

Chronic Training Adaptation of Iron Profile in Male and Female Athletes

S. Srividhya^{1*} Dr. A. Subramanian² Dr. Pralay Majumdar³

¹M.Sc. M.Phil., Junior Scientific Assistant, Department of Biochemistry, Sports Authority of India, Netaji Subhas Southern Centre, Bangalore

²M.Sc. M. Tech Ph.D., Assistant Professor, Department of Biochemistry Raja Serfoji, Government Arts & Science College, Thanjavur

³M.Sc. Ph.D., Senior Scientific Officer, Department of Exercise Physiology, Sports Authority of India, Netaji Subhas Southern Centre, Bangalore

Abstract – The present study is aimed to find out the chronic training adaptation of iron profile in male and female athletes as there are many studies existing in acute training adaptation of iron profile. A total of 361 male athletes and 248 female athletes were volunteered to participate in the present study. Athletes were undergone training in Sports Authority of India. The mean age of the male and female athletes was 23.5±4.0 year and 21.6±4.2 year. The training age of athletes is 5-7 years. Whole blood and the serum were used to analyze hemoglobin, iron, TIBC (Total Iron Binding Capacity), ferritin and transferrin saturation. Athletes were grouped into three categories based on the demand of the energy system of the game that is aerobic game, anaerobic game and aerobic: anaerobic game. Mean value of hemoglobin, iron, ferritin & Transferrin saturation level is less and TIBC is more in female athletes than male athletes. ANOVA results showed that the ferritin, hemoglobin, transferrin saturation level in both the sex and TIBC level in female athletes is significant. Ferritin is the best indicator of the iron parameters and the other parameters gives the additional information of the iron status in the body. Mean ferritin level of male and female athletes in aerobic, anaerobic and aerobic: anaerobic game athletes are 53.7±37.7, 78.5±50.1, 66.1±48.7 and 33.2±15.6, 45.9±1126.8, 29.6±17.2 respectively. Long term of anaerobic training has less impact on iron profile and so the ferritin level is high in anaerobic game athletes when compared with aerobic game and aerobic: anaerobic game athletes. Our study concludes that chronic training adaptation of Iron profile of different sports varies with the demand of energy system of the game.

Key word: Iron Profile, Athletes, Aerobic, Anaerobic

INTRODUCTION

The importance of iron profile in athlete population for their performance has been for several decades. Iron is a micronutrient but plays a major role in the metabolic pathways and it involves in many biochemical reactions such as oxidative phosphorylation, storage and transport of iron, synthesis of neurotransmitters, DNA synthesis etc. In our human body, major part about 65% of iron are present as haemoglobin that involved in the transport of oxygen & carbon dioxide and the next major part of about 20% of iron are used for storage as ferritin & hemosiderin (Clenin *et. al.*, 2016). Deficiency of this micronutrient metal in athletes leads to altered biochemical reactions that impact the performance of an athlete and it is proved by many studies (Zoller, Vogel, 2004).

Negative iron balance will result in iron deficiency anaemia and the cause of negative iron balance may be due to malnutrition, decreased absorption of iron in intestine or increased loss of iron in sweat, urine, faeces during heavy training load (Malczewska-Lenczowska, *et. al.*, 2009). Iron deficiency is the most common nutritional deficiency in the world (Centres for Disease Control and Prevention, 2002). The parameters used in this study to monitor the iron profile are haemoglobin, iron, TIBC (Total Iron Binding Capacity) and transferrin saturation, as because to find out the iron state and the severity of the deficiency more than a single parameter of iron profile should be used (Gibson, 2005). Haemoglobin is a protein present in RBC (Red Blood Cells) that carry oxygen from lungs to various tissues where it is required for energy production and also carry carbon dioxide from tissues to lungs for expiration. Serum

iron informs us the amount of iron circulating in the blood and it is bound with the protein transferrin. Ferritin gives the information about the storage status and TIBC provides the information about the binding capacity of transferrin with iron. Transferrin saturation is the amount of iron actually binded with transferrin molecule.

The energy demand for athletes in different games varies. Hence, our study is aimed to find out the chronic adaptation of these iron parameters who were in training for 5-7 years.

METHODS:

A total of 361 male athletes and 248 female athletes were volunteered to participate in the present study. Athletes were undergone training in Sports Authority of India. The mean age of the male and female athletes was 23.5±4.0 year and 21.6±4.2 year. The training age of athletes is 5-7 years. The study was approved by the ethical committee of Sports Authority of India, India. The written consent was obtained from all participants.

The pre-analytical factors such as an overnight fast, venous blood were collected at 8.30 am with off training session for one day before the sample collection.

Sample collection: Athletes were in a sitting position and the tourniquet was set around the upper arm then the needle was inserted in the vein of antecubital fossa region immediate after the blood was filled in syringe, the tourniquet was removed (Solberg, Petitclerc, 1988). Blood was collected in the serum separator tube, inverted the tube for 5-8 times and allowed the blood to clot for upright position for at least 30 minutes. Then the blood was centrifuged for 15 minutes at 2200-2500 rpm for serum separation and the serum was used to analyze iron, TIBC (Spinreact, Spain) and ferritin (DRG diagnostics, Germany). Hemoglobin was assessed from the whole blood by cyanmethaemoglobin method, ferritin by enzyme immunoassay method, iron and TIBC were assessed by Persjin method. Transferrin saturation was calculated by the formula: Iron/TIBC * 100 (Persijn, et. al., 1971). The instrument used for these estimations were HITACHI U-2000 Double beam UV/Vis spectrophotometer (Japan), ELx800 Absorbance Microplate Reader, Biotek, United States and ERBA Smart wash-III, compact automated ELISA washer, ERBA diagnostics Mannheim GmbH, Germany.

All results are expressed as mean (Standard Deviation). The analysis was performed by the statistical software Statistical Package for Social Sciences (SPSS) MS Windows 9.0. ANOVA (One way analysis of variance) was used to determine the difference exists among the means and LSD post-hoc test was used to determine which means differ. Statistical significance was set at the 0.05 level.

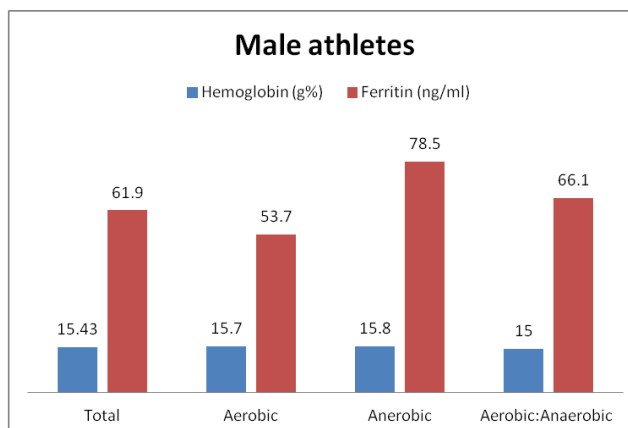
RESULTS:

Table 1: Energy system in different sports

Game	Energy system (11,12,13,14,15,16)		Game
	Aerobic (%)	Anaerobic (%)	
Cycling	80	20	Aerobic
Long Distance Running	90	10	Aerobic
Middle Long Distance Running	80	20	Aerobic
Rowing	80	20	Aerobic
Sprint	20	80	Anaerobic
Swimming	80	20	Aerobic
Walker	98	2	Aerobic
Waterpolo	60	40	Aerobic
Hockey	50	50	Aerobic: Anaerobic
Wushu	50	50	Aerobic: Anaerobic

Table2: Iron profile in Male players

Game	Number of athletes	Hb	Ferritin	Iron	TIBC	Transferrin saturation
Total	361	15.43±1.0	61.9±44.8	119±38	356±76	33±9
Aerobic game	183	15.7±1.0	53.7±37.7	119.9±37.3	350.5±75.6	34.7±9.5
Anaerobic game	47	15.8±0.9	78.5±50.1	116.0±40.8	351.4±77.0	32.8±9.8
Aerobic:Anaerobic game	131	15.0±1.1	66.1±48.7	117.5±39.1	364.9±76.5	32.0±9.0
Cycling	22	16.15±0.7	61.8±33.9	134±40	356±95	39±10
Long Distance Running	20	15.49±0.9	67.3±40.8	114±43	359±69	31±9
Middle Long Distance Running	34	16.03±0.9	73.1±45.6	129±43	355±78	36±11
Rowing	45	15.97±0.8	38.5±25.1	109±23	291±42	38±8
Sprint	47	15.79±0.9	78.5±50.1	116±11	351±77	33±10
Swimming	32	15.19±1.2	42.7±33.3	114±39	383±76	30±8
Walker	20	15.3±1.0	31.3±10.9	144±32	394±56	37±8
Waterpolo	10	15.04±1.3	52.4±37.3	96±24	383±40	25±6
Hockey	111	15.14±1.0	61.2±42.6	126±36	372±80	34±8
Wushu	20	13.94±0.9	91.2±70.0	71±18	329±43	22±6



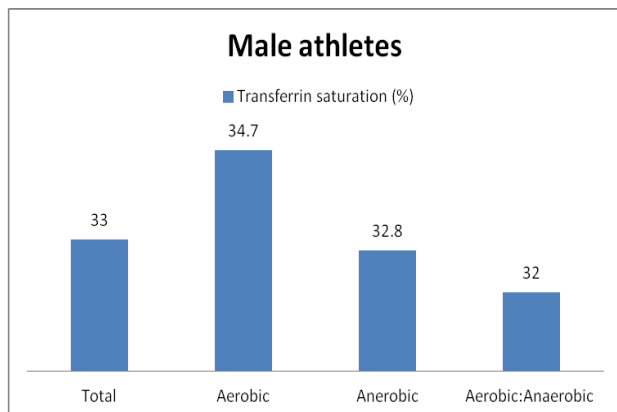
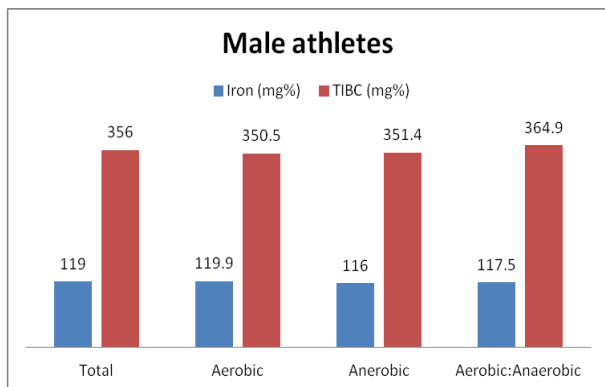
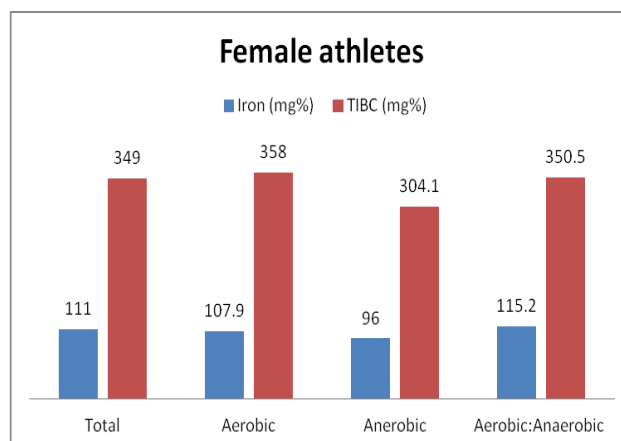
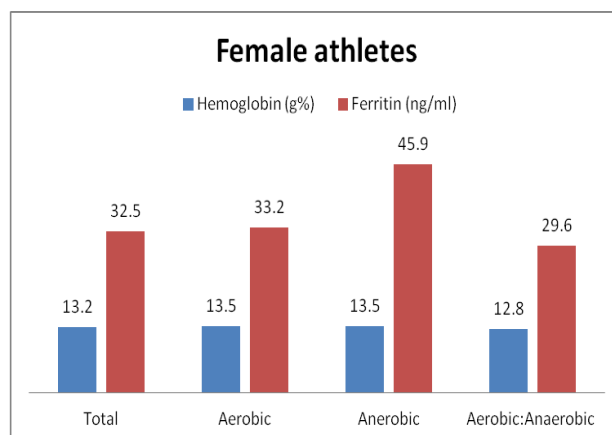


Table 3: ANOVA test of iron profile in male athletes

Parameter		Sum of squares	df	Mean square	F-ratio	Sig.
Hemoglobin	Between groups	47.28	2	23.64	22.37	0.000
	Within groups	378.37	358	1.06		
	Total	425.64	360			
Ferritin	Between groups	25065.91	2	12532.96	6.482	0.002
	Within groups	632285.9	327	1933.60		
	Total	657351.8	329			
Iron	Between groups	800.34	2	400.17	0.271	0.763
	Within groups	515956.5	349	1478.4		
	Total	516756.8	351			
TIBC	Between groups	16212.5	2	8106.3	1.399	0.248
	Within groups	1987148	343	5793.4		
	Total	2003361	345			
Transferrin saturation	Between groups	546.3	2	273.1	3.096	0.046
	Within groups	30345.4	344	88.21		
	Total	30891.7	346			

Table 4: Iron profile in Female players

Game	Number of athletes	Hb	Ferritin	Iron	TIBC	Transferrin saturation
Total	248	13.2±1.0	32.5±18.3	111±42	349±79	32±10
Aerobic game	95	13.5±0.9	33.2±15.6	107.9±37.9	358.0±72.2	30.0±8.6
Anaerobic game	23	13.5±0.6	45.9±26.8	96.0±36.7	304.1±61.0	31.5±7.6
Aerobic:Anaerobic game	130	12.8±0.9	29.6±17.2	115.2±44.8	350.5±83.7	33.5±11.0
Cycling	11	14.1±0.8	40.3±15.2	120±37	343±107	36±7
Long Distance Running	22	13.8±1.2	34.3±19.6	99±40	357±61	27±9
Middle Long Distance Running	22	13.4±1.0	34.3±12.8	123±39	381±76	33±9
Rowing	12	13.3±0.4	28.9±13.6	90±29	314±60	29±7
Sprint	23	13.5±0.6	45.9±26.8	96±37	304±61	31±8
Swimming	18	13.2±0.7	25.4±11.7	116±40	379±64	30±9
Walker	3	13.7±1.1	54.6±7.5	93±30	338±40	27±5
Waterpolo	7	13.3±0.6	29.5±13.1	87±18	337±60	26±7
Hockey	120	12.9±0.9	29.2±17.4	117±46	353±86	34±11
Wushu	10	12.0±0.6	35.6±14.3	90±22	325±54	29±8



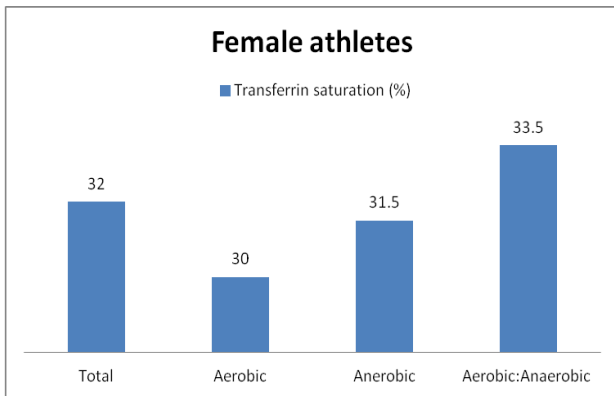


Table 5: ANOVA test of iron profile in female athletes

Parameter		Sum squares	df	Mean square	F-ratio	Sig.
Hemoglobin	Between groups	34.38	2	17.19	22.03	0.000
	Within groups	191.17	245	0.78		
	Total	225.56	247			
Ferritin	Between groups	5220.83	2	2610.42	8.29	0.000
	Within groups	75278.88	239	314.97		
	Total	80499.71	241			
Iron	Between groups	8184.86	2	4092.43	2.375	0.095
	Within groups	406604.79	236	1722.90		
	Total	414789.65	238			
TIBC	Between groups	51883.24	2	25941.62	4.316	0.014
	Within groups	1394603.3	232	6011.22		
	Total	1446486.60	234			
Transferrin saturation	Between groups	654.60	2	327.30	3.403	0.035
	Within groups	22407.71	233	96.17		
	Total	23062.31	235			

DISCUSSION:

Iron is very essential for the function of the various organs of the human body; it plays a major role for the performance of an athlete. Deficient of this micronutrient results in anemic stage which is detrimental to the athlete’s performance. The demand of energy system varies with the type of game such as a long distance runner mainly depends on aerobic system and a sprinter predominates over anaerobic system, the percentage of contribution of energy system in different sports is given in table-1. It is well documented by many studies that the iron profile relates with the aerobic system as haemoglobin protein delivers oxygen to various tissues for the generation of ATP (Adenosine Tri Phosphate) and researchers proved that the maximal oxygen consumption is positively correlated with the iron profile (Kalasuramath *et. al.*, 2015). Hence, the training program of athletes is designed and executed depends on the energy demand of that particular game.

The mean value of haemoglobin, ferritin, iron and transferrin saturation is less in female athletes than male athletes whereas the mean value of TIBC is more in female athletes than the male athletes. Our study supports the findings of Beard *et al* 2000, Constantini *et al* 2000. The cause of low iron profile in female athletes may be due to the high concentration of 17β-estradiol which inhibits the iron regulatory hormone hepcidin expression through an ERE (estrogen responsive element) half-site in the promoter region of the hepcidin gene and the other main cause is menstrual iron loss of about 4-37 mg per period in female athletes (Yang, *et. al.*, 2012. McClung, *et. al.*, 2014. Linder, 2013).

The haemoglobin, ferritin and transferrin saturation level of athletes are significantly different among the various games (Table 3 & 5). Among the other iron parameters haemoglobin, iron, TIBC and transferrin saturation, ferritin value is considered as best marker to know the iron status in the body and the other parameters adds information to it (Harju, *et. al.*, 1984. Hillman, 1998). Hence, in the study, the authors used a ferritin cut off value (<12 ng/ml) to scrutinise the athletes. Results showed that both the male and female athletes from anaerobic game has the highest ferritin level when compared with the aerobic game & the aerobic: anaerobic game counterparts (Table 2 & 4). It also shows that the athletes who were in anaerobic training for long term in the study has the highest ferritin level in turn reveals that the anaerobic training does not induce much loss of iron and it support the findings of Wang *et al* 2012.

Aerobic training has much impact on iron profile when compared with the anaerobic training because in aerobic training, the iron is used for the ATP production in the energy system (haemoglobin carries about 98.5% of total oxygen found in the blood) (Wang, *et. al.*, 2012). Anaerobic training induces a change in iron profile and return to its baseline level after recovery and so the ferritin level is more in anaerobic game athletes than the aerobic & aerobic: anaerobic game athletes. The mechanism behind this is during the anaerobic training, the release of IL-6 stimulates the production of the hormone hepcidin in hepatocytes and in blood, the hormone binds to ferroportin and blocks the intestinal iron absorption. During recovery after anaerobic training the IL-6 return to its baseline level reduces the hepcidin production and thereby enhances the iron absorption and its storage (Wang, *et. al.*, 2012. Skarpan-Stejnborn *et. al.*, 2015)

There are many studies showed the short term effect of aerobic training on iron profile and there are a very limited number of studies on long term adaptation of aerobic training in various games of athletes. The low ferritin value in aerobic game athletes is due to long term adaptations of aerobic training. Long term of aerobic training increase the RBC (Red Blood Cell) turn over by the increased expression of EPO gene in turn which increases the production of erythropoietin

hormone that stimulates the erythropoiesis process (Min, 2012. Smith, 1995). As there is increase in RBC turnover because of reticuloendothelial cell degradation of aged red cells, there is a need of iron molecule for the synthesis of new RBC and in response to the signal of low level of iron in labile iron pool, the iron-rich ferritin is shifted from cytosol to lysosomes by the process of autophagy and the cathepsin degrades the ferritin, releases iron from lysosome to cytosol by diffusion and adds to the labile iron pool. The new iron from the pool is exported into extracellular fluid by Ferro port in. Then the iron binds to transferrin and transported to bone marrow for the development of new RBC Polonifi (Politou, *et. al.*, 2010). Polonifi et al 2010 studied that there are twelve genes that are involved in the iron metabolism of import, storage and export in human body (Calco, *et. al.*, 2008). Long term aerobic training may alter the expressions of these genes on the genetic and molecular basis of adaptation to training (Calco, *et. al.*, 2008). Hence, the ferritin molecule is degraded and used up for the synthesis of new RBC, the level of ferritin is low in aerobic game athletes.

CONCLUSION:

The study concludes that the anaerobic training for long term has a less impact on iron profile of both male and female athletes. The ferritin level of anaerobic game athletes is high when compared with aerobic and aerobic: anaerobic game athletes. Iron profile of different sports varies with the demand of energy system of the game.

REFERENCES:

- Beard J, Tobin B. (2000). Iron status and exercise. *The American Journal of Clinical Nutrition*; 72: pp. 594S-597S.
- Calco CM, Vona G. Vona G. (2008). Gene polymorphisms and elite athletic performance. *Journal of Anthropological sciences*; 86: pp. 113-131.
- Centers for Disease Control and Prevention. (2002). Iron deficiency – United States, 1999-2000. *MMWR. Morbidity and Mortality Weekly report*, 51(40), pp. 897-899.
- Clenin GE, Cordes M, Huber A, Schumacher Y O, Noack P, Scales J, Kriemler (2016). Iron deficiency in sports – definition, influence on performance and therapy. *Consensus statement of the Swiss society of Sports Medicine. Swiss Sports & Exercise Medicine*, 64(1): pp. 6-18.
- Constantini NW, Eliakim A, Zigel L, Yaaron M, Falk B. (2000). Iron status of highly active adolescents: Evidence of depleted iron stores in Gymnasts. *International Journal of sport nutrition and exercise metabolism*; 10: pp. 62-70.
- Fox E, Bowers R, Foss M. (1993). *The physiological basis for exercise and sport*. 5th edition. Madison, WI: Brown and Benchmark.
- Gibson R S. (2005). Assessment of iron states. In: *Principles of Nutritional Assessment*, 2nd Edition, Oxford University Press, pp. 443-476.
- Harju E, Pakarinen A and Larmi T. (1984). A comparison between serum ferritin concentration and the amount of bone marrow stainable iron. *Scandinavian Journal of Clinical and Laboratory Investigation*, 44: pp. 555-556.
- Hill DW, J(1999). *Sports Science, energy system contributions in middle distance running events*.
- Hillman R.S. (1998). Iron deficiency and other hypo proliferative anaemia's. In: *Harrison's Principles of Internal Medicine (14th ed.)*, A. S. Fauci, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL and Longo DL (Eds.). New York: McGraw-Hill, pp. 638-645.
- <http://www.biostat.envt.fr/spip/spip.php?article63>
- <http://www.caasn.com/20-km-walk.html>
- https://etd.ohiolink.edu/!etd.send_file?accession=osu1374168831&disposition=inline
- <https://www.brianmac.co.uk/energy.htm>
- Kalasuramath S, VinodKumar C S, Kumar M, Ginnavaram V, D V Deshpande (2015). Impact of anemia, iron deficiency on physical and cardio respiratory fitness among young working women in India. *Indian Journal of Basic and Applied Medical Research*, 4 (4): pp. 119-126.
- Linder MC. (2013). Mobilization of stored iron in mammals. *A Review. Nutrients*; 5: pp. 4022-4050.
- Malczewska-Lenczowska J, Stupnicki R, Szczepanska B. (2009). Prevalence of iron

deficiency in male elite athletes. *Biomedical Human Kinetics*, 1: pp. 36-41.

Element Half-Site. *Endocrinology* 153: pp. 3170–3178.

McClung JP, Gaffney-Stomberg E, Lee JJ. (2014). Female athletes: a population at risk of vitamin and mineral deficiencies affecting health and performance. *Journal of Trace Elements in Medicine and Biology*; 28 (4): pp. 388-392.

Zoller H, Vogel W. (2004). Iron supplementation in athletes – First Do No Harm. *Nutrition*, 20: pp. 615-619.

Min Hu. (2012). Effects of exercise training on red blood cell production: Implications for Anemia. *Acta Haematologica*; 127: pp. 156-164.

Corresponding Author

S. Srividhya*

Persijn J, Slik W, Riethorst A. (1971). Determination of serum iron and latent iron-binding capacity (LIBC). *Clinica Chemica Acta*, 35: pp. 91-93.

M.Sc. M.Phil., Junior Scientific Assistant, Department of Biochemistry, Sports Authority of India, Netaji Subhas Southern Centre, Bangalore

Polonifi A, Politou M, Kalotychou V, Xiromeritis K, Tsironi M, Berdoukas V, Vaiopoulos G, Aessopos A. (2010). Iron metabolism gene expression in human skeletal muscle. *Blood Cells, Molecules and Diseases*, 45(3): pp. 233-237.

E-Mail – vidhyabiochemistry@gmail.com

Skarpan-Stejnborn A, Basta P, Trzeciak J, Szczesniak-Pilaczynska L. (2015). Effect of intense physical exercise on hepcidin levels and selected parameters of iron metabolism in rowing athletes. *European Journal of Applied Physiology*;115: pp. 345–351.

Smith JA. (1995). Exercise, training and red blood cell turnover. *Sports Medicine*; 19 (1): pp. 9-31.

Solberg HE, Petitclerc C. (1988). Approved recommendation on the theory of reference values. Part 3. Preparation of individuals and collection of specimens for the production of reference values. *Clinica Chemica Acta*. 177: pp. S1-S12.

Suominen P, Punnonen K, Rajamaki A, Irjala K (1998). Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. *Blood*, 92(8): pp. 2934-2939.

Wang L, Zhang J, Wang J, He W, Huang H. (2012). Effects of high-intensity training and resumed training on macroelement and microelement of elite basketball athletes. *Biological Trace Element Research*. 149 (2): pp. 148-154.

Wing Chun Kung- Fu. (1992). A complete guide. Dr. Joseph Wayne Smith, p.no. 90, Tuttle publishing company.

www.hksports.net/hkpe/others/multiapproach-eng.pdf

Yang Q, Jian J, Katz S, Abramson S. B. (2012). Huang X. 17β-Estradiol Inhibits Iron Hormone Hepcidin Through an Estrogen Responsive