Original article

Effect of stabilising amino acids on the digestive absorption of heme and non-heme iron

Nikta Vaghefi[a], Didier Guillochon[b], François Bureau[c],
Philippe Jauzac[c], Dominique Neuville[c], Brigitte Jacob[c],
Pierre Arhan[a], Dominique Bouglé[a]*

[a] Laboratoire de physiologie digestive et nutritionnelle, CHU,
avenue de la Côte-de-Nacre, 14033 Caen cedex, France
[b] Laboratoire de technologie des substances naturelles, IUTA , Université de Lille-I,
BP 179 - 59663 Villeneuve-d'Ascq cedex, France
[c] Laboratoires de biochimie A et d'anatomo-pathologie, CHU,
avenue de la Côte-de-Nacre, 14033 Caen cedex, France

Abstract — We used the Ussing chamber model to study heme iron absorption by rat duodenal mucosa. Heme iron was obtained by enzymic digestion of bovine haemoglobin and concentration of heme (HPH). Its uptake and mucosal transfer was compared to iron gluconate (Gluc), at 100 μM and 1 mM. At 100 μM iron uptake (Qtot), mucosal retention (Qm) and transfer across the mucosa (Qs) was similar for the two sources of iron. Qs was significantly higher at 1 mM for Gluc but not for HPH, and was associated with higher levels of Qm. Addition of L-histidine did not improve iron absorption and indeed it decreased it if iron was provided as Gluc. L-cysteine increased the transfer of iron of both sources. In the in vitro model using rat digestive mucosa, heme iron appeared to be an efficiently used source of iron, which might prevent its accumulation by gut when supplied in excess. © Inra/Elsevier, Paris

heme iron / absorption / Ussing chamber / rat

Résumé — Effet des acides aminés sur l'absorption digestive du fer héminique et non héminique. Le modèle de la chambre de Ussing a été utilisé pour étudier l'absorption du fer héminique par le duodénum de rat ; le fer héminique a été obtenu par hydrolyse enzymatique d'hémoglobine bovine, suivie d'une concentration de l'hème (HPH) ; il a été comparé au gluconate de fer (Gluc) aux concentrations de 100 μM et de 1 mM. À 100 μM, la captation (Qtot), la rétention par la muqueuse (Qm) et le transfert séreux (Qs) du fer ne différaient pas entre les deux groupes. A 1 mM, une augmentation significative de Qs a été observée uniquement pour Gluc et s'est accompagnée d'une augmentation

* Correspondence and reprints: Service de pédiatrie A, CHU Clémenceau, avenue Georges-Clemenceau, 14033 Caen cedex, France

significative de Qm. L'addition d'histidine a diminué l'absorption du gluconate de fer ; la cystéine a augmenté significativement le transfert muqueux de fer à partir des deux sources. Dans ce modèle in vitro utilisant l'intestin de rat, le fer héminique s'est avéré être une source efficace de fer, pouvant protéger contre l'accumulation de fer par la muqueuse en cas d'apport excessif. © Inra/Elsevier, Paris

fer héminique / absorption / chambre de Ussing / rat

1. INTRODUCTION

Iron deficiency is a major problem in developing countries and affects 500 to 600 million people world-wide. Food fortification is generally considered to be the best long-term strategy for combating iron deficiency. However, the common forms of inorganic iron have a low bioavailability, and are subject to nutrient–nutrient interactions [16].

Heme iron is present in meat, fish and poultry and is absorbed by a different pathway than that for inorganic iron: it is absorbed as an intact metalloporphyrin across the intestinal brush border and is cleaved inside the enterocyte by a heme oxygenase. It then enters the inorganic iron pool and enters the bloodstream. Its bioavailability is far higher than that of inorganic iron because heme is soluble at high pH, and is less affected by digestive interactions [4], although it is affected by some [12]; a large range of results has been reported [2, 11, 12, 22, 28], depending on whether haemoglobin or heme is used. Purifying heme increases iron concentration, but is usually associated with lower levels of iron absorption [5, 6].

The aim of this study was to compare the digestive absorption of a concentrated hydrolysate of heme peptides (HPH), produced by enzymic hydrolysis of haemoglobin, with that of inorganic iron in the form of gluconate (Gluc). We used the model of Ussing chamber which makes it possible to differentiate between the various steps of absorption. We also assessed the effects of two amino acids that probably increase iron absorption (L-cysteine, L-histidine) for both forms of iron.

2. MATERIALS AND METHODS

2.1. Animals

Female Sprague-Dawley rats, 200–250 g in weight, were obtained from the Caen University farm. They were housed at 20–22 °C in a room with controlled lighting. The rats had free access to a maintenance diet for adult animals (UAR; Villemoisson-sur-Orge, France; [Fe] = 240 mg·kg⁻¹ diet) and deionised water. Six animals were studied in each group.

2.2. Preparation of the concentrate of heme peptides hydrolysate (HPH)

HPH was prepared at the Laboratoire de technologie des substances naturelles (Lille University, France); bovine blood was first centrifuged and hemolysed by addition of chlorhydric acid (pH = 4); haemoglobin hydrolysis was initiated by addition of pepsin; precipitates were eliminated by continuous centrifugation; pepsin was then inactivated by increasing the pH to 8; heme concentration was realised by ultrafiltration; the resulting concentrate of heme and peptides was spray-dried to produce a water-soluble powder containing 96 % dry matter (95 % protein; 4.6 % heme; 502 mg iron /100 g powder).

2.3. Ussing chamber

The diffusion cell (Marty Technologies, Marcilly-sur-Eure, France) is made of two acrylic half-cells separated by the digestive membrane (1 cm² area); each half-cell contains 3 mL.

Before the experiments, cells were washed with dilute (1/1 000) nitric acid (Merk Labora-

tories, Nogent-sur-Marne) to prevent contamination.

Ringer Lavoisier solution (Laboratoires Chaix et Marais) was used for luminal and serosal solutions; it is made of Na^+: 139 mEq/L, K^+: 2.7 mEq/L, Ca^{++} : 1.8 mEq/L, HCO_3^-: 2.4 mEq/L and Cl^-: 141.4 mEq/L, at a pH of 6.55. Luminal medium was prepared by addition of an adequate amount of iron of 100 µM or 1 mM iron as gluconate (Gluc) or HPH; iron Gluc ($C_{12}H_{22}FeO_{14}$, $2H_2O$) was purchased from Merk Laboratories; L-cysteine and L-histidine (Sigma, Saint Quentin-Fallavier) were added as required, at previously used concentrations: cysteine/iron (w/w) = 105 and histidine/iron = 210 [25]. D-glucose (20 mM) was added to the serosal medium to help maintain tissue viability; mannitol (20 mM) was added to the luminal solution to keep an equal osmolarity of 300 mOsm on each side of the mucosa [10]. pH was adjusted to 6.5–7 by addition of nitric acid to maintain both forms of iron in a soluble state [4]. Solutes were circulated by gas lift controlled by valves (O_2 95 %/CO_2 5 %). A small amount of an antifoaming agent (Silicone 414, Rhodorsil, France) was added to each medium to prevent the development of a foam due to gas circulation.

The diffusion cells and the media were preheated to 37 °C by a block heater, which maintained this temperature throughout the experiment.

2.4. Experimental design and sample analysis

Animals fasted overnight before the experiment. They were killed by intracardiac injection of Pentobarbital (Doléthal, Vétoquinol). A midline incision was made to expose the intestine. A 2 cm segment of duodenum, immediately distal to the first 2 cm measured from the pylorus, was removed and washed in oxygenated Ringer Lavoisier solution. Muscularis mucosa was carefully scraped from the mucosa; the first proximal centimetre of duodenum was opened along its mesenteric border to expose its epithelial surface and was placed on the pins of a half-cell. The matching half-cell was joined to seal the diffusion apparatus [9]. The distal fragment of duodenum was used to measure the basal content of iron of digestive membrane.

At the end of the experiment (2 h) the integrity of the mucosa was checked by histology. Luminal and serosal media were analysed before and at the end of the experiment. The two half-cells were rinsed with diluted nitric acid until no iron could be desorbed from cell walls or digestive membrane.

Pieces of duodenum were dried in an oven until their weight was stable and were digested by incubating with 2 mL nitric acid (65 %) for 24 h.

Iron concentration was measured in luminal and serosal media and in duodenal tissues by atomic absorption spectrometry (Perkin Elmer 1 100B).

The following were calculated:

Qtot: the total amount of iron removed from the luminal medium during the experiment;

Qm: the total amount of iron stored by the mucosa during the experiment (after subtraction of the basal iron concentration of distal duodenum mucosa);

Qs: the total amount transferred across mucosa to the serosal medium during the experiment.

Qm/Qtot and Qs/Qtot ratios were calculated to assess the fate of iron after its uptake by the enterocyte.

2.5. Statistical analysis

Results are expressed as means ± 1 SD.

Student's t-test was used to compare Qtot, Qm and Qs for HPH and gluconate, and to assess differences in these values after addition of L-cysteine and L-histidine. The significance level was set at $P = 0.05$.

3. RESULTS

Figure 1 shows iron absorption from 100 µM and 1 mM solutions. There was no difference at 100 µM between HPH and Gluc iron for Qtot ($t = 0.38$), Qm ($t = 0.18$), or Qs ($t = 0.76$).

There was a significant difference between 100 µM and 1 mM HPH in terms Qtot ($t = 5.3$, $P < 0.001$) and Qm ($t = 5.4$, $P < 0.001$), but not Qs ($t = 0.87$, $P = 0.41$). There was a significant difference between 100 µM and 1 mM Gluc iron in terms of Qtot ($t = 43.8$, $P < 0.001$), Qm ($t = 45.5$, $P < 0.001$) and Qs ($t = 10.1$, $P < 0.001$).
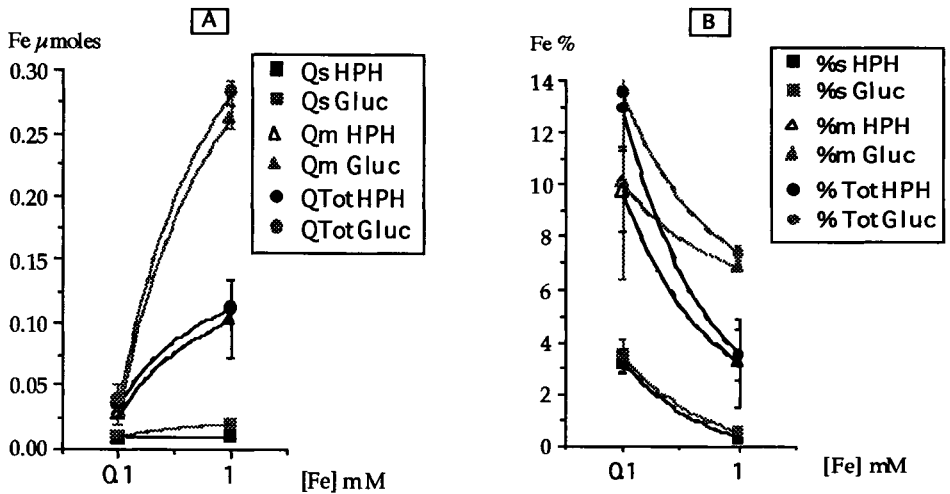
Figure 1. Effect of iron concentration (100 μM versus 1 mM) on its absorption when provided as an hydrolysate of heme peptides (HPH) or as gluconate (Gluc) by rat duodenal mucosa using the Ussing chamber. x ± 1 SD; n = 6/group. **A**, μmoles; **B**, percentage initial luminal content. Qtot, iron taken up from luminal half cell; Qm, iron retained by the mucosa; Qs, iron accumulated in the serosal half-cell. 100 μM: no significant difference between HPH and Gluc. 1 mM: HPH versus Gluc, $P < 0.001$ for Qtot, Qm and Qs. 100 μM versus 1 mM, Gluc: $P < 0.001$ for Qtot, Qm and Qs. 100 μM versus 1 mM, HPH: Qtot $P < 0.001$, Qm $P < 0.001$, Qs $P = 0.41$.

At a concentration of 1 mM, there was a significant difference ($P < 0.01$) between HPH and Gluc in Qtot ($t = 3.5$), Qm ($t = 17.3$) and Qs ($t = 4.9$).

Figures 2 and *3* show the effects of L-histidine and L-cysteine on the absorption of 100 μM HPH and Gluc iron. L-His-tidine significantly reduced Qtot, Qm and Qs for Gluc iron. It did not significantly affect HPH iron absorption. Therefore Qs was significantly higher for HPH than for Gluc iron.

Addition of L-cysteine significantly reduced Qm and significantly increased iron absorption (Qs) from HPH, whereas Qtot did not change. L-Cysteine significantly increased Qtot and Qs for Gluc. The addition of cysteine resulted in significant differences between Qtot for HPH and Gluc.

Neither Qm/Qtot nor Qs/Qtot (*figure 3*) differed between HPH and Gluc, whether used alone or with histidine or cysteine.

4. DISCUSSION

The high prevalence of iron deficiency and the low unpredictable rate of its digestive absorption [4, 16] have led to the search for an efficient, protected form of iron. Haemoglobin iron is naturally protected from digestive interactions [16]. Heme is taken up intact by the enterocyte via a specific pathway after it has been cleaved from globin by digestive enzymes, and is released into the mucosal cell by the action of an heme oxygenase [4, 5]. The bioavailability of heme iron depends on iron stores [4, 5, 11, 13, 27] but is rather independent of the composition of the diet and of the content of the lumen [4, 22]. Increased heme iron intake is associated with an improved iron status in infants [26]. Heme iron has been successfully used for food fortification [15, 32]. It decreases the negative interactions between iron and minerals and trace elements [28]. The main disadvantage of haemoglobin, however, is its very low iron
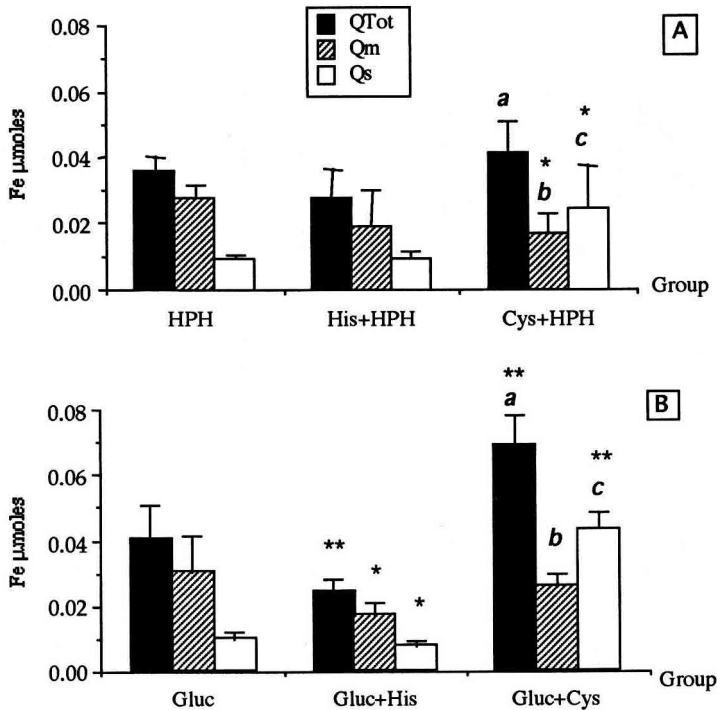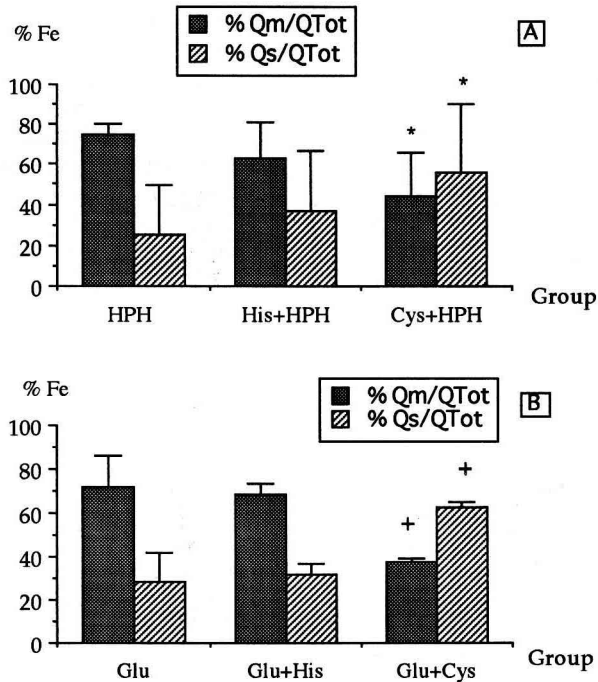
Figure 2. Effect of the addition of L-histidine (His) or L-cysteine (Cys) to the luminal half-cell of the Ussing chamber on the absorption of iron provided as a hydrolysate of heme peptides (HPH) or as gluconate (Gluc). Initial iron concentration: 100 µM; His/Fe (mg/mg): 210; Cys/Fe (mg/mg):105. **A,** HPH; **B,** gluconate iron. Qtot, iron taken up from luminal half cell; Qm, iron retained by the mucosa; Qs, iron accumulated in the serosal cell. Groups with the same superscript are significantly different. Gluconate versus HPH: *a*: $P < 0.01$; *b*: $P = 0.02$; *c*: $P < 0.001$. Effect of amino acids: experimental versus control group (HPH or Gluc):* $P < 0.02$; ** $P < 0.005$.

content. The haemoglobin hydrolysate (HPH) used in this study was concentrated (0.5 % iron versus 0.35 % in native haemoglobin); it has a low peroxidation potential and is stable in response to pH variations in vitro [20].

The Ussing chamber allows the differentiation of uptake from the mucosal step of iron absorption [9]. Contrary to cell cultures, it uses live, fully organised digestive membranes, including the mucus layer, which affects the diffusion of iron from the lumen to the enterocyte [4]. The two iron concentrations used in our model were chosen to mimic the concentrations that occur during digestion of a meal or after the ingestion of a pharmacological supplement.

As expected the rate of iron transfer across the membrane was lower for 1 mM than for 100 µM solutions of both forms of iron, demonstrating that absorption of HPH iron is regulated. A small increase in Gluc iron transfer across the cell was observed. However, it was associated with a large increase in mucosal retention, which could lead to oxidative stress in the mucosa [1, 21]. Mucosa seems to be protected from accumulation of iron when it is provided as HPH [29].

For 100 µM solutions, the absorption of HPH iron was similar to that of Gluc iron which is a highly bioavailable iron source [16].

Figure 3. Effect of the addition of L-histidine (His) or L-cysteine (Cys) to the luminal half-cell of the Ussing chamber on the two intestinal phases of iron absorption from an hydrolysate of heme peptides (HPH) or gluconate (Gluc). Initial iron concentration: 100 μM; His/Fe (mg/mg): 210; Cys/Fe (mg/mg): 105. % Qm/QTot: ratio of iron accumulated by the mucosa to total iron uptake; % Qs/QTot : ratio of iron acumulated in the serosal cell to total iron uptake. Groups with the same superscript are significantly different. Gluconate versus HPH: no significant difference. Effect of amino acids: experimental versus control group; * different from control group (HPH): $P < 0.001$; + different from control group (Gluc): $P = 0.0001$.

The absorption of heme iron is less influenced by environmental factors than that of inorganic iron [4]. However, previous studies have shown that the bioavailability of heme iron varies widely according to the process used to produce heme: intact, nondenatured haemoglobin is better absorbed than pure heme without globin. The rate of absorption of heme hemolysate iron is higher than haemoglobin iron, but decreases with further purification [2, 5, 6, 11, 12, 22, 27, 28, 30]. Presence of meat increases the absorption of heme iron [7, 11, 18, 23, 25, 28]. The mechanisms are not fully understood but may be related to the inhibition of the polymerisation of heme molecules in the presence of protein digestion products, which disappear during purification. Therefore, it is important to note that the amino acid profile of the HPH peptides is quite similar to the composition of globin [20].

Non-heme iron absorption is affected mainly by reducing agents which keep it in a ferrous state and by weak ligands which bind Fe^{3+} and keep it soluble till it reaches mucin [4]. Peptides produced by the digestion of meat are rich in cysteine [31]; cysteine and reduced cysteinyl-glycyl from glutathione increase heme iron absorption [19, 24]. Cysteine and histidine, which are tri-

dentate ions [17], also improve non-heme iron absorption [3, 24, 31, 33]. Histidine, however, has no effect in humans [19, 23]. We confirmed that cysteine increased iron absorption whereas histidine did not. This effect was observed for heme and non-heme iron. As cysteine affects non-heme iron absorption mainly by reduction rather than by increasing its solubility [8, 14, 24], our results suggest that the absorption of heme iron is positively affected by interaction with reducing agents whether provided as haemoglobin or as concentrated heme hemolysate.

Our results showed that amino acids affect both the uptake and the mucosal transfer of iron, which were decreased by histidine and increased by cysteine. Cysteine decreased iron accumulation by the enterocyte which could prevent the production of free radicals induced by iron in the mucosa [1, 21]. The effects of cysteine on the digestive clearance of heme iron suggest that the preparation of HPH was responsible for a partial loss of the stability of heme in vivo, which could be restored by adding the amino acid, as suggested by previous reports [5, 6].

Iron is cleaved from heme within the enterocyte, and enters the common iron pool; thus it could be expected that both forms of iron should display the same metabolism inside the enterocyte and should be similarly affected by amino acids.

Our results are not consistent with previous data from van Campen [3] which showed no significant effect of the transfer of amino acids across the membrane on iron absorption. It is unclear, however, how cysteine improves the transfer of iron to the basal pole of the membrane. It may form a low-molar-mass complex with iron or maintain it in a reduced form, which could favour its transfer across endosome membranes, and, therefore, its binding to transferrin which carries iron into the bloodstream via the basolateral membrane.

5. CONCLUSION

Iron absorption from a concentrated heme hydrolysate was studied with the Ussing chamber model. At low concentration it was similar to iron gluconate absorption; iron absorption was increased by adding L-cysteine which affected both mucosal uptake and the transfer of heme and non-heme iron.

However, at pharmacological levels, the increase in iron absorption from heme was less than that from gluconate, suggesting that mucosa is protected from wide fluctuations of iron intakes when iron is provided as heme.

The high bioavailability of this concentrated heme should be tested by human studies.

ACKNOWLEDGEMENTS

REFERENCES

[1]   Buttke T.M., Sandstrom P.A., Oxidative stress as a mediator of apoptosis, Immunol. Today 15 (1994) 7–10.

[2]   Calvo E., Hertrampf E., de Pablo S., Amar M., Stekel A., Hemoglobin-fortified cereal: an alternative weaning food with high iron bioavailability, Eur. J. Clin. Nutr. 43 (1989) 237–243.

[3]   Campen van D.R., Enhancement of iron absorption from ligated segments of rat intestine by histidine, cysteine, and lysine: effect of removing ionizing groups and stereoisomerism, J. Nutr. 103 (1973) 139–142.

[4]   Conrad M.E., Regulation of iron absorption, in: Prasad A.S. (Ed.), Essential and Toxic Trace Elements in Human Health and Disease: an Update, Wiley-Liss Inc., New York, NY, 1993, pp. 203–219.

[5]   Conrad M.E., Cortell S., Williams H.L., Foy A.L., Polymerization and intraluminal factors in the absorption of hemoglobin-iron, J. Lab. Clin. Med. 68 (1966) 659–667.

[6]   Conrad M.E., Benjamin B.I., Williams H.L., Foy A.L., Human absorption of hemoglobin-iron, Gastroenterology 53 (1967) 5–10.

[7] Garcia M.N., Martinez-Torres C., Leets I., Tropper E., Ramirez J., Layrisse M., Heat treatment on heme iron and iron-containing proteins in meat: iron absorption in humans from diets containing cooked meat fractions, J. Nutr. Biochem. 7 (1996) 49–54.

[8] Glahn R.P., van Campen D.R., Iron uptake is enhanced in Caco-2 cell monolayers by cysteine and reduced cysteinyl glycine, J. Nutr. 127 (1997) 642–647.

[9] Grass G.M., Sweeatana S.A., In vitro measurement of gastrointestinal tissue permeability using a new diffusion cell, Pharmaceutical Res. 5 (1988) 372–376.

[10] Grass G.M., Sweeatana S.A., A correlation of permeabilities for passively transported compounds in monkey and rabbit jejunum, Pharmaceutical Res. 10 (1989) 852–862.

[11] Hallberg L., Bjorn-Rasmussen E., Howard L., Rossander L., A discussion of possible mechanisms for the absorption-promoting effect of meat and for the regulation of iron absorption, Scand. J. Gastroenterol. 14 (1979) 769–779.

[12] Hallberg L., Rossander-Hulthén L., Brune M., Gleerup A., Inhibition of haem-iron absorption in man by calcium, Br. J. Nutr. 69 (1992) 533–540.

[13] Hallberg L., Hultén L., Gramatovski E., Iron absorption from the whole diet in men: how effective is the regulation of iron absorption?, Am. J. Clin. Nutr. 66 (1997) 347–356.

[14] Herlinger E., Jameson R.F., Linert W., Iron catalyzes the autoxidation of cysteine by formation of an oxygen-adduct, in: Collery Ph., Corbella J., Domingo J.L., Etienne J.C., Llobet J.M. (Eds.), Metal Ions in Biology and Medicine, vol. 4, John Libbey Eurotext, Paris, 1996, pp. 139–141.

[15] Hertrampf E., Olivares M., Pizarro F., Walter T., Cayasso M., Heresi G., Llaguno S., Chadud P., Stekel A., Hemoglobin fortified cereal: a source of available iron to breast-fed infants, Eur. J. Clin. Nutr. 44 (1990) 793–798.

[16] Hurrell R.F., Preventing iron deficiency through food fortification, Nutr. Rev. 55 (1997) 210–222.

[17] Laurie S.H., Metal-amino acid complexes, in: Berthon G. (Ed.), Handbook of Metal-Ligand Interactions in Biological Fluids, Bioinorganic Medicine, Volume 2, Marcel Dekker Inc., New York, 1995, pp. 603–619.

[18] Layrisse M., Martinez-Torres C., Model for measuring dietary absorption of heme iron: test with a complete meal, Am. J. Clin. Nutr. 25 (1972) 401–411.

[19] Layrisse M., Martinez-Torres C., Leets I., Taylor P., Ramirez J., Effect of histidine, cysteine, glutathione or beef on iron absorption in humans, J. Nutr. 114 (1984) 217–223.

[20] Lebrun F., Bazus A., Dhulster P., Guillochon D., Influence of molecular interactions on ultrafiltration of bovine hemaglobin hydrolysate with an organic membrane, J. Membrane Sci. 146 (1998) 113–124.

[21] Lund E.K., Wharf G., Fairweather-Tait S.J., Johnson I.T., Increases in the concentrations of available iron in response to dietary iron supplementation are associated with changes in crypt cell proliferation in rat large intestine, J. Nutr. 128 (1998) 175–179.

[22] Martinez-Torres C., Layrisse M., Effect of amino acids on iron absorption from a staple vegetable food, Blood 35 (1970) 669–682.

[23] Martinez-Torres C., Layrisse M., Iron absorption from veal muscle, Am. J. Clin. Nutr. 24 (1971) 531–540.

[24] Martinez-Torres C., Romano E.L., Layrisse M., Effect of cysteine on iron absorption in man, Am. J. Clin. Nutr. 34 (1981) 322–327.

[25] Martinez-Torres C., Leets I., Taylor P., Ramirez J., del Valle Camacho M., Layrisse M., Heme, ferritin and vegetable iron absorption in humans from meals denatured of heme iron during the cooking of beef, J. Nutr. 116 (1986) 1720–1725.

[26] Mira M., Alperstein G., Karr M., Ranmuthugala G., Causer J., Niec A., Lilburne A.M., Haem iron intake in 12–36 month old children depleted in iron: case-control study, BMJ 312 (1996) 881–883.

[27] Olivares M., Hertrampf E., Pizarro F., Effects of iron stores on heme iron absorption, Nutr. Res. 13 (1993) 633–638.

[28] Pallarés I., Campos M.S., Lopez-Aliaga I., Barrionuevo M., Gomez-Ayala A.E., Alférez M.J.M., Hartiti S., Lisbona F., Supplementation of a cereal-milk formula with haem iron palliates the adverse effects of iron deficiency on calcium and magnesium metabolism in rats, Ann. Nutr. Metab. 40 (1996) 81–90.

[29] Roberts S.K., Henderson R.W., Young G.P., Modulation of uptake of heme by rat small intestinal mucosa in iron deficiency, Am. J. Physiol. 265 (1993) G712–G718.

[30] Ros F., Periago M.J., Ros G., Rodrigo J., In vitro and vivo bioavailability in rat of four different iron sources used to fortify dry infant cereal, in: Nève J. (Ed.), Therapeutic Uses of Trace Elements, Plenum Press, New York, 1996, pp. 41–45.

[31] Taylor P.G., Martinez-Torres C., Romano E.L., Layrisse M., The effect of cysteine-containing peptides released during meat digestion on iron absorption in humans, Am. J. Clin. Nutr. 43 (1986) 68–71.

[32] Walter T., Hertrampf E., Pizarro F., Olivares M., Llaguno S., Letelier A., Vega V., Stekel A., Effect of bovine-hemoglobin-fortified cookies on iron status of schoolchildren: a nationwide program in Chile, Am. J. Clin. Nutr. 57 (1993) 190–194.

[33] Wien E.M., van Campen D.R., Ferric iron absorption in rats: relationship to iron status, endogenous sulfhydryl and other redox components in the intestinal lumen, J. Nutr. 121 (1991) 825–831.