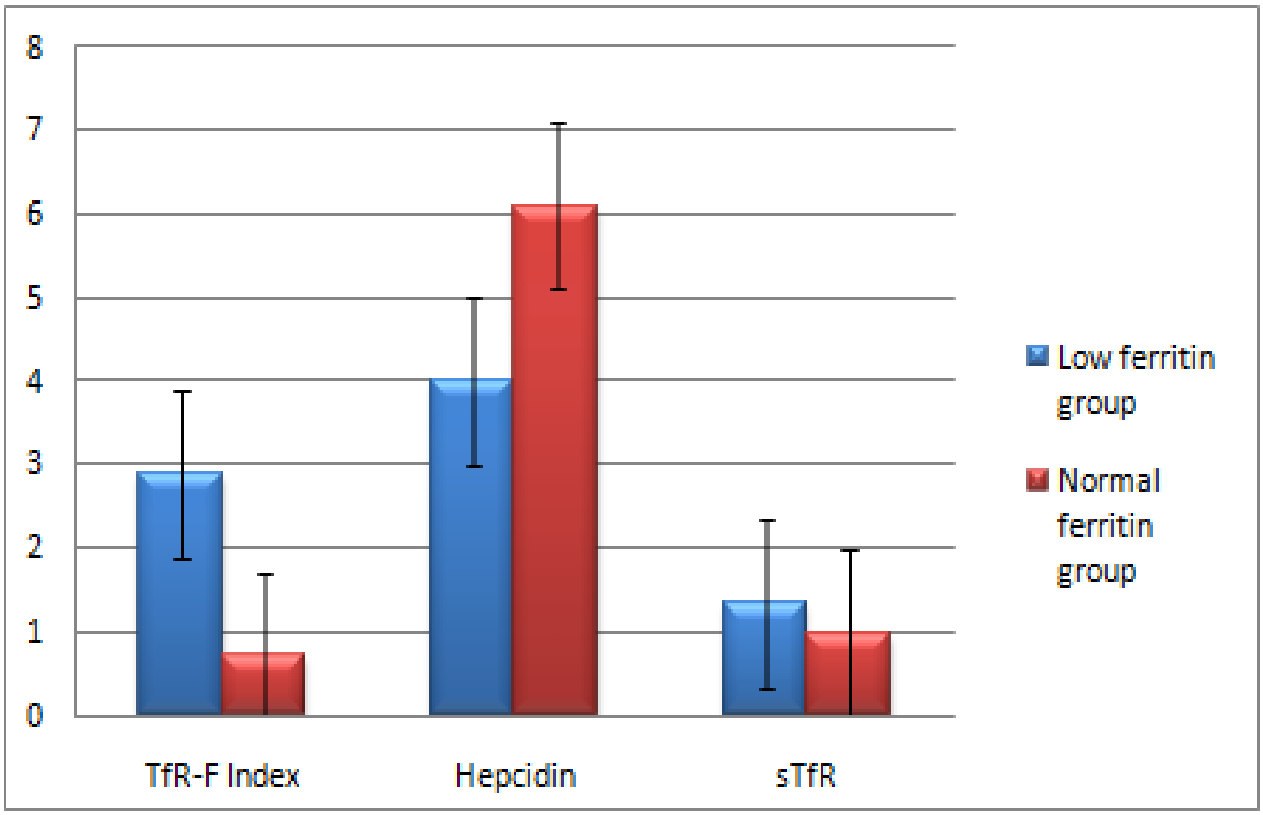

Fig 1: TfR-F index, Hepcidin and sTfR values in the LF and NF group

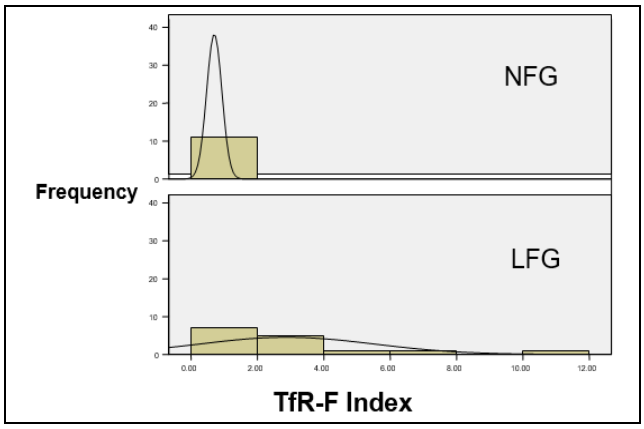

Fig 2: TfR-F Index distribution in the LFG and NFG

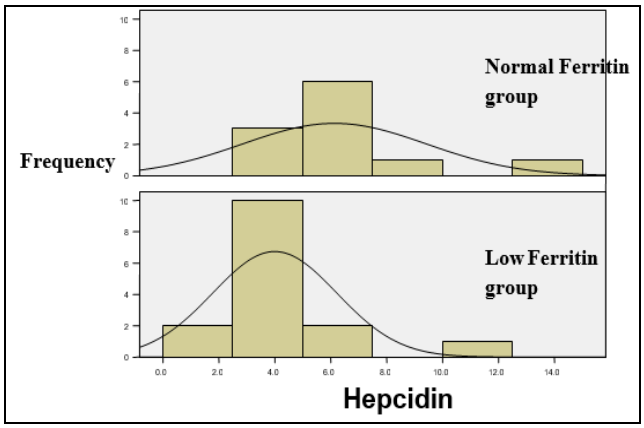

Fig 3: Hepcidin distribution in the LFG and NFG

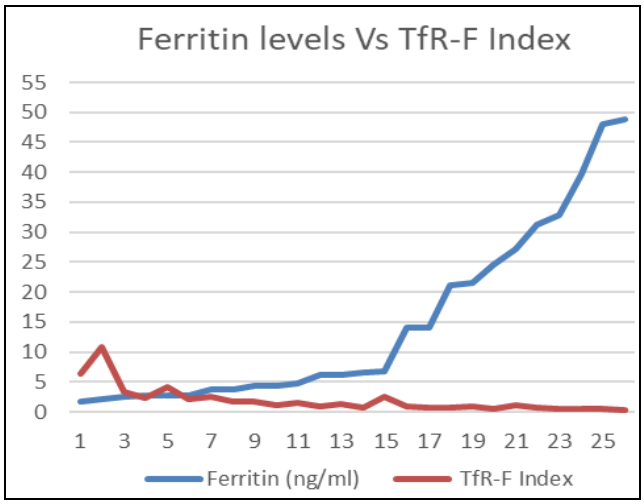

Fig 4: Ferritin levels Vs TfR-F Index in all participants

5. References

1. Magazanik A *et al.* Iron deficiency caused by 7 weeks of intensive physical exercise. Eur. J Appl. Physiol. Occup. Physiol 1988;57:198-202.
2. Majumdar P, Mandai M, Yadav D, Gupta A. Physiological and Biochemical Monitoring of Indian Female Hockey Hayers. Int. J Appl. Sport. Sci 2008;20:1-15.
3. Wang W, Knovich MA, Coffman LG, Torti FM, Torti SV. Serum ferritin: Past, present and future. Biochimica et Biophysica Acta - General Subjects 2010. doi:10.1016/j.bbagen.2010.03.011
4. Abitbol V *et al.* Diagnosis of iron deficiency in inflammatory bowel disease by transferrin receptor-ferritin index. Med. (United States), 2015. doi:10.1097/MD.0000000000001011
5. Peeling P *et al.* Effects of exercise on hepcidin response and iron metabolism during recovery. Int. J. Sport Nutr. Exerc. Metab 2009 doi:10.1123/ijsnem.19.6.583
6. Roecker L, Meier-Buttermilch R, Brechtel L, Nemeth E, Ganz T. Iron-regulatory protein hepcidin is increased in female athletes after a marathon. Eur. J. Appl. Physiol 2005;95:569-571.
7. Auersperger I. *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. doi:10.1123/ijsnem.22.1.55
8. Liu YQ, Chang YZ, Zhao B, Wang HT, Duan XL. Does Hepatic Hepcidin Play an Important Role in Exercise-Associated Anemia in Rats? are with the College of Physical Educa-tion. International Journal of Sport Nutrition and Exercise Metabolism 2011.
9. Kong WN, Gao G, Chang YZ. Hepcidin and sports anemia. Cell Biosci 2014;4:1-11
10. Mena NP, Esparza A, Tapia V, Valdés P, Núñez MT. Hepcidin inhibits apical iron uptake in intestinal cells. Am. J Physiol. - Gastrointest. Liver Physiol 2007. doi:10.1152/ajpgi.00122.2007
11. Brasselagnel C. *et al.* Intestinal DMT1 cotransporter is down-regulated by hepcidin via proteasome internalization and degradation. Gastroenterology 2011. doi:10.1053/j.gastro.2010.12.037
12. Yeh KY, Yeh M, Glass J. Hepcidin regulation of ferroportin 1 expression in the liver and intestine of the rat. Am. J. Physiol. - Gastrointest. Liver Physiol 2004. doi:10.1152/ajpgi.00246.2003
13. Chung B, Chaston T, Marks J, Srai SK, Sharp PA. Hepcidin decreases iron transporter expression *in vivo* in mouse duodenum and spleen and in vitro in THP-1 macrophages and intestinal Caco-2 cells. J Nutr 2009. doi:10.3945/jn.108.102905
14. Nemeth E. *et al.* Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004, 80. doi:10.1126/science.1104742
15. Sireci AN, Kratz A. Iron Deficiency and Overload. Iron Defic. Overload 2010. doi:10.1007/978-1-59745-462-9
16. Thomas C, Thomas L. Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency. Clin. Chem 2002. doi:10.1093/clinchem/48.7.1066
17. Suominen P, Punnonen K, Rajamäki A, Irjala K. Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. Blood 1998;92:2934-2939.