

## IRON DEFICIENCY AND LIPID PEROXIDATION

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### Summary

Iron deficiency is one of the major nutritional problems that affects more than 2 000 millions of persons around the world. Non enough iron ingestion through the foods and the increment of necessities in population groups of risk, as children, women of childbearing age and pregnancy are the principal cause of iron deficiency. Iron catalyzed the Fenton reaction generating hydroxyl radical. Therefore, iron supplementation increases lipid peroxidation meanwhile iron deficiency is believed to decreased lipid peroxidative cell damage. Nevertheless, studies on iron deficiency are contradictory. The aim of this work was show the result of some studies addressed to evaluated lipid peroxidation parameters in iron deficient rats. Thus, when these studies compared iron deficient rats and normal rat regarding to MDA, ethane and pentane level in liver, kidney, plasma or intestinal mucosal., significant increased concentrations were found in iron deficient animals. These studies have suggested that the enhancement of lipid peroxidation in iron deficient rats may be caused by several factors, such as the accumulation of copper ions in liver, and the increase in triglycerides level in plasma and the fragility of mitochondrial membranes. Such a finding identifies other adverse effects of iron deficiency and further emphasizes the need for preventing and correcting it.

Keyword: lipid peroxidation, iron deficiencies

Iron is an essential micronutrient for the survival, replication, and differentiation of animals, plants and almost microorganisms (1). Iron deficiency is one of the major nutritional problems in the world among women of childbearing age, children and infants.(2) This nutritional disorders affects more than 2000 millions of persons around the world.(3) Iron deficiency is associated with and increased risk of poor pregnancy outcomes and impaired cognitive development in young children (2). However, iron in excess is toxic due to excess iron accumulation within tissues, cells and even organelles. This has been associated with pathological disorders such as how Haemochromatosis, Parkinson's and Alzheimer's diseases (1).

The mayor causative factors of iron deficiency is the poor bioavailability of iron in the food intake,(4) and the increased of the requirements in populations groups of risk. The usual treatment of iron deficiency anemia involves oral iron supplementation as ferrous salt. These therapy may induce considerable gastrointestinal side effects, such as nausea, vomiting, heartburn, abdominal pain and constipation (5), that has been associated to the increment of iron-induced oxidative damage in the intestine after oral ingestion of iron supplements. Oral ferrous iron therapy may induce oxidative stress due to its catalyzing role in Fenton chemistry, resulting in a production of highly reactive hydroxyl radicals (6). They cant react with any molecules in their direct environmental, resulting in a cascade of reactions in which lipids, proteins and DNA may get damage (7,8). Both, iron supplementation to prevent iron deficiency and treatment with iron to correct iron deficiency and anaemia increased oxidative stress (9,10). Many studies had demonstrated that iron supplementation increased oxidative stress, but only a few studies exists about the relations between iron deficiency and oxidative stress (6,11). It interesting to note that the results of these studies are contradictories. In some cases studies revealed that iron deficiency increases lipid peroxidation whereas in others iron deficiency decreased lipid peroxidative cell damage. Therefore, the aim of the present work is to review the results of some of these studies and to analyze the factors that influence in such a contradictory results.

### **Iron metabolism and iron deficiency**

Subjects ingest iron in food in two chemicals forms, haem and non haem. Two different receptors exits in the duodenum for the absorption of both types of iron (12,13). When iron has entered in mucosal cell, it has two choices, either to encounter a ferritin molecule and incorporate within the protein shell or to be transported to the basolateral membrane. Iron entering the cells is available for transport across the cell to the basolateral membrane, where a protein of membrane promotes oxidation of  $Fe^{+2}$  to  $Fe^{+3}$ , the Hephaestin. The  $Fe^{+3}$  will be rapidly bound to plasma ligands, essentially to the major iron transporter protein, apotransferrin, except in situations of saturation of the binding capacity of the plasma transporter. In these conditions, it would also bind to other ligands, such as citrate, constituting the so-called non transferring bound iron (12). The main site of body iron stores are the hepatic parenchyma and the reticuloendothelial system (RES), particularly the RE cells of the bone marrow, spleen, and liver (14). Normal iron release does seem to require ceruloplasmin, a multicopper ferroxidase. Ceruloplasmin appears to mobilize iron from storages sites by catalyzing the oxidation of  $Fe^{+2}$  to  $Fe^{+3}$ , with can be incorporated into apotransferrina (15). Gene mapping studies have identified a ceruloplasmin homologue, Hephaestin (16).

In normal conditions when iron is ingested there are many ferritin molecules available. Thus,-most of the iron will be trapped in ferritin, resulting in a low mucosal transfer of iron. In iron deficiency or in conditions of increased of erythropoietin, mucosal cells hardly produce only ferritin and most of the iron is transfer to the apotransferrin. Finally, iron deficiency appeared to result from inefficient released of iron from RES. Iron metabolism is regulated largely by post translational modifications involving cellular iron uptake and storage through interactions of a citoplasmatic iron regulator protein (IRP) with specific

mRNA regulatory sequences termed iron-responsive elements (IRE). These regulatory sequences are located in mRNA that codificated to ferritin or Transferrin receptors. In response to iron deficiency IRP binding to IRE increased. IRP binding to mRNA of Transferring receptors (TRs) protects the IRE from degradation by nucleases, thereby permitting translation of TR and enhanced iron uptake (17).

### **Relations of iron and lipid peroxidation**

Accumulation of iron in biological tissues is associated with cell membrane damage, producing alterations in essentials biomolecules such as DNA and proteins (8,9).  $Fe^{+2}$  can acts as a catalyst in the Fenton reactions to potentiated oxygen toxicity by the generation of a wide range of free radicals species, including hydroxyl radicals (OH $\cdot$ ). Hidroxyl radicals are the most reactive free radicals species known, since it has the ability to react with a wide range of cellular constituents, including amino acids residues purine and pyrimidine bases of DNA, as well as membranes lipids to initiated a free radicals chain reaction known as lipid peroxidation (1). Therefore, iron may be responsible for initiating the peroxidation of lipid and the formation of conjugated diene double bounds. Besides, fluorescent chromolipids, alkoxy and peroxy radicals can propagate lipid peroxidation to form final end products such as pentane, ethane, malondialdehyde, hexanal, isoprostanes, ect. (18,19)

Measurements of these products are used as indicator of lipid peroxidation in organs, plasma, breath and urine. The most commonly used assay is that with detects malonaldehyde (MDA) concentrations through the thiobarbituric acid reactive substances (TBARS). Urinary excretion of several of these metabolites as well as ethane and pentane exhalation rates in breath are usually used to measure total body lipid peroxidation (20). Also, the tissue concentration of these products may reflect total damage. However, the thiobarbituric acid (TBA) test measured MDA present in the sample, plus MDA that is purposefully generated by the breakdown of lipid hidroperoxides during the assays. The TBA-test can also measure various TBARS other than MDA (21). Furthermore, it has been found that unless a strong iron chelator is added to the homogenization buffer large amount of MDA is formed from iron loaded liver, probably through iron catalyzed lipid peroxidation/descomposition that occurs during the homogenization process (11). Besides, several others works have shown that the addition of radical trapping substances such as BHT, can diminish such artefact (19). Therefore, it is quite possible that in some studies the increased of lipid peroxidation (as measured by TBARS) may have occurred during tissue homogenization and not "in vivo" (11).

### **Experimental evidences about relationship between iron deficiency and lipid peroxidation**

To investigate the possible role of membrane peroxidation in iron deficiency anemia Jain et. al., 1983 studied the red blood cell membrane lipids and protein of rats fed with iron deficiency and control diets between 21 and 41 days of age. They showed the presence of phospholipids/MDA adducts and high molecular weight protein complexes in the red blood cell of iron deficiency rats. These provide the evidence that peroxidative damage in the membrane had already take place in vivo. They suggested that peroxidative damage to red blood cell in iron deficiency rats were due to: Reduced glutathioned peroxidase, catalase

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and/or Vitamin E needed to detoxify intracellular peroxide and elevated levels of protoporphyrin and copper with are known oxidants and can generate reactive oxygen species to initiated membrane lipid peroxidation (22).

Rao and Jagadeesan, in 1996 investigated lipid peroxidation and activities of different antioxidant enzymes in iron deficiency to explain the higher risk of tumorigenesis. They evaluated two groups of male weaning Fisher rats maintained an iron sufficient(C), or iron deficient diet for a period of 32 weeks. Each group were subdivided into two subgroups. The carcinogen dimethylhydrazine was fed for two subgroups for 9 weeks, and the other two subgroups served as control. After the experimental period, hepatic assays for MDA levels (as measured by TBARS), and activities of various antioxidant enzymes were measured. They reported that MDA production was elevated by 50% and activity of superoxide dismutase significantly depresses in carcinogen-fed, iron deficient group treated with hydrazine by 28% compared to deficient group (23).

The increased of lipid peroxidation in iron deficiency have been reported for Uehara et al., 1997. They founded that iron deficient rats had increased levels of serum and liver phosphatidylcholine hidroperoxide, as indicator of cell membrane lipid peroxidation. In this study iron deficient rats had copper concentrations in liver mitochondrial and nuclear subcellular fractions that were >8-fold higher than those in normal rats (21).

Srigidhar and Nair in 1998 compare iron deficient female rats supplement for 15 days with FeSO<sub>4</sub> equivalent to 8000 µgFe/day, and compare with iron deficient and iron adequate rats. The levels of intestinal MDA (as measured by TBARS), and protein carbonyls and the activities of various antioxidant enzymes were estimated. They reported that iron supplement in iron deficiency rats intestine had two times more MDA that did unsupplement deficient rats and 1.6 times more MDA than did unsupplement normal control rats. In this some study, the deficient rats that were iron supplemented had liver nonheme iron level 2.9 times higher than those of unsupplemented normal rats. These authors did not measure liver MDA. They suggest that iron deficiency intestine of rat is more susceptible to iron mediated peroxidative damage and functional impairment during correction of deficiency with iron (6).

In another study Knutson compare iron deficiency and iron normal rats fed either 0 or 8000µgFe/day for 21 days. Too they compare deficient rats that were fed either the same supplements daily or once every 3 days for 21 days. They measured lipid peroxidation thought breath ethane and pentane, and tissue and plasma (MDA). Measured of MDA was carried out by GC-MS according to Yeo et al., 1994, but with two modifications: Desferrioxamine was added to the homogenization buffer and the amount of BHT was increased. Author said that these modifications were made to prevent iron catalyzed, *ex vivo* MDA formation that might occur during sample processing and analysis. They measured plasma triglycerides concentration too. They found that iron deficient rats had increased lipid peroxidation: ethane and pentane liver MDA and kidney MDA were all significantly increased in these rats. Plasma triglycerides concentration in deficient rats was increased in five-fold. (11)

We considered that the level of MDA is an important determination to consider if iron deficiency increased or not lipid peroxidation, but is very important analysed the method of MDA determination that was utilized. We supposed that the results of Knutson et al., 2000 about the increased of MDA concentrations are more reliable that the report by other author due to the methodology used for measured MDA concentrations in this work (11).

Another important aspect for analysed if iron deficiency increased lipid peroxidation are the doses of iron administration to the rats, and the extension of the period of iron supplementation. In the most of these work author employed similar doses for iron deficiency rats (8000 µgFe/day) and for normal rats 800 µgFe/day. These doses are to those often given to pregnant women in the developing world, of 120 mg Fe/day (24).

About the explanation of why iron deficiency increased lipid peroxidation is similar by the different author. In the most of them they considered the increased in level copper in liver. Some studies have been demonstrated that in iron deficiency rats rapidly accumulate liver copper (25,26). Excess of copper can also catalyse lipid peroxidation (27). Other author suppose that high concentration of triglycerides provide more lipid substrate for lipid peroxidation, and these may have contributed to the high levels of liver and kidney MDA.(11) Another factors that the investigations had showed that could increased lipid peroxidation is due to increase fragility of mitochondrial membranes of iron deficiency rats tissue mitochondrial, with have been described as being greatly enlarged and swollen (28,8). We are agree with all these explanations, but we consider that another important element about iron metabolism that could increase iron peroxidation during iron deficiency is the increase in intracellular iron trafficking from iron stores in ferritin and hemosiderin (17). This effect may account for some early increased in the potential for iron availability to catalyzed some of the oxidant induced damage.

As we can see some studies had showed some experimental evidences that iron deficiency increase lipid peroxidation in biological tissue, and this is another elements about the importance of to prevent and treatment iron deficiency and anaemia in man.

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