HEME IRON IN MEAT AS THE MAIN SOURCE OF IRON IN THE HUMAN DIET

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Abstract

Iron is a trace element involved in many cardinal metabolic processes of almost all living organisms. It is well known that iron participates in oxygen transport as well as it is a cofactor in many fundamental enzymatic and nonenzymatic processes. Accordingly, disturbances of iron homeostasis can cause serious clinical consequences. In humans, dietary iron can enter the body in two main forms: heme and nonheme. The former is a component of many hemoproteins (including myoglobin, hemoglobin, cytochromes b and c) and is easily absorbed in the duodenal enterocytes. Red meat is an excellent source of heme iron, while the less bioavailable nonheme form is found in large amounts in milk products and vegetables. For this reason, consumers of meat have a better iron status than vegetarians and vegans. Heme iron found in muscle protein should be supplied to humans to prevent iron deficiency, which can lead to anemia. It is easily absorbed and its main source is red meat. In addition, heme iron, which is mainly found in myoglobin in meat, contributes to the desirable bright red color and to the most undesirable brown color of meat. Both heme and nonheme iron are catalysts of lipid oxidation in meat. This process lowers the nutritive value through oxidation of polyunsaturated fatty acids, which produces an undesirable flavor and aroma. The aim of this paper was to discuss the role of heme iron in the human diet.

Keywords: iron, meat, lipid oxidation, metabolism, meat color.
INTRODUCTION

Iron is crucial for many of the body’s metabolic processes, such as oxygen transport and storage, and electron transfer. Iron is also necessary for normal development of the central nervous system, cell proliferation, synthesis and the repair of genetic material. As a component of catalase and peroxidase, iron represents a protective mechanism against reactive oxygen species (Martínez-Navarrete et al. 2002, Papanikolaoua, Pantopoulos 2005, McAfee et al. 2010, Słomka et al. 2012). For this reason, prenatal and postnatal iron deficiency may affect adversely the cognitive processes and thus lead to neuropsychological disorders (Conrad, Umbreit 2000, Tapiero et al. 2001, Mancini, Hunt 2005). Iron deficiency is one of the most common nutritional deficiencies, affecting around 20% of the world’s population. Deficiency of this element is more prevalent in less industrialized countries (20-50% of the population) than in more developed countries (2-28%) (Martínez-Navarrete et al. 2002). The most significant consequence of iron deficiency is sideropenic (iron deficiency) anemia. It is mostly caused by insufficient dietary intake of iron, often when the demand is high. The disease is most commonly found in infants and in women of childbearing age, often as a result of heavy menstrual bleeding. It is also stressed that iron deficiency can induce many abnormalities in fetal and neonatal development (Słomka et al. 2012). In adolescence the demand for iron increases, due to the growth and development of muscles and increasing blood volume. This results in an intensification of erythropoiesis, for which iron is necessary to produce myoglobin and hemoglobin (Mesías et al. 2013). Although iron plays a significant role in the human body, an excessive intake of this element may contribute to intestinal mucosal damage and be a risk factor in cardiovascular diseases, neurodegenerative diseases, infections and colorectal cancer (Pereira, Vicente 2013). The results of the meta-analysis suggest that the intake of heme is associated with an increased risk of coronary heart disease. In individuals who consume more heme iron a 31% increase in the risk of the disease was found compared with those with a lower consumption of this type of element (Yang et al. 2014).

The present review is focused on role of heme iron, which is mainly found in meat and is the principal source of iron in the human diet.

Iron as a component of meat myoglobin

Myoglobin (17 kDa) is the main proteins of muscle tissue sarcoplasm, containing a centrally located heme (Figure 1), which is a complex of protoporphyrin IX with Fe(II). Globin is a single chain of 153 amino acid residues with 80% of the polypeptide chain existing in α-helical conformations, which makes protein structure highly compact. Heme in the hydrophobic pocket of this molecule is protected from oxidation to Fe(III) (Brewer 2004, Mancini, Hunt 2005, Papanikolaoua, Pantopoulos 2005). The fifth coordination position of the iron atom is occupied by the imidazole nitrogen atom of histidine resi-
due (His 93). The sixth coordination position of the iron atom on the opposite side of the heme is the main binding site for oxygen and also for nitric oxide and carbon oxide. In addition, differences in the occupancy of the sixth coordination position enable myoglobin to exist in three physiological forms. In deoxymyoglobin, this position remains empty; in oxymyoglobin, it is occupied by oxygen, and in ferrimyoglobin, the site is occupied by water (BREWER 2004, MANCINI, HUNT 2005).

The myoglobin content of skeletal muscles can be influenced by an animal’s breed, age and muscle activity (KOLCZAK 2008). Red meat owes its dark red color to the presence of large amounts of heme, the content of which in this meat is 10-fold higher than in white meat (BASTIDE et al. 2011). Beef has the highest amount of myoglobin per gram of fresh meat (15 mg) compared to mutton (10 mg), pork (5 mg), poultry and rabbit meat (< 5 mg) (VALENZUELA et al. 2011). Heme iron content in beef may depend on the type of muscle, breed and storage time (RAMOS et al. 2011). Physical activity of animals may increase the amount of heme iron, especially in muscles having high oxidative activity. A greater degree of physical fitness increases the muscle oxidative capacity by increasing the number of mitochondria in the white fibers, hence turning them into red fibers (CASTELLINI et al. 2002). The muscles of heifers, young bulls and steers contain less myoglobin than the muscles of cull cows, while calf muscles have less pigment than steer muscles (KOLCZAK 2008). In addition, the content of total iron, heme iron and myoglobin in the muscles of animals on low level feeding is higher than in those on high feeding level (PURCHAS, BUSBOOM 2005, CALKINS, HODGEN 2007). Furthermore, the above parameters of iron homeostasis are lower in older animals than in younger ones (CALKINS, HODGEN 2007).

**Effect of iron on meat color**

Meat color is primarily determined by its content of myoglobin, but also hemoglobin and cytochrome c. The binding of an oxygen molecule to myoglo-
bin makes it oxygenated (oxymyoglobin), thus giving the desired bright red color of meat. During the oxygenation, iron’s valence does not change and the sixth coordination position is occupied by an oxygen molecule. In addition, the distal histidine (His 64) in myoglobin interacts with bound oxygen, altering the oxygen’s structure and stability. As exposure to oxygen increases, the oxymyoglobin penetrates deeper beneath the meat’s surface. The depth of oxygen penetration depends on the meat’s temperature, oxygen partial pressure, pH and competition for oxygen by other processes that require this cofactor (Mancini, Hunt 2005, Kołczak 2008).

Under anaerobic conditions, most often during the vacuum packaging of meat, oxymyoglobin is converted to deoxymyoglobin, because the sixth coordination position for oxygen is unoccupied and iron is present in the form of Fe(II). In this case, meat becomes purple red or purple pink, because very low oxygen partial pressure (<1.4 mm Hg) maintains myoglobin as deoxymyoglobin (myoglobin without oxygen).

Metmyoglobin is a molecule between the oxymyoglobin present on the surface of meat and the deoxymyoglobin present in meat, as a result of a change in valency of from Fe(II) to Fe(III). Metmyoglobin is formed inside meat and extends towards the surface, producing the undesirable brown color of the meat. Metmyoglobin formation depends on many factors, including oxygen partial pressure, temperature, pH, the reducing activity of meat components and in some cases the growth of microorganisms. Metmyoglobin reduction is essential to meat color and largely depends on the enzymatic activity and pool of NADHs (reduced nicotinamide adenine dinucleotide), which are constantly depleted with the increasing time after slaughter. Lactate dehydrogenase, which catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD+, injected postmortem into meat was found to oxidize lactate to pyruvate and to reduce the amount of metmyoglobin, thus improving the color of meat (Mancini, Hunt 2005, Kołczak 2008).

**Iron as a catalyst of lipid oxidation in meat**

During postmortem changes, the natural antioxidant system that prevents living cells from lipid oxidation becomes increasingly weaker, which accelerates the lipid oxidation processes. The cessation of blood circulation in the body shifts metabolism from oxidative to glycolytic during the conversion of muscle to meat. This results in lactic acid accumulation, which reduces pH from 7.4 to approx. 6.0-5.5. This is when the structural integrity of muscle cells is compromised as a result of proteolysis and protein denaturation, releasing iron ions from hemoproteins and low-molecular-weight compounds, which may become catalysts. Iron initiates lipid oxidation by generating, via the Fenton reaction, reactive oxygen species capable of abstracting a proton from unsaturated fatty acids. Free iron ions may bind to negatively charged phospholipids (such as phosphatidylcholine) in cell membranes and catalyze
the breakdown of pre-formed lipid hydroperoxides. The lipid hydroperoxides are further decomposed oxidatively to form peroxide radicals or, by reduction, to produce alkoxyl radicals. These radicals may initiate new chain reactions, and alkoxyl radicals may further decompose to produce aldehydes and other secondary products of lipid oxidation (Carlson et al. 2005, Min, Ahn 2005, Orino, Watanabe 2008). One such short-chain aldehyde is malondialdehyde (Tapiero et al. 2001, Hesz, Korczak 2007, Daneshyar 2012). This compound is often used to evaluate the degree of lipid peroxidation and may produce a rancid flavor and aroma, undesirable for the consumer (Tapiero et al. 2001, Faustman et al. 2010, Daneshyar 2012). Malondialdehyde may induce DNA damage, leading to mutations, and can react with DNA to form adducts with deoxyguanosine, deoxyadenosine and deoxycytidine. The major DNA adduct formed by the reaction of malondialdehyde with DNA is 1,N2-malondialdehyde-deoxyguanosine (M1dG) (Bastide et al. 2011). In addition, heme proteins that initiate lipid oxidation in biological membranes may impair membrane function, decrease fluidity, inactivate membrane-bound receptors and enzymes, and increase permeability to ions such as Ca\(^{2+}\) (Baron, Andersen 2002).

Free iron ions released from heme and ferritin may be considered as the main catalysts of lipid peroxidation in both raw and cooked meat. Ferritin is a ubiquitous intracellular protein (consisting of 24 protein subunits) that stores iron and releases it in a controlled fashion. Ferritin releases iron in the presence of reducing agents such as superoxide anion and ascorbate (Min, Ahn 2005). Hydrogen peroxide, present in the muscle cell cytosol, releases free iron from heme as a result of oxidative cleavage of the porphyrin ring, whereas ascorbate releases iron ions from ferritin, which catalyzes lipid peroxidation in meat. Ascorbic acid can exhibit both antioxidant and prooxidant properties, depending on its concentration and the amount of iron present. In low concentrations, ascorbic acid most often contributes to lipid peroxidation in muscle tissue by reducing iron, and in high concentrations it transforms some of peroxide radicals directly into lipid hydroperoxides, as a result of breaking the free radical reaction by donating a hydrogen atom to the free radical. Ascorbic acid also regenerates \( \alpha \)-tocopherol in biological membranes (Min, Ahn 2005).

In addition, iron in muscle can be chelated by low-molecular-weight compounds, such as organic phosphate esters, inorganic phosphates, amino acids and organic acids. Low-molecular-weight, water-soluble iron-chelating agents may be responsible for catalyzing the oxidation of tissue lipids by iron. What is more, the conversion of ferritin to hemosiderin in the body is biologically beneficial because it decreases the availability of iron for promotion of lipid peroxidation. Therefore, the body’s iron metabolism should be strictly regulated by iron-binding proteins to ensure that no free iron exists (Min, Ahn 2005). Ferritin is a ubiquitous and highly conserved iron storage protein, which plays a major role in iron metabolism by storing iron ions and protec-
ting tissues from its toxic effect. Furthermore, owing to the enzymatic activity, ferritin molecules are able to oxidize Fe(II) to Fe(III) (Orino, Watanabe 2008, Słomka et al. 2012). Non-transferrin-bound iron (NTBI) can be highly toxic because it facilitates reactive oxygen species generation, which contributes to the damage of all biomolecules. Non-transferrin-bound iron may be involved in the pathogenesis and progression of many hematological disorders, as well as in the etiopathogenesis of the central nervous system in neonates, neurodegenerative diseases and diabetes (Słomka et al. 2011, Koba et al. 2013).

The susceptibility of raw meat to lipid oxidation depends on the animal species, muscle type, activity of antioxidant enzymes, fat content, fatty acid profile and other factors (Min et al. 2008). Muscles with a higher number of red fibers (type I or type IIA) are more sensitive to lipid oxidation because they contain more iron and phospholipids compared to muscles that mostly contain white fibers (Faustman et al. 2010, Samuel et al. 2012). Muscles with more red fibers generate more hydrogen peroxide during auto-oxidation of this protein compared to meat with a lower content of this pigment. Hydrogen peroxide may react with metmyoglobin to form ferrylmyoglobin, which initiates lipid oxidation by abstracting a hydrogen atom from polyenoic fatty acids and generates lipid hydroperoxides. Additionally, heating reduces the activation energy for lipid peroxidation and breaks down initially formed hydroperoxides to free radicals, which stimulate auto-oxidation processes and the development of undesirable flavor and aroma (Min et al. 2008). Nonheme iron plays a greater role in this process, especially in an acidic environment and in cooked meat. In turn, heme iron may initiate lipid oxidation in both raw and cooked meat (Hęś, Korczak 2007). Additionally, the meat cooking process inhibits the activity of antioxidant enzymes and releases iron from heme, leading to its oxygenation.

**Source of iron in the human diet**

Iron in the human diet has two forms: heme iron (II), which is easily absorbed, and nonheme iron (III), which is much less readily absorbed by the body (Carpenter, Mahoney 1992, Beard, Han 2009, Valenzuela et al. 2009, Schonfeldt, Hall 2011). Red meat, in particular beef, mutton and goat meat, is regarded as a richer source of heme iron (Table 1) than poultry meat and fish (Tapiro et al. 2001, Umbreit 2005, Valenzuela et al. 2009, McAfee et al. 2010, Schonfeldt, Hall 2011, Zotte, Szendro 2011). Heme iron, although consumed in smaller amounts, is two- or threefold more easily absorbed (50-87%) than nonheme iron. Nonheme iron, which is found mainly in vegetables and milk products, forms the majority of all dietary iron (approx. 60%), but its absorption is low (2-20%) (Carpenter, Mahoney 1992, Benito, Miller 1998, Valenzuela et al. 2009, 2011). The absorption rate of nonheme iron depends on the presence of dietary components that increase or inhibit its bioavailability (Tapiro et al. 2001, Umbreit 2005, Schonfeldt, Hall 2011, Pereira et al.
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Table 1

<table>
<thead>
<tr>
<th>Type of meat</th>
<th>Iron content (mg kg(^{-1}))</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veal</td>
<td>5.5-23</td>
<td>(Schonfeldt, Hall 2011, Zotte, Szendro 2011)</td>
</tr>
<tr>
<td>Mutton</td>
<td>33</td>
<td>(Zotte, Szendro 2011)</td>
</tr>
<tr>
<td>Lamb</td>
<td>9.6-26</td>
<td>(Schonfeldt, Hall 2011, Hoffmann et al. 2010)</td>
</tr>
<tr>
<td>Goat</td>
<td>21-44</td>
<td>(Webb et al. 2005)</td>
</tr>
<tr>
<td>Game</td>
<td>33-47</td>
<td>(Sales, Kotera 2013, Triumf et al. 2012)</td>
</tr>
<tr>
<td>Pork</td>
<td>5.5-17</td>
<td>(Min et al. 2008, Zotte, Szendro 2011, Hoffmann et al. 2010)</td>
</tr>
<tr>
<td>Poultry</td>
<td>5.9-20</td>
<td>(Min et al. 2008, Zotte, Szendro 2011, Buzala et al. 2014)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1.1-3</td>
<td>(Zotte, Szendro 2011, Cygan-Szczegielniak et al. 2012)</td>
</tr>
<tr>
<td>Fish</td>
<td>1.2-5</td>
<td>(Stanek et al. 2012, Grela et al. 2010)</td>
</tr>
</tbody>
</table>

2013). Its absorption is facilitated by factors such as ascorbic acid, citric acid and some amino acids. Citric acid enhances the absorption of nonheme iron through chelation, preserving it in the solution of ascorbic acid and some amino acids such as cysteine, which reduce iron to a more readily available form of Fe(II), thus facilitating its intestinal absorption (CarPenter, Mahoney 1992, Benito, Miller 1998, Conrad, UMBREIT 2000, UMBREIT 2005, Beard, Han 2009, Pereira, Vicente 2013). Other organic acids, such as malic and tartaric acid, can also improve the absorption of iron (Lim et al. 2013). Infants and approx. one-third of the elderly have low secretion of hydrochloric acid by gastric parietal cells, which may lead to iron malabsorption (Benito, Miller 1998). The absorption of nonheme iron is inhibited by factors such as phosphates, phytates, dietary fiber, lignins, polyphenols and tannins. These inhibitors generally bind most iron ions into complexes and make them unavailable to transport proteins. In turn, heme iron, which is easily soluble in the alkaline environment of the duodenum, is immediately absorbed by enterocytes (Conrad, Umbreit 2000, 2005, Beard, Han 2009). Minerals such as zinc, calcium, copper and manganese can also inhibit the bioavailability of iron by competing for the same carrier in enterocytes, modifying the oxidation state of iron or interfering with metabolism (Mesías et al. 2013). Studies have shown that calcium chloride also inhibits the absorption of heme iron (López, Martos 2004). As a result, iron in a diet which is high in cereal products and thus has a substantial fibre content will be less bioavailable than in a diet containing meat, regardless of other factors affecting iron absorption in the human body (Benito, Miller 1998, Umbreit 2005, Beard, Han 2009).
Iron metabolism in the human body – short presentation

The average intake of meat is 41.72 g day\(^{-1}\) in men and 24.2-45.5 g day\(^{-1}\) in women, which supplies the body with 14.5±5 mg and 20.2±28 mg of iron, respectively (Pereira, Vicente 2013). Daily dietary iron requirements are approx. 8 mg for adult men and approx. 18 mg for adult women with menstrual iron losses (Ganz, Nemeth 2012). During pregnancy, total iron requirement is approx. 1.040 mg (Słomka et al. 2012). However, iron deficiency often occurs in young women in Poland (Hamulka et al. 2011, Waskiewicz, Sygnowska 2011). Consequences of iron deficiency and iron overload in humans are shown in Figure 2.

Iron is stored mainly in the liver, containing around 60% of the body’s iron pool, of which around 95% is stored as ferritin in hepatocytes. Small amounts of iron (around 5%) can also be stored as hemosiderin, apparently a degradation product of ferritin, which is mainly found in Kupffer cells. The amount of ferritin, an easily available source of iron, is mainly regulated by iron regulatory proteins (IRP1 and IRP2). These proteins are key regulators of iron cell homeostasis in higher eukaryotes. A single ferritin molecule can hold up to 4,500 iron atoms, which may participate in erythropoiesis. Ery-

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### Iron disorders

1. Iron deficiency anemia (IDA) and its main clinical symptoms (fatigue, pallor, pica syndrome, glossitis).
2. Material ID: poor intrauterine growth, increased risk of preterm births and low birth weight, higher perinatal and infant morbidity and mortality.
3. Consequences during infancy and childhood: behavioral, cognitive, motor, and language deficits (e.g.: wariness, hesitance, less positive affect, and less social interaction).

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### Consequences of iron deficiency (ID)

- Hereditary hemochromatosis (primary iron overload)
- Secondary iron overload

Consequences: organ dysfunctions due to reactive oxygen species (ROS) overproduction: liver, heart, the central nervous system, pancreas, and thyroid damage.

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Fig. 2. Summary of the consequences of iron deficiency and iron overload in human (Kohgo et al. 2008, Falkowska, Ostrowska 2010, Słomka et al. 2012)
thrombopoiesis requires around 30 mg of iron/day, and erythrocytes contain around 80% of the body’s total iron. The remaining content of iron in the body (around 40%) is found in muscle tissue myoglobin, epithelial reticular cells, cytochromes and iron-containing enzymes (catalase, peroxidase). Only very small amounts of iron are excreted and the body’s iron cycle is nearly closed. A human with around 4 g of iron stored in the body loses only 1 mg per day (0.025% of the body’s iron) and consumes a similar amount daily with food. Body iron is lost, among others, through iron found in sloughing epidermal and intestinal epithelial cells, and as a result of menstrual bleeding in women (CONRAD, UMBREIT 2000, TAPIERO et al. 2001, PAPANIKOLAOUA, PANTOPOULOS 2005, UMBREIT 2005, BEARD, HAN 2009, SŁOMKA et al. 2012).

Iron ions in food are absorbed mainly in the duodenum. The transport of iron ions into enterocytes occurs through the divalent metal transporter DMT-1, which is located at the apical membrane of intestinal enterocytes. DMT-1 works in concert with duodenal cytochrome b (Dcytb) (GANZ, NEMETH 2012). Heme iron passes through the apical membrane of the duodenal enterocytes with the help of the heme transporter HCP1 protein (heme carrier protein 1). Next, heme is released from the porphyrin ring by the action of the heme oxygenase enzyme and divalent iron is released. The porphyrin ring may be responsible for the high absorption of heme iron (LÓPEZ, MARTOS 2004). In turn, globin degradation metabolites facilitate the absorption of nonheme iron. Next, iron leaves the cell through the basolateral membrane of the enterocyte and enters the bloodstream via ferroportin, which works in combination with hephaestin. Ferroportin is a key iron transporter found on duodenal enterocytes. It is also subject to molecular control. After binding to hepcidin, ferroportin decreases the export of iron from cells to the blood. Hepcidin is a peptide hormone that inhibits duodenal iron absorption and its synthesis is regulated by iron. Large amounts of iron in the hepatocytes (inflammation) contribute to higher hepcidin production in the liver, thus reducing iron absorption and release in the body. In turn, iron deficiency decreases or inhibits hepcidin production by hepatocytes, allowing more iron to enter blood plasma (LÓPEZ, MARTOS 2004, GANZ, NEMETH 2012). Following release from enterocytes, the ferrooxidase enzymes hephaestin and ceruloplasmin, which contain atoms of copper, can oxidize Fe(II) to Fe(III). In the blood, two atoms of oxidized iron bind to transferrin, which is a 78 kDa glycoprotein. This protein is responsible for transporting iron to most cells and normally about 25-30% of transferrin is saturated by iron. The transferrin-Fe(III) complex in blood is taken up by transferrin receptors (TfR1 and TfR2) located in the target cell membrane. The rate and site of iron uptake from plasma depends on the number of transferrin receptors, which are highly expressed in all diving cells, especially erythroid cells. Transferrin receptor 1 binds two molecules of transferrin, as a result of which iron ions are released into the cell cytoplasm. The role of the second receptor (TfR2) in iron metabolism has received less attention. Probably, this receptor is responsible for binding non-transferrin-bound iron (NTBI). Iron ions are
transported into the cell via endocytosis. In the resulting endosome, which maintains an acidic pH, iron is separated from transferrin. Next, iron enters the cytoplasm via DMT-1 present in the endosome membrane. Iron ions in the cytosol are incorporated into ferritin and hemosiderin to form a storage pool or are used for producing heme and nonheme proteins. After release of iron, apotransferrin is recycled to the membrane, leaves the cell and enters circulation to transport the next iron ions again (Benito, Miller 1998, Conrad, Umbreit 2000, Tapiero et al. 2001, Umbreit 2005, Beard, Han 2009, Słomka et al. 2012).

**SUMMARY**

Heme iron found in muscle protein should be supplied to humans to prevent iron deficiency, which can lead to anemia. Heme iron, whose main source is red meat, is easily absorbed by the human body. In addition, heme iron, which is mainly found in myoglobin in meat, contributes to the desirable bright red color and to the most undesirable brown color of meat. Both heme and nonheme iron are catalysts of lipid oxidation in meat. This process lowers the nutritive value through oxidation of polyunsaturated fatty acids, which produces an undesirable flavor and aroma.

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