Review

Mineral nutrient interaction: Improving bioavailability of calcium and iron

Leif Horsfelt Skibsted*

Department of Food Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

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*Corresponding Author
Tel/Fax: +45-3533-3221
E-mail: ls@food.ku.dk

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Abstract Insufficient uptake of essential metals leads to serious malnutrition, which is a worldwide problem. Low bioavailability of iron and calcium may lead to anemia and osteoporosis, respectively, even in individuals with a high dietary intake. For iron, fractionation of meat proteins was studied in order to increase iron bioavailability from other meal components, and uptake of iron was found to increase with minimal risk of increasing oxidative damage. Calcium binding to peptides was found to prevent formation of insoluble calcium salts otherwise hampering absorption particularly in combination with calcium hydroxycarboxylates, entailing spontaneous supersaturation. Based on a review of results from different strategies available for increasing bioavailability, safe iron fortification is suggested to be supported by calcium, with modulation of iron as a prooxidant.

Keywords: mineral bioavailability, nutrient interaction, spontaneous calcium salt supersaturation, iron bioavailability/prooxidative balance

Introduction

Calcium, zinc, and iron are essential nutrients. A very low dietary intake of calcium, zinc, and iron will lead to serious malnutrition causing osteoporosis, high risk of infection, and anemia, respectively. Calcium and iron may be present in food and beverages in sufficient amounts to meet daily requirements; however, low bioavailability of these metal ions affects a large part of the world's population by causing serious calcium and iron malnutrition. Osteoporosis, often caused by age-dependent calcium malabsorption, affects 75 million people worldwide and has predominant incidence in north-western Europe (1). Iron deficiency ranks as the most common nutritional disorder affecting probably one-third of the world's population and may lead to iron deficiency anemia, apathy, and mental retardation (2,3).

Metal deficiency for calcium and iron may be treated through supplementation, whereas for iron, the load on the gastrointestinal tract of both heme iron (from meat, offal, and blood) and simple iron ions (from supplements) possess the risk of oxidative stress and free radical damage, initiating serious pathological conditions (4). Combinations of iron salts and ascorbate may even increase such a risk by causing Fenton-type reactions during food digestion (5). Interaction of iron with other metals (such as calcium and zinc) and other essential nutrients may in contrast lower such a risk through synergistic binding to common metal ion carriers in the digestive tract; however, it may also decrease iron absorption (6). Calcium may

bind to heme iron and more simple iron coordination compounds, thus modifying their redox potential, as was demonstrated for the binding of calcium to iron(III) gluconate, and accordingly modulate metal-catalyzed formation of free radicals during food digestion and for optimal combinations may make iron supplementation safer (7). Such an interaction between ions of calcium and iron during digestion is poorly understood. Moreover, other essential nutrients such as selenium, polyphenol antioxidants, and carotenoids may be involved (6,8,9). A better understanding of the effect of calcium and natural antioxidants on redox active metal ions such as iron may open up for safer fortification strategies, but will clearly depend on a more quantitative description of metal speciation in the complex and changing matrix of various foods during digestion, as well on an understanding of the effects of metal binding to natural antioxidants on their radical scavenging efficiency (10–12). Understanding the dynamic aspects of metal speciation throughout digestion is also required to allow for better utilization of minerals already present in food through optimized processing in order to optimize bioavailability of these essential nutrients (12). Even calcium, without direct redox activity, presents a challenge for optimizing mineral nutrition, although a better understanding of the physicochemical aspects of calcium speciation may lead to improvements (13). Calcium bioavailability from different salts has not been explained based on their equilibrium solubility in the intestines. Understanding the dynamics of (i) precipitation of metastable calcium salts under these alkaline conditions following the dissolution of food components in the acid

environment of the stomach and (ii) ligand assisted dissolution spontaneously forming supersaturated calcium salt solutions also needs to be considered (14,15).

Calcium

Calcium during food digestion: Calcium binding in non-dairy food and beverages is labile, and calcium, under the acidic conditions of the stomach, may dissociate into simple calcium ions causing a risk of precipitation of calcium salts at the increasing pH of the intestines, hampering calcium absorption from such foods (13,14). Calcium bioavailability from foods combining plant oil and dairy products also decreases due to precipitation of calcium from milk product by palmitate from partly digested plant oils in the intestines (14). Calcium from calcium citrate and calcium gluconate as supplements, which will also dissociate in the stomach due to protonization of the carboxylic ligands, has a higher bioavailability than most other calcium salts used for fortification, which may depend on kinetic factors such as rate of dissociation (16). For citrate, even higher bioavailability of calcium was found for certain “solubilized” forms of calcium citrate when this sparingly soluble salt was prepared in a metathesis reaction from calcium hydroxide and citric acid, further indicating that speciation of calcium is crucial for non-saturable calcium absorption (17,18), although no simple explanation for this unusual observation has been presented. Calcium absorption relies on the active transport across intestinal cells, which further depends on the presence of vitamin D, but more significantly on paracellular diffusion in a non-saturable vitamin D independent process (19). For the non-saturable process, a calcium concentration gradient from the partly digested food in the intestines to the extracellular fluids will control the calcium flux. Precipitation by phosphate, phytate, or fatty acid anions from the food may at least partly be prevented by complex binding of calcium by various ligands, in effect determining the free calcium concentration in the intestines thereby further controlling this absorption path by diffusion (20). The importance of supersaturation of calcium salts in the intestines for increasing absorption has not yet been addressed; however, calcium gluconate as a salt of an oxidized sugar was recently shown to form supersaturated calcium gluconate solutions spontaneously by isothermal dissolution of calcium lactate in an already saturated aqueous solution of calcium lactate in the presence of sodium gluconate under conditions relevant for the intestines (15). Supersaturation of calcium salts increases the driving force for calcium diffusion in the non-saturable absorption and may even be required to exceed a critical concentration limit for a spontaneous process. The absorption of calcium in the non-saturable process could also involve specific calcium-peptide or calcium-hydroxycarboxylate complexes as vehicles (21,22); similar effects from complexation are known for iron absorption (23).

Calcium absorption and excretion: Calcium absorption occurs mainly in the intestines as the result of two parallel processes: (i) a transcellular, saturable transport mediated by cells and through cells,

which is regulated by vitamin D and (ii) a paracellular, non-saturable transport through the intercellular space between the cells controlled by diffusion and mucosal permeability (19). Transcellular transport involves entry across the brush border membrane, intracellular movement, and secretion into the extracellular liquid across the basolateral membrane (19). Calcium is accumulated in the skeleton together with phosphate mainly as hydroxyapatite, while soluble calcium is found extracellularly in the aqueous phase mainly bound to proteins (24). Unabsorbed calcium is excreted as insoluble salts through feces. Excess of absorbed calcium will be excreted through urine, although higher intake of calcium from both non-dairy and dairy sources paradoxically seem to be associated with a lower risk of calcium salt precipitation in the urine, evident as kidney stones (25).

Calcium bioavailability: Calcium absorption in an acidic stomach, where most calcium salts are dissociated into simple calcium ions is minimal. Calcium absorption mainly occurs in the intestines where the acid of the partly digested food, chyme, becomes neutralized. The increasing pH of the intestines carries the risk of precipitation of calcium as oxalate, phosphates, phytates, or other salts with low solubility, hampering absorption although some of these processes may be slow (14). Complex binding by natural ligands such as partly hydrolyzed proteins, peptides, and hydroxycarboxylates from fermented sugars will compete with precipitation and may enhance absorption although strong complexation of calcium also will lower the free calcium concentration. During fermentation of milk, lactate is formed, lactate, the oxidation product of glucose, gluconate, and the oxidation product of lactose from milk, lactobionate, all form moderately soluble calcium salts, which also find use as calcium supplements (20). These and other hydroxycarboxylates like citrate bind calcium in complexes (Fig. 1). Binding of calcium to these hydroxycarboxylates is endothermic and binding is entropy-controlled, which particularly for calcium gluconate results in rather robust supersaturated aqueous solutions with almost constant calcium ion activity for increasing concentration even when the concentration is largely exceeding the equilibrium solubility (22). Such supersaturation was found to occur spontaneously during isothermal dissolution of sodium gluconate in a saturated solution of calcium lactate, reaching a factor of 7 in supersaturation of calcium gluconate (15). This unprecedented example of continuing dissolution of a salt against saturation was concluded to result from dissolution overshooting as a kinetic phenomenon, where the hydroxycarboxylates ligand reacts with the solid surface of the precipitate (Fig. 2).

Notably, from the supersaturated calcium hydroxycarboxylate solution, only the least soluble calcium salt, i.e., gluconate, was found to precipitate in a slow process compared with the dissolution rate for lactate, as the more soluble calcium salt.

The presence of such metastable states with selective dissolution and precipitation could be detected in the chyme during further digestion in the intestines, as such states may provide better bioavailability of calcium and explain the surprising effect of hydroxycarboxylates, such as lactate, gluconate, and citrate, on

Fig. 1. Endothermic binding of calcium to L-lactate, D-gluconate, and D-lactobionate: DFT-optimized geometry from PCM solvent model (water), based on (22). K_{calc} is the stability constant for the complexes relative to the lactate complex as obtained from the DFT-calculation, whereas K_I , the thermodynamic association constant, is from electrochemical measurements determined together with ΔH° and ΔS° from temperature dependence of complex formation.

Fig. 2. Spontaneous isothermal supersaturation during transformation of a saturated aqueous solution of calcium L-lactate and solid sodium D-gluconate to a saturated solution of calcium D-gluconate in aqueous sodium L-lactate, based on (15).

increasing calcium absorption compared to calcium chloride despite the fact that all these calcium salts dissociate completely or almost completely in the acid of the stomach (13). Recombination in the intestines to form calcium hydroxycarboxylate complexes seems faster than precipitation, explaining the high bioavailability from calcium hydroxycarboxylates. Biomineralization processes may also benefit from this unusual phenomenon involving spontaneous supersaturation, resulting in high mineral mobility in bone tissue. Clearly, here is a need for new research initiatives, which also may help to explain the important but largely unexplained good bioavailability of calcium from mixtures of calcium hydroxide and citric acid despite the low solubility of calcium citrate (17). Moreover, the solubility of calcium citrate tetrahydrate decreases with increasing temperature, which further hampers dissolution under physiological conditions (26), so-called inverse solubility. However, similar to gluconate, citrate may react with other precipitated calcium salts,

overshooting the equilibrium solubility and making paracellular absorption compete efficiently with a slow secondary precipitation of calcium salts in the intestines.

Calcium precipitation in the intestines has been described for anions of saturated fatty acids such as palmitate (14,27). The so-called calcium soap formed, calcium palmitate or calcium hydroxyl palmitate as its metastable precursor, will hamper calcium absorption and calcium utilization from infant formula which has been enriched with highly nutritive plant oils (Fig. 3). The precipitation of metastable calcium hydroxyl palmitate rather than the thermodynamically stable calcium palmitate presents an example of Ostwald's rule of stages (28). The metastate, i.e., initially formed calcium precipitate, may remain available for ligand assistant dissolution during further digestion. A positive effect of the precipitation of saturated fatty acid anions seems, however, to have been recognized in weight control by dairy products for adults. Peptides from protein hydrolysis in dietary

Fig. 3. Calcium is precipitated in the intestines by anions of saturated fatty acids such as palmitate from partly digested triglycerides (triacylglycerols, TAG). In plant oil, R and R'' are most often saturated fatty acids, whereas R' is a polyunsaturated fatty acid. This is in contrast to milk fat with a more random fatty acid distribution. Pancreas lipase will hydrolyse R and R'' (1,3 selective). Plant oil may accordingly lower calcium bioavailability, since calcium hydroxyl palmitate will precipitate as a metastable precursor for insoluble calcium palmitate (14).

Fig. 4. The passive paracellular absorption of calcium in the intestines depends on a calcium ion concentration higher than mM to allow free diffusion across the epithel. The free calcium is controlled by competitive precipitation and complex formation. Modified from (20).

supplements and infant formula may counteract such precipitation of calcium by the partly digested lipids and also by phosphate and phytate in the intestines. Peptides found in fermented milk and in fish protein hydrolysates bind calcium depending on the structure. Peptides with phosphorylated serin are among the stronger calcium binders, but surprisingly, the non-phosphorylated peptide SerSerSer GluGlu has an even higher affinity for calcium (29). A whey protein hydrolyzate chelated with calcium and transformed into a nano-composite material was shown to deliver increased calcium bioavailability compared to both calcium gluconate and calcium chloride in Caco-2 cell studies (30). Notably, this nano-material was found resistant to both acidic and basic conditions and may through the whole digestive tract serve as a vehicle for calcium transport, increasing the bioavailability of calcium through prevention of formation of calcium salts of low solubility in the intestines.

The balance between precipitation of calcium under the neutral to

slightly alkaline conditions in the intestines and complex binding of calcium controls the concentration of calcium ions in the partly digested food in the intestines. For most ligands, L⁻, a simple equilibrium seems to account for the binding of calcium (31):

This binding of calcium is coupled with the precipitation equilibrium of calcium salts, resulting in a low free calcium concentration:

The concentration of free calcium should be in the mM range in order to allow for the spontaneous, non-saturable calcium absorption (Fig. 4). Thermodynamic data for the calculation of the critical equilibrium concentrations of calcium are becoming available as complex constants corresponding to the equilibrium of Eq. (1) and as solubility products corresponding to the equilibrium of Eq. (2) in Table 1. The equilibrium concentration of free calcium will be lower than the critical concentration for absorption, and absorption may accordingly depend on kinetic factors allowing supersaturation or on specific absorption of calcium complexes of peptides or hydroxycarboxylates (Fig. 2). In order to obtain a better understanding of the solubilization of calcium salts, theoretical calculation should be used to predict calcium binding affinity to specific amino acid sequences in proteins and peptides, thus identifying sequences in peptides enhancing calcium binding and calcium absorption. Such Density Functional Theory (DFT) calculations, already available for some hydroxycarboxylates (22), seem promising as they gave results qualitatively in agreement with experimental values for the binding constants (Fig. 1). Aspartate was shown to bind calcium stronger than glutamate. For small peptides such as GluGlu and AspGlu, calcium binds stronger when compared to binding by the combinations of two amino acids, showing synergism (34). The potential of peptide calcium chelates for dietary supplements has been demonstrated for the aspartate containing peptides TyrAspThr and PheAsp. Also, the

glutamate containing peptides GluGly and GlyTyr as obtained from whey hydrolysates bind calcium efficiently (35-38). Marine algae and soybean have been used as a non-dairy source for protein hydrolyzates with peptides binding calcium efficiently for calcium transport and delivery under both acidic and alkaline conditions, as characterized by calcium binding efficiency and calcium releasing percentage, respectively (39,40).

Calcium from milk and milk products is acclaimed for a high bioavailability. Caseins, the phosphoproteins of milk, bind calcium and phosphate. During digestion, casein phosphopeptides, CPP, are formed by enzymatic hydrolysis, and CPP binds calcium, preventing the precipitation of calcium salts in the intestines (41). An amino acid sequence typical for CPP, Ser[P]-Ser[P]-Ser[P]-Glu-Glu, where Ser[P] is a phosphorylated serine, is highly negatively charged and binds calcium strongly (42). Peptides such as CPP increase calcium absorption by preventing precipitation of calcium salts in the intestines and may also more actively participate in the paracellular absorption. In contrast, calcium in the homogeneous serum phase of milk is found as simple calcium ions, but also loosely coordinated to peptides and other smaller molecules and to the whey proteins and may precipitate in the intestines (34).

A number of calcium salts including calcium lactate and calcium gluconate and their mixtures are used as calcium supplements or for fortification of juice, soy milk, or even milk with calcium (43). In milk, calcium and phosphate are distributed between the micelles formed by the caseins and the homogeneous serum phase. In the micelles, calcium is present as colloidal calcium phosphates, CCP. CCP nanoparticles interact with the phosphoserine residues of the caseins, which are relatively small, hydrophobic proteins with low levels of secondary and tertiary structure. CCP are integrated in the casein structure and stabilize the sub-micelles (44), but may dissolve in the serum phase on cooling, since CCP shows "reverse" solubility similar to calcium citrate, or by pressure treatment (45). The distribution of calcium between the micelles and the serum phase further depends on pH, and a decreasing pH, resulting from fermentation of milk, releases calcium and phosphate from the micelles (46). The caseins are carriers of calcium and phosphate in neonates, but the increasing acidity in the stomach during digestion releases calcium from the micelles with the risk of precipitation in the intestines (41,42). For milk, fortification with calcium chloride, calcium lactate, and calcium gluconate has been compared with normal milk with pH 6.6, and the highest amount of calcium remained in the serum phase for calcium chloride and the highest transfer to caseins was observed for calcium lactate (16). The bioavailability of calcium from calcium lactate and calcium gluconate was found to be higher than that for calcium chloride for milk fortified with these salts, further confirming the role of caseins as mineral carriers during digestion also for added calcium salts (16).

For calcium lactobionate, the distribution between the serum and the micelles of calcium was found to be strongly dependent on pH as calcium remained in the serum phase for acidified milk of pH 5.4, but

Table 1. Solubility products and complex constants for 1:1 complexes of calcium at 25°C together controlling free calcium ion concentration in chyme in intestines

Solubility products	K_{sp} (mol ³ /l ³)	Reference
Calcium palmitate, Ca(C16) ₂	$8 \cdot 10^{-18}$	27
Dicalcium phosphate, CaHPO ₄ , 2H ₂ O	$2 \cdot 10^{-7}$	32
Calcium carbonate, CaCO ₃ (amorphous)	$4 \cdot 10^{-7}$	33
Calcium gluconate: Ca(Gl) ₂	$5 \cdot 10^{-5}$	22
Calcium lactate: Ca(Lact) ₂	$9 \cdot 10^{-4}$	22
Calcium lactobionate: Ca(Lacto) ₂	$2 \cdot 10^{-3}$	22
Complex constants (1:1)	K_{ass} (l/mol)	
Calcium, citrate	$8 \cdot 10^4$	31
Calcium, lactate	49	22
Calcium, gluconate	88	22
Calcium, lactobionate	140	22
Calcium, Asp	7	22
Calcium, Glu	3	34
Calcium, Asp-Glu	27	34
Calcium, Asp-Asp	23	34
Calcium, Glu-Glu	26	34
Calcium, Glu-Asp	23	34
Calcium, o-p-Ser	22	34
Calcium, phosphopeptide	38	29
Calcium, SerSerSerGluGluGlu	131	29
Calcium, hydrogenphosphate	$5 \cdot 10^2$	31

was partly transferred to the micelles for normal milk (47). This implies that pH control is important for fortified milk and also that the changes in calcium distribution during acidification in the stomach are not followed by full redistribution in the intestines, since lactate and gluconate showed higher bioavailability in a rat model (16). The kinetics of the redistribution was not investigated but may be important for bioavailability of these calcium hydroxycarboxylates from fortified milk.

A number of calcium salts such as calcium citrate and some calcium phosphates show reverse solubility, as the solubility in water decrease with increasing temperature (26). Cooling of milk accordingly increases free calcium concentration in the serum phase of milk (20,47). For calcium citrate, a supersaturated aqueous solution may be obtained by saturation at low temperature and subsequent heating, whereas for calcium gluconate, the opposite procedure with initial heating and subsequent cooling is required (22,26). Both solutions will, however, remain supersaturated without precipitation if undisturbed for considerably long periods of time. For calcium gluconate precipitation is, moreover, known to be inhibited by an initial dissolution of a small amount of calcium saccharate, an even less soluble calcium salt of an oxidized sugar (48). The mechanism behind this protection against precipitation of calcium gluconate from an even strongly supersaturated solution remains unexplained, but seems to depend on an initial heating. However, similar phenomena should be looked for, in the intestines and also for calcium salts in chyme as they may be important for calcium bioavailability. The observation that the mixtures of calcium

Fig. 5. Catalytic activity of myoglobin is coupled to the color cycle of meat with metmyoglobin, MbFe(III), as a common intermediate in the two cycles.

hydroxide and citric acid provide better bioavailability than calcium citrate seems also to depend on significant faster dissolution rates compared to precipitation rates (17). The slow precipitation rate of some (but not all) calcium salts may also explain the surprising observation that bassanite, calcium sulfate demihydrate, precipitates from aqueous solutions not saturated in this compound but saturated in gypsum, calcium sulfate dihydrate (49).

Iron

Iron during food digestion: In contrast to calcium, iron is redox active. Iron may induce free radical processes in food by one-electron cycling between iron(II) and iron(III) for simple iron ions as in the Fenton reaction (4),

or between iron(V) and iron(IV) or between iron(IV) and iron(III) in hypervalent heme compounds such as myoglobin. For myoglobin, the oxygen storage molecule of muscles, this pseudo-peroxidase activity in meat is coupled to the color cycle as outlined in Fig. 5. Electron transfer is also important for the bioavailability of iron from food. For the non-heme iron, mainly iron(II) is absorbed, and reduction of iron(III) by ascorbate or other reducing components of food and beverages becomes important. Heme from meat is an important dietary iron source in numerous societies. Current dietary recommendations are, however, in favor of reducing the intake of red meat and processed meat due to the increased risk of gastrointestinal cancers for individuals with high red meat intake (2). Iron in food of vegetable origin is mainly non-heme iron with very low bioavailability, which moreover is further reduced by phytate from cereals and polyphenols present in coffee, tea, and vegetables, all precipitating iron under neutral to alkaline conditions. A future decrease in meat availability resulting in a reduced intake of meat for most populations is to be expected for environmental reasons with the increasing world population. This development will make iron deficiency an even more common condition.

Both iron deficiency and iron overload is, however, of health

Fig. 6. Proteins promote non-heme iron absorption. Other promoters are ascorbic acid through reduction of iron(III) to iron(II) and citrate through complex binding and salt-soluble meat proteins through an unknown mechanism. Inhibitors for non-heme absorption are phytate, polyphenols, and calcium, and hepcidin from the liver reduces iron uptake.

concern (50). Excessive iron absorption is genetically determined, and iron overload resulting in hemochromatosis induces free radical processes and formation of reactive oxygen species that primarily damage the liver. The balance between iron absorption and iron excretion seems to have had great impact on evolutionary adaption for the human race (51).

Iron bioavailability: Heme iron from meat, offal, and blood is absorbed efficiently although the heme iron content of meat seems to be negatively affected by aging of meat (52). Absorption of non-heme iron depends on a protein factor and meat enhances absorption of non-heme iron from a meal comprising both meat and vegetable, as shown by a double isotope labeling technique in human intervention studies (Fig. 6) (23,53,54). Absorbed iron is stored in ferritin and is available for heme synthesis (3). Uptake of iron in mammals is regulated by the protein hepcidin expressed mainly in the liver (55), and when iron stores are low, hepcidin production is down-regulated to increase uptake (6). With the perspective of a reduced availability of meat for human consumption, the factor from meat active in facilitating non-heme absorption from other meal components

Fig. 7. Meat enhances the absorption of non-heme iron as shown for meat labeled with ^{59}Fe and ^{55}Fe using a double isotope technique. The different meat protein fractions (water soluble, salt soluble, or insoluble) behave differently as seen from enhancement of non-heme absorption relative to a meal without meat protein. Data from (23,53,54). The prooxidative effect is in contrast large for the water soluble protein fraction from meat (56).

needs to be identified. This “meat factor” seems to be associated with the salt-soluble protein fraction of meat rather than the water-soluble or insoluble proteins (Fig. 7) (23). The “meat factor” is not characterized by high cysteine content and numerous free thiol groups, since heating meat and reducing the number of free thiol groups were not found to reduce the uptake of non-heme iron from phytate-rich meals (53). Proteins with similar properties promoting non-heme iron uptake could be made available otherwise from plants or cell cultures to be used as food additives.

The prooxidative effect of iron presents a health problem when iron in food becomes more available for absorption. While the salt-soluble protein fraction from meat is the most effective in enhancing absorption of non-heme iron, the water soluble fraction is the most prooxidative (56). The prooxidative effect of the water-soluble protein fraction of meat is related to iron proteins soluble in water such as myoglobin and iron enzymes, which may gain increased pseudo-peroxidase activity upon digestion in the stomach (Fig. 8):

Activation of myoglobin partly proteolyzed during digestion probably results from reaction with lipid hydroperoxides, resulting in compound I type hypervalent iron heme pigments (8,9,57). Such perferryl compounds are strongly oxidizing and may even oxidize the otherwise inert carbon monoxide to carbon dioxide (58) as in Table 2. Antioxidants such as the water-soluble carotenoid crocin and quercetin are efficient reductants for these hypervalent iron species. Accordingly, spices such as saffron and vegetables such as onion may counteract the damaging effect of the activated meat pigments (8,9). It seems to be an important observation that the protein fraction from meat, which promotes iron absorption from non-heme iron in

Fig. 8. Proteolyzed heme iron species are prooxidative and enhance lipid oxidation. Myoglobin proteolyzed by pepsin: (A) SE-HPLC profiles of MbFe(III) in a neutral solution, (B) MbFe(III) at pH=3.9 for 15 min at 37°C, (C) MbFe(III) subject to pepsin proteolysis at pH=3.9 for 15 min at 37°C. Oxygen consumption as determined electrochemically for a methyl linoleate emulsion with pH=5.8 at 25°C in the presence of native MbFe(III), MbFe(III) treated with HCl, or MbFe(III) proteolyzed with pepsin corresponding to (A), (B), and (C). Modified from (57).

Table 2. Rate constants for perferrylmyoglobin; $^{\circ}\text{MbFe(IV)}=0$, and ferriylmyoglobin, MbFe(IV), myoglobin activated during meat digestion by lipid hydroperoxides, oxidizing food related substrates in neutral aqueous solution at 25°C

	$^{\circ}\text{MbFe(IV)}=0$ $\text{l}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$	MbFe(IV)=0 $\text{l}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$	Reference
Carbon monoxide	330		58
Crocin	$4\cdot 10^5$ ²⁾	183	63
Ascorbate		$16^{(1)}$	64
Epicatechin		93	64
Quercetin		$279^{(1)}$	64
Cysteine anion		0.31	65

¹⁾At 10°C

²⁾Upper limit

meals combining meat and vegetables, is not the same protein fraction from meat associated with prooxidative activity (56). It should be possible to use meat protein fractionation to promote iron absorption without inducing severe oxidative damage.

Calcium and iron interaction: Calcium modulates the prooxidative effect of iron. This was shown for the iron(III) complex of gluconate, which binds calcium, resulting in a lower standard reduction potential (7). Calcium, accordingly, makes iron less damaging to epithel in the intestines during food digestion; whether a similar effect is observed for calcium and heme pigments remains to be investigated. It is, however, known that zinc as a Lewis acid binds to heterocyclic nitrogen acting as an antioxidant, an effect which could also be important in protecting against heme-iron radical formation (59). Similar to calcium, during digestion, zinc could protect against iron induced oxidative damage. The tendency of free radical formation in meat increases on moderately heating meat, but again decreases on heating to higher cooking temperatures, probably reflecting a balance between heme iron and free iron (60), which could also be affected by the presence of other metal ions.

Calcium has been shown in several studies to decrease iron absorption from food, whereas zinc seems to increase iron absorption (6,61,62). The mechanism behind the interaction between calcium and iron is not clear, since a high calcium intake does not correlate with iron deficiencies over a longer time period (61). For iron, a divalent metal transporter, DMT1, transports simple iron ions across the luminal membrane (62). Calcium may inhibit this transport (6), while zinc does not. Better calcium and iron bioavailability from food is an important aspect of human nutrition. Current theories do not provide full explanations for the apparent paradoxes related to essential metal nourishment. Increased knowledge of fundamental aspects of calcium and iron chemistry will clearly help to develop better food fortification strategies and to make traditional food more nutritious without the risk of inducing iron dependent free radical processes.

In summary, insufficient uptake of essential metals such as calcium and iron leads to serious malnutrition. Osteoporosis caused by calcium malabsorption, even for individuals with a good dietary intake of calcium, affects 75 million people worldwide. Paracellular calcium absorption, quantitatively more important than transcellular, vitamin-D dependent absorption, is non-saturable, but depends on free calcium ion concentration in the intestines and competes with calcium precipitation. The slow precipitation rate under alkaline conditions to form metastable calcium salt phases in agreement with Ostwald's rule of stages or the spontaneous isothermal formation of supersaturated solutions by ligand assisted dissolution seem to correlate better with bioavailability than equilibrium solubility. Clearly, a better understanding of the inorganic chemistry of calcium salts in the digestive tract may also provide explanations to some of the paradoxical behaviors known about calcium nutrition such as the high frequency of osteoporosis for some population segments with a high calcium intake. Iron deficiency ranks among the most common nutritional disorders affecting one third of the world's population. Increased intake of iron with high bioavailability from red meat carries, however, the risk of inducing free radical processes and oxidative damage in the digestive tract, entailing serious health problems. Fractionation of meat proteins has shown that high bioavailability and damaging prooxidativity of iron are associated with different protein fractions, and safe iron fortification could be based on protein fractionation. Identification of specific proteins as vehicles for iron may also hold the perspective of avoiding iron overload. Interaction of calcium with bioavailable iron seems to lower prooxidative activity of iron. Dietary combinations of iron and calcium should accordingly be given more attention, since fortification with calcium seems without negative effects on long-term iron status.

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