Nutritional Sciences 9(4), November, 2006, pp.295-300

Clinical Study on the Iron Absorption from Heme-Iron Polypeptide and Nonheme-Iron*

Tae-Sik Nam, Ji Yeon Shim, Byung-Ju Kim, So Young Rah, Kwang-Hyun Park, Seon-Young Kim, Eun-Gyeng Mun[1], Young-Ju Jeong[2], Myung-Kwan Han[3], Youn-Soo Cha[1], Soo-Wan Chae[4], Mie-Jae Im and Uh-Hyun Kim[4§]

Department of Biochemistry, Chonbuk National University, Jeonju 561-182, Korea
[1]Department of Food Science & Human Nutrition, Chonbuk National University, Jeonju 561-182, Korea
[2]Department of Obstetrics, Chonbuk National University, Jeonju 561-182, Korea
[3]Department of Microbiology, Chonbuk National University, Jeonju 561-182, Korea
[4]Institute of Cardiovascular Research, Chonbuk National University, Jeonju 561-182, Korea

Iron bound to heme appears to be more bioavailable than iron salts. A clinical study was performed to investigate the absorption efficiency of heme-iron and iron-salt products available. Heme-iron and nonheme-iron supplements have become available in Korea. We performed iron absorption studies to compare the absorption of heme-iron polypeptide (HIP) products made from digested hemoglobin, produced in Korea (HIPk) and imported from Japan (HIj), with that of iron salts. In the study, 80 subjects were divided into 5 groups (n=56): placebo group; 12 mg glucose, HIPk group; 12 mg iron as HIPk, HIj group; 12 mg iron as HIj, iron-salt group 1; 12 mg of iron as ferrous aminoacetate, and iron-salt group 2; 100 mg iron as ferrous aminoacetate. Changes in serum iron levels were measured at 3 and 5 hours post ingestion. Absorption of iron in HIPk was higher compared to HIj, iron-salt or placebo. There was a significant inverse correlation between low serum iron levels (<80 μg/dl) and iron absorption from HIPk. These results demonstrated that HIPk was more bioavailable, even taken with a meal, and would have potential advantages over iron salt or HIj as an iron supplement. Our results indicate that heme-iron absorption is regulated by iron status through a heme receptor, whereas iron-salt absorption is unregulated.

Key words: Heme-iron, Iron salt, Iron absorption, Iron supplement, Iron deficiency

Received October 18, 2006; Revised November 3, 2006; Accepted November 14, 2006

INTRODUCTION

Iron is one of the most important essential elements for all living organisms and is involved in a wide variety of key physiological processes such as oxygen transport, DNA synthesis, and electron transport.[1)] Iron deficiency is a most common nutritional problem worldwide and a common cause of anemia. Pregnant women, young women during their reproductive years, and children tend to be at the highest risk of iron deficiency.[1)] Iron is absorbed from the diet in two forms: one is inorganic iron (nonheme-iron) mainly released from vegetables and plant-based foods; the other is heme-iron obtained from the pharmaceutical breakdown of hemoglobin (Hb) or myoglobin present in animals.[2)] Pharmacological iron is usually a ferrous salt that is insoluble in solutions with a pH greater than 3 whereas heme-iron is soluble in solutions with a pH greater than 7 and below pH 3.[3)] Therefore, it is well known that absorption of heme iron is far greater than nonheme-iron: The absorption rates of heme-iron and iron-salt are 37% and 5%, respectively.[4)] Ferrous ion must be solubilized and chealated in the stomach to be available for absorption in the more alkaline duodenum. Heme-iron is absorbed through a receptor while iron-salts are transported into the body through non-selective iron channels called divalent metal transporters.[5)] Other metal ions can therefore inhibit iron-salt absorption by competing for the use of the same channel. Moreover, the iron channel is not regulated, suggesting that iron-salt intake needs to be strictly controlled to prevent iron overload.

Body concentrations of iron are maintained primarily

* This study was supported by a grant of the Ministry of Health & Welfare, Repubilic of Korea.(HMP-00-VN-02-21100-0050)
§ To whom correspondence should be addressed.
(E-mail : uhkim@chonbuk.ac.kr)

by the regulation of dietary iron absorption. A man with a 4 g body store of iron will lose only 1 mg daily whereas women during the childbearing years will lose approximately twice that amount due to menstruation and childbirth.[6,7] Since Hb contains 3.46 mg of iron per gram of Hb, each ml of blood loss (Hb 15 mg/dl) results in the depletion of a half mg of iron.[8] In addition, iron overload due to excessive intake from diet or supplement also has serious health consequences due to generation of reactive oxygen species by free iron.[9,10]

Due to these well characterized side effects of iron, consumers have been interested in a safer iron supplement other than iron-salt products, such as heme-iron product. Very recently, we have reported that a heme-iron product produced in Korea is soluble over a wide-range of pH, whereas a heme-iron product imported from Japan is insoluble, and that the two products have different physicochemical properties.[11] As a continuation of that study, a clinical study was performed to compare the absorption efficiency of heme-iron and iron-salt products available in the Korea market.

MATERIALS AND METHODS

1. Materials

Heme-iron polypeptide (HIP) products were obtained from Advanced Biochemicals, Inc (Jeon-Ju, Korea) and Ito Chemical Co. (Japan). Ferrous aminoacetate was purchased from Hyundai Pharmaceuticals Corp. (Korea) and glucose (placebo) from Ashland Nutritionals (Irvine, CA). All preparations were put into opaque gelatin capsules.

2. Subjects

The study design was approved institutional review board of Chonbuk National University. Subjects (24 males and 32 females) were all healthy volunteers, ranging between 22 and 58 years of age. After reading and signing informed consent, subjects fasted from 8 p.m. in the evening prior to testing, and upon arrival in the morning, 5 ml of blood was drawn. They immediately thereafter consumed a standard meal and test dose of iron. The basal iron content of the meal was not determined. After eating the meal, the subjects continued fasting and blood was drown after 3 and 5 hours. Each subject received, in a double blinded randomised fashion, 12 mg of HIPk iron or 12 mg of HIj iron, 12 mg of iron as ferrous aminoacetate, 100 mg of iron as ferrous aminoacetate or placebo (12 mg glucose). In the separate experiments, either female subjects (32 females), aged 22-35 years old, were pre-screened to determine serum iron levels and the subjects who had low level of serum iron (<80 μg iron/dl) were separately grouped in a blinded and randomised fashion to evaluate iron absorption efficiency (Table 1).

Table 1. General characteristics of subjects

		HIPk[1]	HIj[2]	Fe[3], 12mg	Fe[3], 100mg	Placebo	Total
Sex	Male	7	0	6	5	6	24
	Female	7	4	7	7	7	32
	Total	14	4	13	12	13	56
Age	20~29	3	2	3	3	5	16
	30~39	8	2	8	7	6	31
	40~49	1	0	1	1	1	4
	50~59	2	0	1	1	1	5
	Total	14	4	13	12	13	56

[1] Heme-iron polypeptide product in Korea
[2] Heme-iron product in Japan
[3] Ferrous amminoacetate

3. Measurement of Serum Iron Concentration

For assessing iron absorption, we modified the method of serum iron technique.[12,13] The time intervals for drawing blood were selected on the basis of the reports by Ekenved *et al.*.[14,15]

Blood samples were allowed to clot in siliconized tubes, centrifuged, and then the serum was collected. Serum iron was measured at 0, 3, and 5 hours post ingestion using a standard assay (Sigma Chemical, St. Louis, MO).

4. Statistical Analysis

Data from individual experiments were expressed as the mean ± standard deviation. All statistical analyses were performed using SPSS software. Differences within the groups were separated using Duncan's multiple test and student t-test at $p<0.05$.

RESULTS

1. Intake of HIPk, but not Iron-Salt, Increases Serum Iron Levels

Figure 1 shows the average changes in serum iron concentrations of two groups. The HIPk group exhibited a gradual increase in serum iron levels up to 5 hours after the intake of 12 mg iron from HIPk. In contrast, the levels of serum iron in the placebo group were sharply decreased with time. The iron levels in the iron-salt group (12 mg ferrous aminoacetate) were maintained the initial level for the first 3 hours and then

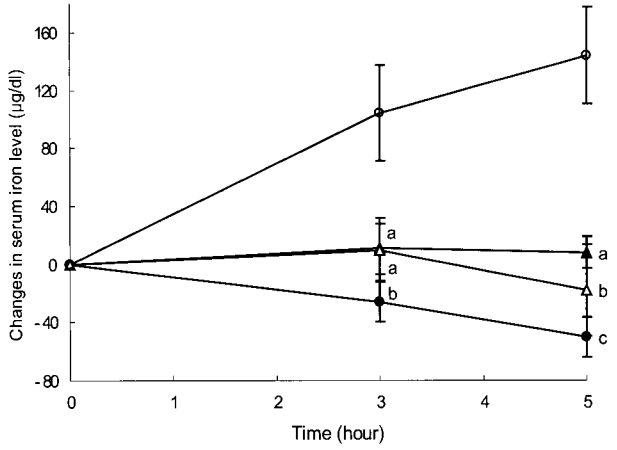

Fig. 1. Average change in serum iron for all subjects at 3 and 5 hours

Values are mean ± standard deviation.
Symbols with different superscript letter are significantly different at $p<0.05$ by Duncan's multiple-range test.
Closed triangles (▲); HIPk 12 mg, Open triangles (△); ferrous aminoacetate 12 mg, Closed circles (●); placebo, Open circles (○); ferrous aminoacetate 100 mg

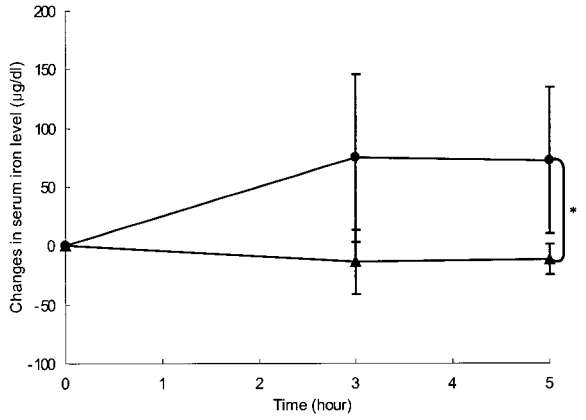

Fig. 2. Average change in serum iron for subjects of the normal status and the low status at 3 and 5 hours following intake of HIPk (12 mg)

* significant difference at $p<0.05$ by student's *t*-test
Normal status (▲; closed triangles); 80-200 μg/dl (n=4), Low status (●; closed circles); less than 80 μg/dl (n=3)

decreased after 5 hours. These results suggest that HIPk absorption is far better than iron-salt absorption and is gradually absorbed throughout the experimental period. Interestingly, the average of initial serum iron level in male subjects was approximately 185 μg/dl whereas the average iron level in female subjects was 95 μg/dl. Approximately 33% of female and 4.5% of male subjects had low serum iron levels (<80 μg/dl). On the other hand, the iron-salt group which took 100 mg iron as ferrous aminoacetate showed variable results: serum iron level was slightly increased (n=3), 5- to 8-fold or 2- to

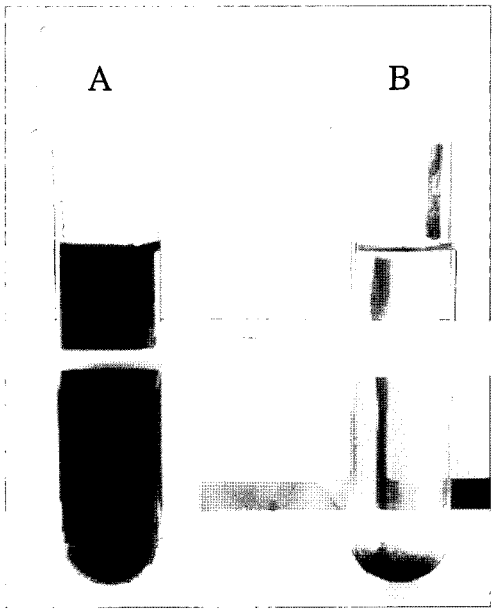

Fig. 3. Comparison of solubility of HIPk (A) and HIj (B)
Heme iron products (10 mg) were mixed with 10 ml water. Photos were taken at 10 min after shaking

3-fold (n=2) as compared to the initial serum iron levels.

2. Iron Absorption Correlates with Serum Iron Level

Heme-iron is absorbed through the binding to a specific heme receptor and the absorption is regulated depending on the iron saturation in the body.[16] Therefore, we compared the iron absorption efficiency in subjects with low serum iron level (<80 μg/dl) and subjects with normal serum iron level (>100 μg/dl). The subjects with low initial serum iron levels had dramatically increased serum iron levels at 3 hours after HIPk intake and the iron level was maintained up to 5 hours. The subjects who had normal initial iron levels did not show any significant increase in serum iron levels, and maintained the initial serum iron levels (Figure 2).

3. Solubility of HIP is an Important Factor for Iron Absorption from Heme-Iron

We have very recently reported that the solubility of HIPs available in the Korea market vary in water-based solutions with wide range of pHs.[11] HIPk made in Korea was soluble and HIj imported from Japan was not. Therefore, we decided to compare the iron absorption efficiency of these two products. Female subjects (aged 25-35 years old) were pre-screened for serum iron levels at 4 hours after taking the same meal provided in the previous study. The 12 subjects with low serum iron

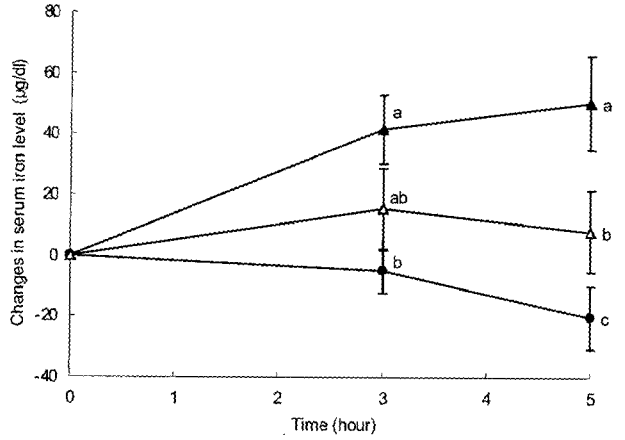

Fig. 4. Average change in serum iron for all subjects after 3 and 5 hours

Values are mean ± standard deviation.
Symbols with different superscript letter are significantly different at $p<0.05$ by Duncan's multiple-range test.
Closed triangles (▲); HIPk 12 mg, Open triangles (△); HIj 12 mg, Closed circles (●); placebo

levels (≤80 μg/dl) were divided into 3 groups as 12 mg iron of HIPk (n=4), 12 mg iron of HIj (n=4), and placebo (12 mg glucose, n=4). Consistent with the previous findings,[11] HIPk was soluble in water but HIj was not (Figure 3). Iron absorption of the women (20 to 35 years old) who had low levels of serum (<80 μg/dl) was significantly greater for HIPk than for HIj (Figure 4).

DISCUSSION

A very recent study demonstrated that a specific receptor for heme is expressed in the duodenum and liver[16] and that the receptor regulates heme-iron absorption. Thus, when the iron levels in the cells are saturated, iron absorption is limited. On the other hand, iron-salts are also absorbed in the duodenum through non-selective ion transporters, which cannot regulate metal ion absorption.[16] In this study, we investigated the efficiency of iron absorption from HIPk, HIj or ferrous aminoacetate using same dose of iron (12 mg/dose). The results have revealed that iron absorption from HIPk was significantly greater than that from HIj and iron-salt, and that the iron absorption in the subjects with low serum iron levels was significantly higher in subjects who were given HIPk, but not HIj or iron-salt than the subjects with normal serum iron levels. In contrast, no differences were found with the iron salt. We have also found that the serum iron levels were higher in men than in women (men 185 μg/dl, women 95 μg/dl). Our results also reviewed that in women, approximately 33-40% of the subjects had serum iron levels <80 μg/dl. Moreover, low serum iron levels were more severe in young women (<40 years old) than aged women (>50 years old). These observations indicated that women need to take precautions against iron deficiency.

One of the interesting findings was that iron absorption from HIPk was controlled depending on iron status. After taking HIPk, the serum iron level was greatly increased in subjects with low level of serum iron, but not in subjects with normal level of serum iron. Supporting our observations, studies using HIP products have shown that administration of 6-7 mg of iron in HIP per day increases iron parameters including ferritin values in the subjects who are at risk for iron deficiency.[17-19] Previous studies using radiolabeled Hb have suggested that iron absorption from these preparations is also controlled by iron stores, but not inhibited when taken with a meal.[20] Taken together with our findings, we suggest that the regulation of iron absorption from HIPk does occur and thus, continual supplementation of HIPk does not lead to iron overload.

Our results have indicated that the absorption of iron from iron-salt was inefficient and was not controlled. Our results are supported by a previous report that showed 5% absorption from iron salt.[4] Our results also showed that although the intake of 100 mg iron as iron aminoacetate showed increased serum iron levels, in some subjects, the level was increased more than 5-fold, reaching above 400 μg/dl. Therefore, we suggest that the overuse of iron-salt supplements may cause iron-overload. A number of studies have demonstrated that iron-overload can produce reactive oxygen species.[9,10,21,22] The inorganic iron supplements are transported through divalent metal transporter, which transports many metal ions nonspecifically.[5] Iron-salts are relatively inexpensive but side-effects of these iron supplements are well recognized. The side effects include constipation, nausea, and gastrointestinal disturbance.[23] It is also known that taking iron-salts with a meal decreases iron bioavailability, due to interactions with numerous food components.[24-27]

Heme-iron products are generally produced by the digestion of Hb with proteolytic enzymes. Depending on the digestion conditions, enzymes used, and drying method, the properties of heme-iron can be altered.[28,29] Especially, the solubility of the heme-iron product is greatly affected by the conditions mentioned above and determined by peptides associated with the heme-iron molecule. Our very recent studies with HIP products

available in Korea have revealed that HIP produced in Korea is soluble in aqueous solutions with wide pH ranges.[11] In contrast, HIP imported from Japan is precipitated in these aqueous solutions. Our results, obtained from subjects with low level serum iron levels, revealed that HIPk was absorbed efficiently whereas HIj was not. The heme-iron molecule and heme moiety are very hydrophobic and thus do not dissolve in water-based solution: heme-iron molecule has been mixed with arginine or amino acids or BSA to make it soluble in aqueous solution.[30,31] Heme-iron is transported into cells by a specific transporter expressed mainly in duodenum and liver.[30] Thus, a heme-iron product should be soluble to be transported into cells through the heme receptor following binding to the receptor. Accumulation of iron in the digestive track could cause the generation of free radicals that induce gastrointestinal discomfort or inflammation.

In summary, the absorption of iron in HIP is far more efficient than iron salt. It also appears that the solubility of HIP is critical for absorption since iron uptake from water soluble HIPk is far greater than water insoluble HIj. Moreover, our results indicate that heme-iron absorption is regulated by iron status through a heme receptor, whereas iron-salt absorption is unregulated. Therefore, controlling iron salt intake may be necessary to prevent iron-overload.

Acknowledgments

We deeply appreciate volunteers for their enthusiastic participations. We also thank members in the Clinical Trial Center for Functional Foods for assisting our work.

Literature Cited

1) Crichton RR. Inorganic biochemistry of iron metabolism, pp.29-58, Ellis Horwood Ltd, West Sussex, 1991
2) Carpenter CE, Mahoney AW. Contributions of heme and non-heme iron to human nutrition. *Crit Rev Food Sci Nutr* 31:333-367, 1992
3) Conrad ME, Umbreit JN, Moore GE. Regulation of iron absorption: proteins involved in duodenal mucosal uptake and transport. *J Am Coll Nutr* 12:720-728, 1993
4) Bjom-Rasmussen E, Hallberg L, Isaksson B, Arvidsson B. Food iron absorption in man. Applications of the two-pool extrinsic tag method to measure heme and nonheme iron absorption from the whole diet. *J Clin Invest* 53:247-255, 1974
5) McKie AT, Barrow D, Latunde-Dada GO, Rolfs A, Sager G, Mudaly E, Mudaly M, Richardson C, Barlow D, Bomford A. An iron-regulated ferrous reductase associated with the absorption of dietary iron: a novel duodenal iron-regulated transporter. *Science* 291:1755-1759, 2001
6) Conrad ME, Umbreit JN, Moore EG. Iron absorption and transport. *Am J Med Sci* 318:213-229, 1999
7) Finch CA, Ragan HA, Dyer IA, Cook JD. Body iron loss in animals. *Proc Soc Exp Biol Med* 159:335-338, 1978
8) Hurrell RF. Prevention of iron deficiency through food fortification. *Nutr Rev* 55:210-222, 1997
9) Balla G, Vercellotti GM, Eaton JW, Jacob HS. Iron loading of endothelial cells augment oxidant damage. *J Lab Clin Med* 116:546-554, 1990
10) Zacharskia LR, Glenn S, Gerhard GS. Atherosclerosis: a manifestation of chronic iron toxicity? *Vasc Med* 8:153-155, 2003
11) Kim SY, Shin YM, Im MJ, Kim UH. Physicochemical properties of heme iron products in the Korean market. *J Med Food* 9:231-236, 2006
12) Seligman PA, Caskey JH, Frazier JL, Zucker RM, Podell ER, Allen RH. Measurements of iron absorption from prenatal multivitamin-mineral supplements. *Obstet Gynecol* 61:356-362, 1983
13) Jensen NM, Brandsborg M, Boesen AM, Yde H, Dahlerup JF. Low-dose oral iron absorption test: establishment of a reference interval. *Stand J Clin Lab Invest* 58:511-519, 1998
14) Ekenved G, Norrby A, Solvell L. Serum iron increase as a measure of iron absorption-studies on the correlation with total absorption. *Stand J Haematol Suppl* 28:31-49, 1976
15) Ekenved G. Absorption from different types of iron tablets-correlation between serum iron increase in total absorption of iron. *Stand J Haematol Suppl* 28:51-63, 1976
16) Shayeghi M, Gladys O, Latunde-Dada, Oakhill JS, Laftah AH, Takeuchi K, Halliday N, Khan Y, Warley A, McCann FE, Hider RC, Frazer DM, Anderson GJ, Vulpe CD, Simpson RJ, Mckie AT. Identification of an intestinal heme transproter. *Cell* 122:789-801, 2005
17) Kazuhiro S, Takashi K, Hatsuko A, Iwao U, Kaori I, Takashi I. A study of the preventative effects of highly-absorbable iron on anemia. *The Society of Physical Fitness, Nutrition and Immunolog* 6:47-56, 1996
18) Suzuki S, Ito H, Kiyokawa H. The effect of BioIron-150 on pregnant women and women with females associated bleeding disorders. *The World of Obstetrics and Gynecology* 42:33-42, 1990
19) Ito H, Yamamoto E, Tamura T, Furuya H. The effect of "BigIron-150" on female students. *The Japanese Journal of Clinical Nutrition* 78:841-846, 1991
20) Conrad ME, Benjamin BI, Williams HL, Foy AL. Human absorption of hemoglobin-iron. *Gastroenterology* 53:5-10, 1967
21) Afanas'ev IB. Superoxide and nitric oxide in pathological conditions associated with iron overload: the effects of antioxi-

dants and chelators. *Curr Med Chem* 12:2731-2739, 2005
22) Lippi G, Schena F, Franchini M, Salvagno GL, Guidi GC. Serum ferritin as a marker of potential biochemical iron overload in athletes. *Clin J Sport Med* 15:356-358, 2005
23) Hallberg L, Ryttinger L, Solvell L. Side-effects of oral iron therapy. A double-blind study of different iron compounds in tablet form. *Acta Med Stand Suppl* 459:3-10, 1966
24) Whiting SJ. The inhibitory effect of dietary calcium on iron bioavailability: a cause for concern? *Nutr Rev* 53:77-80, 1995
25) Lynch SR, Dassenko SA, Merck TA, Beard JL, Cook JD. Soy protein products and heme iron absorption in humans. *Am J Clin Nutr* 41:13-20, 1985
26) Reddy MB, Hurrell RF, Juillerat MA, Cook JD. The influence of different protein sources on phytate inhibition of nonheme-iron absorption in humans. *Am J Clin Nutr* 63:203-207, 1996
27) Hurrell RF, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD. Soy protein, phytate, and iron absorption in humans. *Am J Clin Nutr* 56:573-578, 1992
28) Garcia MN, Martinez-Torres C, Layrisse M. Heat treatment on heme iron and iron-containing proteins in meat: iron absorption in humans from diets containing cooked meat factors. *J Nutr Biochem* 7:49-54, 1996
29) Tenhunen R, Tokola O, Linden IB. Heme arginate: a new stable heme compound. *J Pharm Phamacol* 39:780-786, 1987
30) Ekenved G, Arvidsson B, Solvell L. Influence of food on the absorption from different types of iron tablets. *Stand J Haematol Suppl* 28:79-88, 1976
31) Frykman E, Bystron M, Hensen T. Side effects of iron supplementation in blood donors: superior tolerance of heme iron. *J Lab Clin Med* 123:561-564, 1994