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Iron, Oxidative Stress and Health

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1. Introduction

Iron is an element of crucial importance to living cells. It has incompletely filled 'd' orbitals and exists in a range of oxidation states, the most common being ferrous [Fe II (d^6)] and ferric [Fe III(d^5)] forms. By virtue of this unique electrochemical property, iron is an ideal redox active cofactor for many biological processes and fundamental biochemical activities in all cells. It can associate with proteins; bind to oxygen (O_2), transfer electrons and mediate catalytic reactions. Enzymes of the citric acid cycle – succinate dehydrogenase and aconitase are iron-dependent. Iron is a critical component of heme in hemoglobin (Hb), myoglobin, cytochromes as well as iron–sulfur complexes of the electron transport chain. Iron is also required for activity of ribonucleoside reductase, the rate-limiting enzyme of the first metabolic reaction committed to DNA synthesis. Therefore, iron plays an important role in metabolic processes including O_2 transport, electron transport, oxidative phosphorylation and energy production, xenobiotic metabolism, DNA synthesis, cell growth, apoptosis, gene regulation and inflammation (Zhang and Enns, 2006; Cairo and Recalcati, 2007; He et al., 2007; Outten and Theil, 2009; Wang and Pantopoulos, 2011). In the central nervous system, iron is required for myelogenesis and myelin maintenance by oligodendrocytes. It is also a necessary cofactor for the synthesis of neurotransmitters, dopamine, norepinephrine and serotonin (He et al., 2007). Therefore deficiency of iron can result in myriad disorders. Even mild iron deficiency can adversely affect cognitive performance, behavior, physical growth of infants, preschool and school age children and physical work capacity and work performance of adults (Brabin et al, 2001; Haas and Brownlie, 2001).

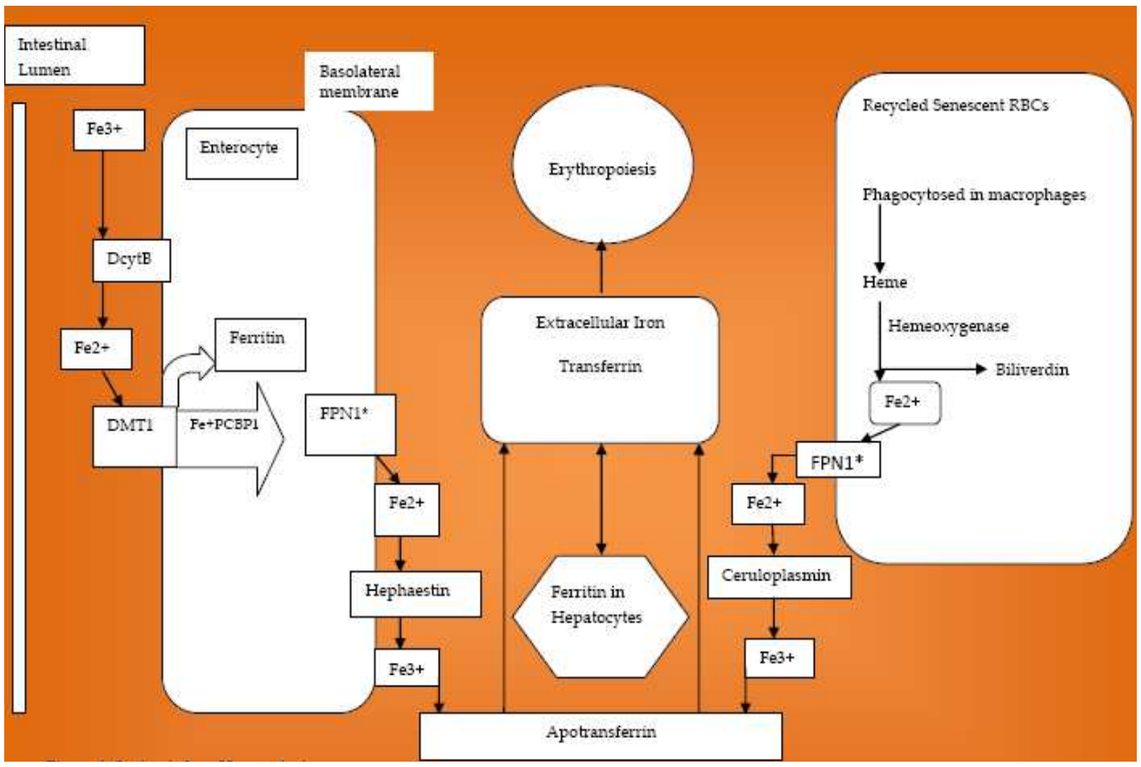
2. Iron homeostasis

The redox reactivity of iron makes it extremely useful but the same property makes it a toxic entity, because of its propensity to generate free radicals if it is not tightly bound and/or it is present in excess. Due to this dual nature, the human body possesses elegant and elaborate control mechanisms to maintain iron homeostasis by coordinately regulating iron absorption, iron recycling and mobilization of stored iron. Disruption of these processes can result in deviation from normal iron levels both systemically and at cellular level, which can lead to deleterious consequences.

Homeostasis of all essential metal ions share common features. However, compared to other metals, the body contains much higher levels of iron (3.5 - 4 g of iron versus 100 mg of

copper), aqueous ferric ions exhibit low solubility and unlike other metals, excess iron is not actively excreted via the kidney. Also free iron can catalyze formation of reactive oxygen species (ROS) and must be safely bound by specialized proteins. Therefore additional iron-specific homeostatic features are required (Theil and Goss, 2009; Wessling-Resnick, 2010). Iron homeostasis must be tightly controlled at both systemic and cellular levels to provide optimum amounts of iron at all times and yet maintain the delicate balance between iron nutrition and toxicity, especially because the human body does not possess mechanisms for getting rid of the excess metal.

(a)Systemic homeostasis: Maintenance of stable extracellular iron concentration requires the coordinated regulation of iron transport into plasma from (i) dietary iron absorbed in the intestines (ii) recycled senescent red blood cells (RBC) and (iii) storage in hepatocytes (Figure 1). A major indicator and determinant of systemic iron homeostasis is the saturation of plasma transferrin (Tf). Saturation levels are predominantly determined by the amount of iron entering from the above three sources and the amount utilized for erythropoiesis (Mucken et al., 2008).



DcytB- Ferric Reductase Enzyme- Duodenal cytochrome B. DMT- Divalent Metal Transporter. PCBP- Poly(rc)-binding proteine. FPN- Ferroportin
*regulated by Hepicidin

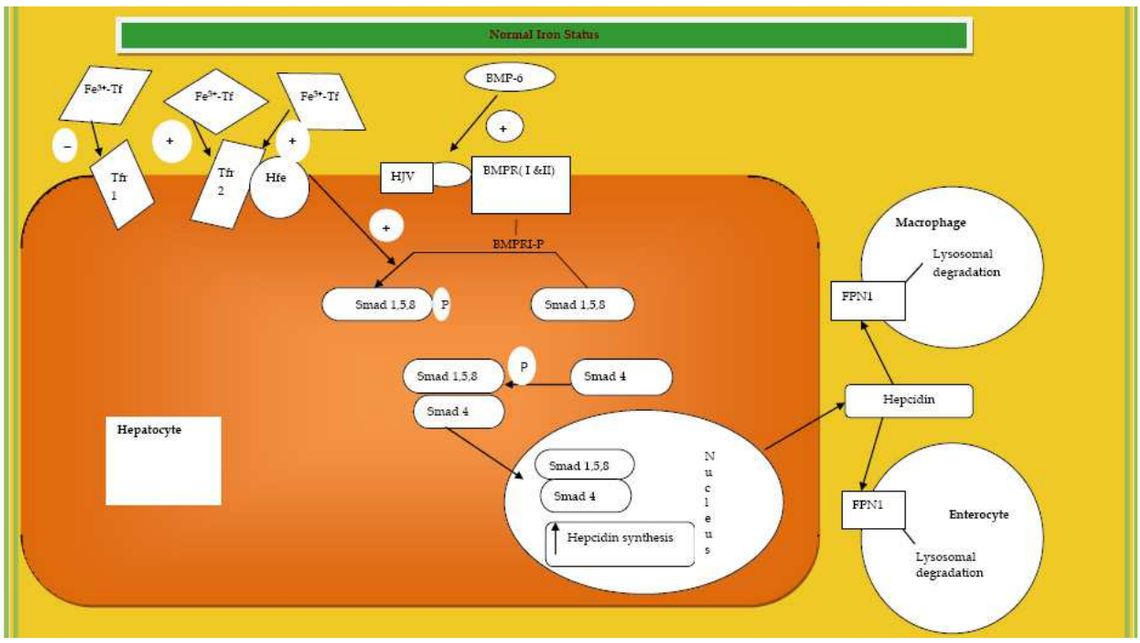
Fig. 1. Systemic Iron Homeostasis

Intestinal absorption of dietary iron involves transport across the apical membrane followed by translocation through the cytoplasm and across the basolateral membrane into portal circulation. Non-heme iron which comprises a major component of dietary iron is taken up

from the lumen via the transmembrane protein divalent metal transporter (DMT1) located at the enterocyte apical membrane and actively transported as Fe (II) ion. Prior to transport, dietary Fe (III) is reduced by the ferric reductase enzyme – duodenal cytochrome B (DCytb) (Knutson, 2010). The percentage of iron absorbed from the amount ingested is generally higher in case of heme than non-heme iron. As the iron intake increases, the percent of ingested dose that is absorbed reduces; however, the absolute amount absorbed and likely to enter systemic circulation is more when intakes are high as encountered in supplementation and perhaps with fortified foods. Within the enterocyte, due to its reactive nature, iron is bound to carrier proteins- poly(r c) -binding protein (PCBP-1) which acts as a cytoplasmic chaperon in delivering excess iron to ferritin (Shi et al., 2010). Apparently PCBP1 translocates iron to the basolateral membrane where iron is transported out of the enterocytes by the iron export protein ferroportin (FPN1). Hephaestin is thought to oxidize Fe (II) to Fe (III) which is rapidly sequestered by apotransferrin (Knutson, 2010; Muchenthaler, Galy and Hentz, 2008).

Intestinal sources provide only 1-2 mg of iron per day. The daily demand for 20 mg of iron for erythropoiesis is largely met by recycled iron from senescent erythrocytes processed by the reticuloendothelial system (RES) consisting of specialized macrophages present mainly in the liver, spleen and bone marrow. Within macrophages, heme derived from phagocytized RBCs is catalyzed by hemeoxygenase (HO). The liberated iron is released via FPN1, donated to Tf and reutilized (Kohgo et al., 2008; Tanna and Miller, 2010). The iron that is not used for metabolic purposes is stored in ferritin. The RES represents a major iron storage compartment. Ferritin messenger RNAs (mRNAs) are among the most abundant mRNAs in monocyte-derived macrophages. Liver has a high iron storage capacity and accumulates iron mostly in the periportal regions with a decreasing gradient towards the centrilobular areas. Iron stores are depleted in case of increased requirements or excessive iron loss (Muckenthaler, Galy and Hentz, 2008).

Iron export from duodenal enterocytes, macrophages and hepatocytes through FPNI appears to be a limiting step, modulated by the peptide hormone, hepcidin. Binding of hepcidin to FPN1 triggers the internalization and degradation of the receptor-ligand complex. Once internalized, the hepcidin-FPN1 complex is degraded in lysosomes and cellular iron export ceases (Nemeth and Ganz, 2009; Knutson, 2010). Hepcidin expression is regulated by a number of proteins in response to both extracellular and intracellular iron (Figure 2). Two main iron-related signaling pathways, one involving bone morphogenetic proteins (BMPs – these comprise a group of at least 20 soluble molecules belonging to the transforming growth factor β superfamily) and the other dependent on transferrin receptor II (TfRII) regulate hepcidin regulation. Iron sensing by BMP pathway is mediated through BMP-6, as their levels in mice increased with dietary iron overloading and decreased with deficiency (Kantz et al., 2008). BMP-6 binds to BMP receptor (BMPR) complex comprised of BMPRI and BMPRII. After binding of BMP-6, BMPRII phosphorylates BMPRI which then propagates the signal by catalyzing phosphorylation of cytoplasmic Smad1/Smad5/Smad8. Its association with the common mediator Smad4 is followed by translocation to the nucleus where they act as transcription factors. BMP signaling is modulated by coreceptor hemojuvelin (HJV) a cell surface protein (Knutson, 2010). All these proteins are important for hepcidin expression. Disruption of Smad 4 and HJV in mice have been reported to result in iron overload (Nemeth and Ganz, 2009).



HJV- Hemojuvenil, BMP Bone Morphogenic Protein, BMPR- Bone Morphogenic receptor, FPN-ferroportin

Fig. 2. Regulation of Hepidin Expression

Hepcidin regulation by the TfRII dependent pathway requires holotransferrin and high-Fe gene (HFE) which shuttles between TfRI and TfRII depending on the holotransferrin concentration. In high iron conditions, holotransferrin binds to TfRI, displaces HFE and allows it to interact with TfRII. Gao et al., (2000) suggested that interaction between TfRII and HFE is required for hepcidin induction in response to iron. There appears to be crosstalk between the BMP and TfRII dependent signaling converging into a common pathway for hepcidin expression in response to iron (Nemeth and Ganz, 2009; Knutson, 2010). Hepcidin expression is apparently regulated by iron stores, although the mechanism of intracellular sensing is unclear. Hepcidin is down regulated in response to iron deprivation but how this occurs remains to be fully elucidated. Cell surface HJV appears to be cleared by Matriptase-2, thereby reducing BMP signaling. Matripatase deficiency leads to iron overload phenotypes (Knutson, 2010).

Hepcidin expression is suppressed in anemias with ineffective erythropoiesis, mediated by proteins produced by erythroid precursors. Hypoxia also decreases hepcidin, being modulated by hypoxia-inducible factor. However hepcidin synthesis rapidly increases during infection and inflammation (Nemeth and Ganz, 2009). Inappropriate products of hepcidin have been implicated in pathogenesis of various iron disorders.

(b) Cellular homeostasis: The cytosolic iron pool is highly regulated because it is an important source of iron for numerous cytosolic and nuclear iron proteins, as well as being a source from which mitochondria and other organelles derive iron (Rouault and Cooperman, 2006). Cellular iron levels are balanced by divergent yet coordinated regulation of proteins involved in iron uptake (TfR), export, storage (ferritin) and distribution. Within cells, iron is stored in ferritin. Also, labile iron pool (LIP) is present within cells. Most LIP is free iron

bound to small organic chelators - citrate or adenosine phosphate, carboxylate, polypeptides, (Kohgr et al., 2008; Jiang et al., 2009). LIP is biologically active in intracellular metabolism but toxic if present in excess. Maintenance of appropriate LIP levels is critical for homeostasis (Mackenzie, Iwasaki and Tsuji, 2008).

Iron regulatory proteins (IRP) are critical components of a sensory and regulatory network controlling iron homeostasis by exerting genetic control at multiple steps (Goforth et al.,2010). The expression of TfR and ferritin are coordinately regulated upon binding of IRP1 and IRP2 to iron regulatory elements (IRE) in the untranslated regions (UTRs) of their respective genes. IRPs are activated by iron deficient conditions, bind to their target IREs and modulate translation of mRNA (Figure 3). The location of IREs in target mRNA determines whether regulation is positive or negative. In case of TfR1 mRNA, five IREs are located in 3' UTR whereas the mRNA coding for ferritin contains a single IRE in the 5' UTR. When iron is depleted, IRE-IRP interaction at the 3' UTR of TfR1 stabilizes mRNA, while imposing a steric blockade to ferritin mRNA translation. As a result, TfR1 is upregulated and stimulates acquisition of iron from plasma Tf to counteract low iron levels. Simultaneously ferritin synthesis is inhibited as storage of iron is not needed.

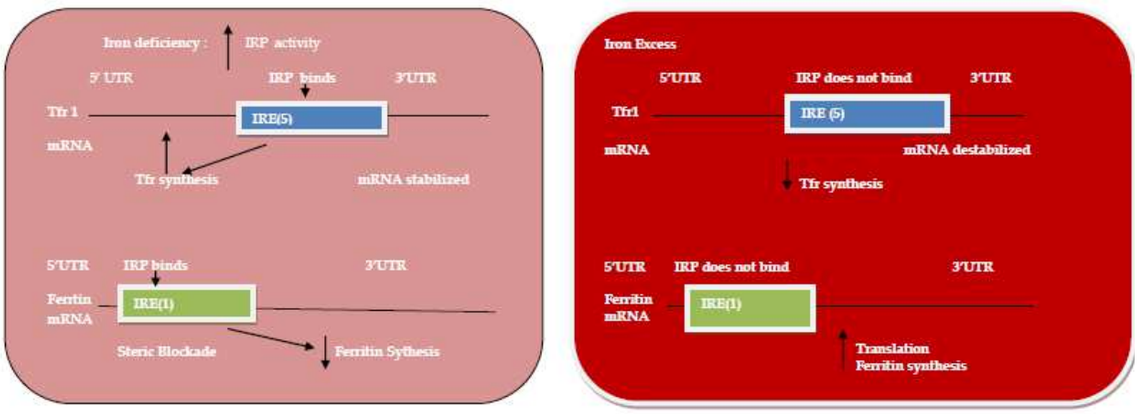


Fig. 3. Iron Status and IRP Regulation of Transferrin Receptors and Ferritin Synthesis

Conversely, when iron concentration is high, IRPs are inactivated resulting in degradation of TfR1 mRNA while translation of ferritin mRNA occurs. Thus when iron levels exceed the cellular needs, the IRE/IRP switch minimizes iron uptake and promotes iron storage (Mackenzie, Iwasaki and Tsuji, 2008; Wang and Pantopoulos, 2011). However, in erythroid progenitor cells which require high amounts of iron for heme synthesis, TfR1 stability is uncoupled from iron supply and TfR1 level is transcriptionally regulated by erythroid active element in its promoter region (Wang and Pantopoulos, 2011). Also TfR1 is regulated in hypoxic conditions, wherein dimerized hypoxia-induced factors (HIF-1α and HIF 1β) bind to hypoxia response element located upstream of the transcription start site (Mackenzie,Iwasaki and Tsuji, 2008).

IRPs, in addition to modulating ferritin and TfR1 levels can regulate the mRNAs or other proteins required for iron utilization (erythroid 5-aminolevulanata synthase), iron uptake (DMT1) and export (FPN1). DMT1 mRNA has IRE in its 3' UTR and is upregulated by iron deficiency while the other two proteins have IREs in their 5' UTR and are therefore

upregulated under high iron conditions (Cairo and Recalcati, 2007; Wang and Pantopoulos, 2011). It appears that the functional significance of IRE/IRP system is beyond cellular iron uptake and storage. The regulation of FPN1 at the basolateral surface of enterocytes by both IRE/IRP and hepcidin indicates that the two regulatory systems are interconnected (Muckenthaler et al., 2008). Similar mechanisms may apply to other cells and work is warranted to understand the functional interconnection between hepcidin and IRE/IRP systems.

3. Iron as a pro-oxidant

A variety of mechanisms tightly regulate iron homeostasis in the human body. However, iron can be toxic if its level and/or distribution are not carefully regulated. Iron toxicity is developed through the production of (ROS). ROS are formed during normal cellular metabolism. Superoxide anion ($O2^{\bullet -}$) is generated continuously by the mitochondrial electron transport system, myeloid cells as well as during several cellular oxidase catalyzed reactions. Mitochondrial electron transport system is not the only electron transport system producing $O2^{\bullet -}$. Non mitochondrial electron transport chains such as mono oxygenase system (microsomal or P_{450}), photosynthetic electron transport chain and others are critically important. Besides xanthine oxidase, NADPH oxidase is one of the major sources of $O2^{\bullet -}$, while other oxidases mainly produce hydrogen peroxide (H_2O_2). H_2O_2 is generated as a result of enzymatic (superoxide dismutase) and non-enzymatic destruction of superoxide anions. Both $O2^{\bullet -}$ and H_2O_2 are found in extracellular spaces and in blood. Since H_2O_2 is an uncharged compound of low molecular mass, it is believed that it may be capable of freely crossing biological membranes and diffusing considerable distance from its site of production. However, further work is warranted to confirm the same (Branco et al., 2004; Lushchak, 2011). $O2^{\bullet -}$ being negatively charged is unable to cross the biological membrane whereas its protonated form $HO^{\bullet}$ can. These molecules are not particularly reactive by themselves. It is the interaction of these partially reduced forms of oxygen with transition elements including iron, which lead to the production of highly damaging radicals. The most important reaction of H_2O_2 with free or poorly liganded Fe(II) is the Fenton reaction, generating the highly reactive hydroxyl ions.

$$Fe\ (II) + H_2O_2 \longrightarrow Fe\ (III) + OH^- + HO^{\bullet} \quad (1)$$

Superoxide can also react with ferric iron in the Haber – Weiss reaction to produce Fe (II) again thereby effecting redox cycling.

$$O_2^{\bullet -} + Fe\ (III) \longrightarrow O_2 + Fe\ (II) \quad (2)$$

Further, ascorbic acid has a number of known interactions with metal ions. Ascorbate can replace $O_2^{\bullet -}$ within the cell for reducing the Fe (III) to Fe (II), thereby facilitating the Fenton reaction. Also, metal ion catalysis of oxidation of ascorbic acid leads to formation of H_2O_2, ultimately generating hydroxyl ions. This generation of hydroxyl ions from the simple system containing metal ions, ascorbic acid and oxygen has potentially deleterious consequences.

Hydroxyl radical ($HO^{\bullet}$) is highly toxic among the partially reduced oxygen species. It reacts with all kinds of biological macromolecules including cellular DNA, protein, carbohydrates and lipids. In the nucleus, hydroxyl radicals cause DNA damage, especially double strand

breaks as well as chemical changes in deoxyribose, purines and pyrimidines. Hydroxyl radical can be added onto C-8 of guanine leading to guanine modification. 8-hydroxy-deoxyguanosine is an important indicator of DNA damage caused by iron-mediated Fenton reaction. Many of the damaged proteins are enzymes, hence critical cellular functions including ATP generation are adversely affected. Fe (II) reacting with unsaturated fatty acids in the presence of H_2O_2 can initiate lipid peroxidation cascade in biological membranes and lipoproteins by production of HO•. Lipid peroxidation increases membrane fragility of cell organelles such as mitochondria, lysosomes and endoplasmic reticulum leading to impaired cell function (Fardy and Silverman, 1995; Casanueva and Viteri,2003; Kell, 2009 and de La Rosa et al., 2008). Thus HO˙ mediate many higher level manifestations of tissue damage, disease and organ failure and ultimately death.

Normally, the potential toxicity of iron is minimal because it is complexed with Tf in plasma and with ferritin which "minimizes opportunities for uncontrolled iron dioxygen chemistry and oxidative stress", within the cells (Kapralov et al, 2009). In addition, there are several chaperones and iron-binding proteins, reducing the chances of free or unligated iron becoming available to participate in the Fenton reaction. However, any small amount of 'free iron' due to elevated iron levels or released due to specific mechanisms that operate during iron deficiency or dysregulation of iron homeostasis, can increase risk of iron-induced oxidative stress.

3.1 Iron deficiency and oxidative stress

In iron-deficiency anemia, the lifespan of the RBCs is reduced. There is increased membrane stiffness and a decrease in deformability, thus making it relatively difficult for the RBCs to pass through the spleen. Thus in iron deficiency there is suicidal death of RBCs. This will liberate iron which increases the potential for oxidative stress. In turn, the presence of oxidative stress increases membrane stiffness and decreases deformability, setting up a vicious cycle. Further, there is increased lipid peroxidation, combined with depletion of antioxidant enzymes such as glutathione peroxidase, exacerbating the production of ROS. With less number of RBCs and reduced Hb, there is lower partial pressure of oxygen. The partially oxygenated Hb increases autooxidation of Hb which is converted to methemoglobin and superoxide is generated. Heme degradation has been reported to correlate with immunoglobulin G binding to the RBC membrane, which in turn is associated with denaturation of Hb. Denatured Hb and heme degradation products as well as free iron, promote removal of RBCs from circulation. Since the ROS concentration can be increased near the membrane, not only are the RBCs themselves damaged, ROS can be released to the vasculature and neighboring tissues (Nagababu et al., 2008).

3.2 Elevated body iron and oxidative stress

Iron stores tend to increase with age (Kell, 2009). Hofera et al., (2008) studied the iron content and RNA oxidation in skeletal muscle of 6m and 32m old Fischer 344/Brown Norway rats. They observed that with age, non-heme iron levels increased by 233% and RNA oxidation by 85%. Simultaneously, HJV and TfR1 decreased by 37% and 87%, respectively. There appears to be dysregulation of iron homeostasis with age, favoring increased intracellular free iron which in turn can induce oxidative stress. It has been shown that lysosomes are a source of rapidly mobilized, chelatable iron, with accumulation of iron

being higher with ageing. Lipofuscin, an age-related iron rich pigment is non-degradable and not hydrolyzed by lysosomal enzymes. As a result, lipofuscin accumulates in the lysosomes. Lysosomes do not have the capacity to remove intralysosomal iron bound to lipofuscin, leading to excessive iron accumulation. This accumulation is evident in the RES, hepatocytes and post-mitotic cells such as neurons. Low internal pH i.e. 4 to 5 , presence of reducing equivalents, such as cysteine, ascorbic acid and glutathione favour intralysosomal Fenton reaction (Kurz et al., 2008). Further, iron is rapidly taken up into mitochondria by calcium uniporters where it catalyzes the ROS cascade (Uchiyama et al., 2008). ROS formation initiates mitochondrial permeability, depolarization, uncoupling of oxidative phosphorylation, mitochondrial swelling followed by membrane rupture, ultimately leading to necrosis or apoptosis(de La Rosa et al., 2008; Uchiyama et al., 2008). Thus the process of ageing and the associated morbidity depends on the oxidative stress-related alterations occurring in long-lived post-mitotic cells.

In women, besides the normal process of ageing, menopause also contributes to iron accumulation. Three-fold higher ferritin values have been reported in post-menopausal women compared to pre-menopausal women (Crist et al., 2009). Ferritin sequesters potentially reactive iron and is cytoprotective. Ferritin is autophagocytosed and degraded through lysosomal pathway to liberate iron which is then transported to the cytosol (Kurtz et al., 2004). Aberrant release of ferritin iron may lead to production of ROS (Mackenzie et al., 2008). Jiang, Pelle and Huang (2009) hypothesized that this increase in body iron may have a role to play in postmenopausal hot flashes since they found that serum ferritin levels were higher among African-American, white, and Hispanic women who reported more hot flashes. Iron is involved in heat production. Poor temperature regulation and thereby intolerance to cold has been observed in iron deficient persons, due to impaired heat production.

Iron status may be an important determinant for occurrence of breast cancer. In young women, deficiency may be linked to breast cancer recurrence whereas in postmenopausal women, higher iron may contribute to breast cancer (Jiang, Pelle and Huang, 2009). Hemochromatosis has been linked with high risk of osteoporosis. Osteopenia has been observed in patients with iron overload although vitamin D status and parathyroid hormone levels were normal. Similarly in patients with sickle cell disease, osteoporosis and osteopenia have been reported and the low bone mineral density was found to be associated with lower Hb and higher levels of ferritin. Iron status may also affect bone health. In animal models, both deficiency and excess of iron have been found to affect bone. D'Amelio et al., (2008) observed that patients with osteoporosis were iron deficient, had lower serum iron and higher Tf levels. A case report suggests that frequent phlebotomies normalized serum while the bone mineral density of the lumbar spine increased.

Iron and oxidative stress may be involved in the dryness, thinning and wrinkling of skin during menopause. It has been suggested that iron accumulation in the skin increases oxidative stress when the skin is exposed to UV rays. This in turn affects downstream genes and increased body iron stores may cause damage and photoaging of skin as well as make the skin more susceptible to damage by UV rays. Prolonged exposure to sun and UVrays was found to be associated with accumulation of non heme iron while treatment with iron chelators reduced the amount of skin damage.

Besides ageing, iron supplementation can expose the body, especially mucosal cells to very high levels. In this situation, large proportion of iron is retained in the mucosal cell as ferritin. However there may be accumulation of small amounts of iron, increasing the risk of oxidative stress. Iron supplementation has been shown to generate free radicals in a murine model, increasing intestinal susceptibility to oxidative stress (Ianotti et al., 2006). Iron accumulation and mucosal abnormalities such as reduction of microvillus height, necrosis have been reported in rats having high iron dose, especially those who were previously anemic (Casnueva and Viteri, 2003). In addition to local effects, iron supplementation can also affect other organs. Studies conducted in rats have indicated that there is continuous absorption of small proportion of supplemental iron at a rate higher than the normal, probably by passive diffusion. Under such circumstances, iron levels may exceed the binding capacity of Tf, giving rise to non-transferrin bound iron (NTB1). NTB1 is taken up by liver and has been shown to contribute to iron loading and hemochromatosis (Mackenzie et al., 2008). Unlike Tf–bound iron, cellular uptake of NTBI is not dependent on TfR and therefore the resulting iron is diffusely distributed throughout the organ (Kohgo et al., 2008). Whether long-term iron supplementation will have adverse effects in humans is also determined by one's antioxidant status.

Conversely, oxidative stress itself influences iron metabolism and iron proteins. Intracellular iron levels are increased in response to oxidative stress. There is reduction in ferritin synthesis, increased expression and uptake of TfR and upregulation of HO-1(Deb et al., 2009). Hb can act as a peroxidase in the presence of H_2O_2. Normally, tight regulation prevents this from happening, except in case of severe inflammation or generation of superoxide radicals by immune cells. Peroxidase activity of Hb may induce self-oxidation of proteins, with covalent cross-linking and aggregation of Hb being consequences which can induce oxidative stress in plasma and macrophages (Kapralov et al., 2009).

Oxidative stress and iron have been linked to several pathological states. Herein we review the role of ROS and iron in reproduction, the central nervous system and liver.

4. Reproduction, oxidative stress and iron

Human reproduction is a complex but not very efficient process, as less than one –third of fertilized human embryos have a chance of surviving upto a term delivery (Ruder et al., 2008). The reproductive process in males and females is regulated by myriad factors-nutritional and non-nutritional, with ROS having an important role. Excess generation of ROS and oxidative stress can adversely affect normal reproductive processes and supplementation with antioxidants may improve menstrual cycle regularity, prevent ovulatory disorders as well as enhance fertility (Ruder et al., 2009).

4.1 Sperm and fertility

Spermatogenesis is a very active process and about 1000 sperm are generated per second (Aitken and Roman, 2008). Corresponding to this high rate of cell division, the germinal epithelium mitochondria utilize oxygen. However, poor vascularization of the testes and the associated low oxygen tension may provide protection to spermatogenesis and Leydig cell steroidogenesis which are susceptible to ROS. Iron-induced lipid peroxidation, protein carbonyl expression and depletion of lipid - soluble antioxidants in testicular tissue results in disruption of spermatogenesis.

Human spermatozoa produce ROS which enhance tyrosine phosphorylation through the suppression of tyrosine phosphorylase activity by H_2O_2 and stimulation of cAMP production, that play a role in regulating signal transduction pathways that control sperm capacitation. A low, steady state of ROS production appears to be important for gamete cell stability (Hsieh et al., 2006) and capacitation (Baker and Aitken, 2005). Seminal fluid is an important source of antioxidants (the enzymes superoxide dismutase, catalase, glutathione peroxidase and vitamin C, vitamin E, hypotaurine, taurine, L-carnitine and lycopene) which are important for protecting spermatozoa from oxidative injury, especially because sperm do not possess much cytoplasmic fluid which generally contains the antioxidant enzymes. Hence the antioxidant capacity of sperm is relatively poor (Zini et al., 2009). In the event of excessive ROS production, a state of oxidative stress ensues. Since spermatozoa contain relatively high amounts of unsaturated fatty acids vis-a-vis the low antioxidant capacity, they become susceptible to oxidative stress. Persistent infertility has been linked to lipid peroxidation of unsaturated fatty acids in the sperm membrane (Hsieh et al., 2006). ROS have been associated with impaired metabolism, morphology, motility and male infertility (Baker and Aitken, 2005; Hsieh et al., 2006), retention of excess residual amorphous cytoplasm that human spermatozoa are not capable of remodeling or discharging. Also, oxidative stress results in DNA damage i.e. strand breaks, formation of DNA base adducts such as 8-hydroxyl-2′-deoxyguanosine O 6-methylguanine that have been reported to inhibit methylation of adjacent cytosine residues which in turn results in DNA hypomethylation (Tunc et al., 2009). This adversely affects the sperm's ability to fertilize.

ROS may be produced by sperm that are immobile, morphologically abnormal, or morphologically normal but functionally abnormal. Hsieh et al.,(2006) reported that sperm motility or concentration were negatively correlated with malondialdehyde concentrations, an indicator of lipid peroxidation. Zribi et al., (2011) recently reported a positive correlation between oxidation and DNA fragmentation in a study wherein 21 nonasthenozoospermic (sperm motility ≥ 50%) were compared with 34 asthenozoospermic (sperm motility < 50%). DNA fragmentation was found to be negatively correlated with several parameters of sperm motility and vitality.

Administration of high dose of iron to male mice was reported to induce a dominant lethal effect of male mice characterized by high embryonic loss in females that were mated with male mice exposed to iron. The authors postulated that this embryonic loss was due to non-viable embryos. Lucesoli et al., (1999) exposed male rats to varying doses of iron dextran (250 to 1000 mg per kilogram of body weight) administered intraperitoneally. Iron was found to accumulate in the sperm and other testes cells. In the group administered the highest dose, spermatogenesis was markedly lower and concentrations of antioxidants (alpha-tocopherol, ubiquinol-9, and ubiquinol-10) in the testes were inversely correlated with oxidation. Oxidative protein products viz protein thiols and protein-associated carbonyls were higher in rats exposed to the high iron dose (Doreswamy and Muralidhara, 2005). Supplementation with alpha–tocopherol partially mitigated the oxidative damage caused by chronic iron intoxication.

Wise and coworkers (2003) observed in boars that concentrations of iron and ferritin were higher in small testes, with a decrease as testes weight increased. With increasing testicular iron concentrations, sperm production declined. These authors cited reports in the literature that human males with β-thalassaemia major have decreased pituitary function from Fe

overload, and serum ferritin is highly correlated with the presence of hypogonadism. Doreswamy and Muralidhara (2005) administered acute sub-lethal doses of iron dextran to adult mice (50, 100 and 200 mg per kilogram of body weight). Multiple doses induced increase in lipid peroxidation in mitochondrial and microsomal fractions. There was significantly higher DNA damage in testes indicated by increased single strand breaks. Although sperm count remained unaffected, there was a 3- to 7-fold increase in abnormal sperm. The authors attributed the genotoxic consequences to early oxidative damage in testis due to iron overload. Thus, iron overload leads to oxidative stress that damage sperm DNA, making the embryos non-viable(Aitken and Roman, 2008). Therefore, several investigators have examined the effect of antioxidant supplements. Some investigators have observed that short term supplementation with antioxidants to men with high levels of sperm damage, resulted in better fertility potential and improved pregnancy rates (Zini et al., 2009). Tunc et al., (2009) compared 45 men with known male factor infertility with 12 fertile controls who were sperm donors. They were given a capsule containing 500 µg of folate 100 mg of Vitamin C, Vitamin E - 400 IU, Lycopene - 6 mg, zinc -25 mg, selenium - 26 µg, and garlic oil -33 µg for three months in order to cover one full cycle of spermatogenesis. The authors reported that the supplement significantly improved sperm DNA integrity and methylation. Recently, 690 infertile men with primary or secondary male factor infertility for at least one year, were given a daily supplement of 200 µg with vitamin E(400 units) for more than 3 months by Moslemi et al.,(2011). The investigators observed that in about half the subjects, there were improvements in sperm motility, and/ or morphology. In one-tenth of the cases, spontaneous pregnancy occurred compared to none in the control group.

As in case of males, ROS play a role in the entire lifecycle of females, affecting various physiological functions from oocyte maturation, modulation of ovarian germ cell, stromal cell physiology, transcription factors, ovarian steroidogenesis, corpus luteal function and luteolysis, implantation, formation of blastocyst, pregnancy, parturition to perhaps even menopause. Iron nutriture during pregnancy is of concern since iron requirements during pregnancy are significantly higher than in the non- pregnant state and iron deficiency during pregnancy has been linked to maternal morbidity and mortality, low birth weight and infant mortality (Ramakrishna, 2002). Aune et al., (2004) studied 19 healthy pregnant women given 26 mg of iron (as ferrous aspartate) per day. After 4 weeks, total antioxidant activity and conjugated diene levels (indicative of lipid peroxidation) were raised. Casanueva and Viteri(2003) reported that supplementation of pregnant women with 100 mg iron and 500 mg vitamin C in the third trimester of pregnancy was associated with higher levels of serum iron and thiobarbituric acid-reactive substances than in the control group. Vyas (2005) observed an increase in lipid peroxide levels with an increase in serum iron of 85 pregnant Indian women from lower socio-economic strata. Women who delivered at term tended to have slightly higher levels of lipid peroxides than those who delivered preterm. However, there are few studies in the literature on iron deficiency and oxidative stress, *per se* particularly related to pregnancy. Many investigators have reported on iron proteins and their roles in oxidative stress, which is reviewed in this section.

4.2 ROS and pregnancy

Oxidant status may influence early embryonic development. Pregnancy is characterized by increased basal oxygen consumption and susceptibility to oxidative stress, although little data is available on oxidative stress in the preconception and early conception period. Fetal

development and survival depend on the placenta as it plays a pivotal role by anchoring the conceptus, providing an interface between the mother and fetus, transfers nutrients, is important for exchange of gases, synthesis of a number of hormones and provides an immune barrier. Any problems associated with placental growth and differentiation ultimately affect its function and therefore fetal growth and development. The placenta itself produces ROS since it is rich in mitochondria, is hemomonochorial and allows electrons to pass that are converted into O2•- radicals (Casanueva and Viteri, 2003; Fuji, et al., 2005; Agarwal et al., 2005). The half lives of these ROS differ which influences their diffusion across the placenta. Superoxide is unable to diffuse across beyond the cell of its origin/synthesis because of short half life whereas the nitric oxide has a higher diffusion distance and can be a paracrine mediator in adjacent cells and be potentially more damaging (Myatt, 2010).

Proliferation and differentiation of trophoblasts and invasion by them is essential for placentation. It is important that trophoblasts colonize the endometrium and sequester the blood supply that is essential for the developing placenta. There is also considerable vascular remodeling in order to provide for the requirements of the developing fetus. In the early stages of pregnancy, a low oxygen milieu prevails. This placental hypoxia in the first trimester is physiological, regulates both morphogenesis and functioning of the placenta and is important for normal embryonic development. Maternal blood flow to the intervillous spaces commences only around 11 weeks of gestation (Pringle et al, 2010). This increase in oxygen concentration is vital for active placental transport and synthesis of proteins required by the rapidly developing fetus in the second and third trimesters (Burton, 2009). Jauniaux and coworkers (2000) studied 30 women at 7-16 weeks of gestation and observed a rapid increase in oxygen tension within the placenta at the end of the first trimester. Maternal blood flow starts at the periphery of the placenta and then gradually extends towards the centre, accompanied by an increase in the oxygen tension. This is associated with increased oxidative stress in the placenta and especially the synctiotrophoblast. Physiologically this is necessary for trophoblast differentiation. A deviation from this normal phenomenon such as early onset of blood flow would result in premature rise in oxidative stress since the placenta at early stages of pregnancy (8-10 weeks) does not have adequate antioxidant enzymes.

Further, the low levels of oxygen in the uterus are advantageous in terms of having relatively lower metabolic rates and thereby minimizing the production of ROS. However, impairment of these processes or hypoxia at later stages in pregnancy is pathological. Insufficient trophoblast invasion, defective vascular remodeling in the first trimester have been found to increase blood flow, damage villous architecture, increase the risk of spontaneous vasoconstriction and ischemia - reperfusion which can result in oxidative stress (Burton et al., 2009). Higher intensity of oxidative stress can have adverse effects on the developing embryo, and can lead to embryo fragmentation (Jauniaux et al., 2000). In studies on *in vitro* fertilization, fluid from follicles whose oocytes did not fertilize contained higher activity of superoxide dismutase than those that fertilized. Also, higher levels of 8-hydroxy-2-deoxyguanosine, an indicator of DNA damage caused by oxidative stress, have been observed in granulosa cells of patients with endometriosis and were associated with lower fertilization rates and poor embryo quality (Agarwal et al., 2005).

Most studies reported in the literature relate oxidative stress to pregnancy complications and some to the outcome of pregnancy. The focus in most investigations has been on the role of oxidative stress in preeclampsia.

4.2.1 Pregnancy complications – Preeclampsia

During early gestation, the syncytiotrophoblast has low concentrations of antioxidant enzymes compared to other villous tissues, making it susceptible to oxidative stress (Jauniaux et al., 2000). Hence, disturbances in oxygen tension and ROS can result in pregnancy complications. Preeclampsia, miscarriages, preterm labour, birth defects, endometriosis and tubal factor infertility, intrauterine growth retardation and stillbirth have been linked to insufficient trophoblast invasion, development, vascular remodeling and impaired placental development and function (Jauniaux et al., 2003, Szczepanska et al., 2003, Pringle et al, 2010) In the event of placental hypoxia, due to defective placentation, ischemic-reperfusion injury occurs and the ensuing oxidative stress stimulates the release of cytokines and prostaglandins. This has been implicated in endothelial cell dysfunction and possible development of preeclampsia (Agarwal et al., 2005). In patients with preeclampsia, antioxidant nutrient levels as well as antioxidant response was lower while lipid peroxidation was higher (Takagi et al, 2004; Aydin et al, 2004 and Mikhail et al, 1994). Also, free 9-isoprostane levels that are produced by non-enzymatic random oxidation of tissue lipids by ROS were found to be significantly higher in women with preeclampsia as compared to healthy pregnant women (Zhang et al., 2008).

Placentas obtained from women with preeclampsia had higher lipid peroxides and xanthine oxidase activity as well as nitrotyrosine residues. Burton (2003, 2009) proposed that complications such as preeclampsia and intrauterine growth restriction may be related to fluctuations in oxygenation and not hypoxia alone due to inadequate trophoblastic invasion which in turn result in spontaneous constriction of the spiral arteries. Cindrova-Davies et al., (2007a) observed in an *in vitro* study that placenta explants exposed to hypoxia and reoxygenation, exhibited increased oxidative stress while addition of vitamin E and C mitigated it.

Several events during pregnancy from placentation, maintenance of uterine quiescence, regulation of hemodynamic control within the uterus and placenta, protection against preeclampsia, regulation of apoptosis and inflammatory cascades in trophoblast cells among others are influenced by HO and its degradative products viz biliverdin, carbon monoxide and ferrous ions. HO-1 has antioxidative, anti-inflammatory and anti-apoptotic effects. Centlow et al., (2008) observed that placental tissue from preeclamptic women exhibited increased expression of Hbα and Hbγ mRNA than placentas from normal pregnant women. Increased number of cells expressed Hb in the preeclamptic patients' placentas suggesting that either the cells were migrating into or out of the vessels or there may be binding sites on the vessel walls. Also, the placental vessels in preeclamptic patients had high numbers of Hb+ nucleated cells, which were probably fetal erythroblasts migrating in response to the poor perfusion and low oxygen levels in the pre eclamptic placenta. The authors postulated that if the turnover of Hb producing cells is high, these cells could well release heme into the placental blood vessels and extra villous space as evidenced by their observations of high Hb levels in the blood vessel lumen of pre eclamptic placentas. In contrast, this phenomenon did not occur in the normal control placentas.

Free heme generates ROS and thus can oxidize lipids including low density lipoproteins into cytotoxic peroxides that will damage the endothelium, oxidize membrane proteins resulting in increased membrane permeability and lysis of cells. Normally, HO degrades free heme but in preeclampsia, it has been observed that there is decreased expression of

this enzyme and the activity is also less. This may result in accumulation of heme in the placenta. To further exacerbate the situation, carbon monoxide production from heme by HO may be less, thus contributing to reduced placental blood flow, inducing inflammatory response, activation of nuclear factor (NF)-κB transcription factors by the heme-generated ROS. This in turn may stimulate production of adhesion molecules and cytokines, recruitment of leukocytes, migration of neutrophils as well as their activation and induce further increasing risk of damage. Heme can also induce secretion of tumor necrosis factor-α and activate toll like receptor 4 leading to an immune response.

Gandley and coworkers(2008) reported that levels of placental myeloperoxidase, a hemoprotein produced and released by activated monocytes and neutrophils, were elevated in 27 placentas obtained from women with preeclampsia compared to normal gestationally - matched placentas. Myeloperoxidase may be involved in the generation of potent ROS and has been associated with mediation of oxidation of lipoproteins, catalyzing nitration of tyrosine residues, depleting endothelially derived nitric oxide, vascular inflammatory diseases and utilization of antioxidants.

4.2.2 Pregnancy outcome

Zhaoa et al., (2009) observed that early embryonic death in mice occurred when the enzyme HO-1 was deficient, indicating a possible role in early placentation or embryonic development. The authors suggested that deficiency of this enzyme may also affect complement activity and play a role in fetal resorption. Deficiency of this enzyme was associated with rise in diastolic blood pressure, and less dilated spiral arteries in the placenta.

Exposure to ROS during organogenesis can increase risk of birth defects. Burton (2009) reported that in animals with genetically impaired antioxidant enzyme activity and in diabetic rats in whom ROS levels were increased, the offspring had congenital abnormalities (Hagay et al. 1995; Eriksson, 1999; Nicol et al. 2000). Slonima and coworkers (2009) compared differences in gene expression between euploid fetuses and second trimester Down's syndrome fetuses, in uncultured amniotic fluid supernatant samples and suggested that oxidative stress may have a significant role in Down's syndrome. Prater and colleagues (2008) used methylnitrosourea to induce oxidative stress in C57BL/6 mice. They observed that several important placental proteins such as Hgf, Kitl, IFNα4, Ifrd, and interleukin (IL)-1β which are important for development of the placenta and fetal skeleton were altered. Also there was damage to placental endothelial cells and trophoblasts. In the group given methylnitrosourea with quercetin, an antioxidant, the levels of these proteins were normalized. Also, in the group treated with methylnitrosourea, the pups had disproportionately short limbs as well as distal limb malformations which were not observed in the quercetin supplemented group. The authors suggested that oxidative stress could alter normal placental osteogenic signaling and skeletal formation in the fetus.

Guller (2009) reported that in preeclamptic condition, there may be reduced nutrient supply to the fetus, resulting in intrauterine growth retardation. Levels of malondialdehyde were higher in small for gestational age newborns compared to adequate for gestational age controls. Markers of oxidative stress were significantly correlated with maternal Hb. Further, SGA newborns had higher number of "unprotected' erythrocytes i.e. content of

antioxidant protectants were lower. This would make the erythrocytes more susceptible to lysis which would in turn release heme and increase risk of ROS generation

During pregnancy, iron supplements are routinely recommended, but there is little direct evidence to determine whether iron supplementation would contribute to oxidative stress in either direction i.e. mitigate the stress by reducing anemia which leads to oxidative stress or produce iron overload which may increase ROS generation (Casaneuva and Viteri, 2003).

5. Iron and central nervous system

Neurons are long-lived postmitotic cells which are rarely replaced through division and differentiation of stem cells. This permits biological waste materials to accumulate in these cells with ageing, possibly because lysosomal degradation is not adequate or unsuccessful, resulting in functional decay and ultimately cell death. Among the waste materials that can be accumulated are lipofuscin, irreversibly damaged mitochondria and aberrant or abnormal proteins with consequent selective loss of neurons which may be age-dependent. Abnormal proteins that accumulate are Ab-amyloid neuritic plaques and neurofibrillary tangles in Alzheimer's disease (AD), α-synuclein, and Lewy bodies in Parkinson's disease (PD) (Gaeta and Hilder, 2005). Ageing of such long lived post mitotic cells has been attributed ROS formed mostly within the mitochondria. Due to its high content of poly unsaturated fatty acids, the brain is very sensitive to attack by ROS. Lipid peroxidation and its aldedydic products, 4-hydroxynonenal (4 HNE) and acrolein have been implicated in neurological ailments. Due to oxidative stress, there may be cytoskeletal damage, mitochondrial dysfunction and altered signal transduction. Neurons stressed and injured by free radicals are rendered non-functional, function and transport of mitochondrial to synaptic regions is impaired and synaptic function decreases resulting in neurodegeneration. The superoxide radical is the most abundant ROS and has been reported to play a role in brain edema and hippocampal neuronal death (Ansari et al, 2008). Diseased neurons can remain viable for 10 years or longer and as such must have sufficient protective mechanisms to maintain normal homeostasis. However, at some point in a neurodegenerative disorder, the oxidative insults may overwhelm cellular antioxidant defense systems leading to cellular dysfunction and death (Siedlak, 2009). In fact oxidative stress is regarded as one of the earliest changes in the pathogenesis of AD that may be present several years before the disease overtly manifests itself (Smith et al., 2010).

5.1 Iron, ROS and neurodegeneration

The brain is vulnerable to oxidative stress because, not only does it have a high content of polyunsaturated fatty acids (George et al., 2009) but also contains substantial amounts of iron (Gaeta and Hilder, 2005). The blood brain barrier serves to limit entry of iron into the brain, thus protecting it from overload (Wang and Michaelis, 2010). Within the brain, there are mechanisms to regulate the cellular iron level ensuring adequate supply to allow normal function of the cells and nervous system while protecting them from toxicity. Iron deficiency affects cell division of neuronal precursor cells, astrocytes, and oligodendrocytes. However, when the amount of iron exceeds what can be safely sequestered by ferritin, heme proteins and iron-sulfur clusters; oxidative stress results especially in regions containing higher amounts of iron – globus pallidus, substantia nigra, red nucleus, putamen, caudate nucleus. It has been suggested that excess iron or "iron invasion" may be the primary event and

cause degeneration of axons as well as neuronal cell death. Evidence from studies on inherited disorders of iron metabolism indicates that neurodegeneration occurs with iron accumulation (Mills et al., 2010).

Olivares et al., (2009) reported that iron accumulation is probably a primary event and not a consequence of degenerative diseases. In a rat model, when $FeCl_3$ was injected into the *substantia nigra,* there was selective decrease in striatal dopamine suggesting that iron is responsible for dopaminergic neurodegeneration. The phenomenon was prevented by infusion of desferrioxiamine, an iron chelator. Mutations in the gene that codes for a ferritin subunit result in a disease similar to PD. Catecholamine neurons are selectively degenerated by 6-hydroxydopamine which is oxidized to cytotoxic chatecholaminergic semiquinones and quinines along with hydrogen peroxide and hydroxyl radicals. Iron deficient rats were resistant to toxicity induced by 6-hydroxydopamine. Further, its toxicity was reversed by an iron chelator, desferal. Similarly in iron models of stroke, use of iron chelating agents could reduce the infarct size and brain injury as well as improve neurologic outcome (Stankewicz et al, 2007). In mice, deletion of the gene encoding for IRP2 which interferes with regulation of iron metabolism has been found to result in abnormal iron deposition in the brain, as well as ataxia, tremors and neurodegeneration. Similarly mutation in the gene coding for a ferritin subunit and polymorphisms in genes related to iron homeostasis have been associated with iron deposits, neuronal degradation and sometimes PD (Olivares et al, 2009).

Another possible mechanism is the ready binding of iron to advanced glycation end-products that accumulate with age in the endothelial internal elastic lamina or basement membrane. Iron and copper can scavenge nitric oxide and thus prevent its action on the smooth muscle resulting in prolonged vasoconstriction (Eaton and Qian, 2002). If this occurs in the blood vessels of the brain, it could contribute to neurodegeneration and may therefore contribute to the efficacy of the iron chelator desferroxiamine in AD (Stankiewicz et al., 2007).

Lysosomal (autophagic) degradation is rapid and effective but is not completely successful. Even under normal conditions, some iron-catalyzed peroxidation occurs intralysosomally (lysosomes are rich in redox-active iron), resulting in oxidative modification of autophagocytosed material and yielding lipofuscin, a polymeric compound slowly accumulating within ageing postmitotic cells at a rate that is inversely correlated to the longevity across species (Kurz et al, 2008). When iron-rich, non-degradable substances like lipofuscin and hemosiderin (which is not degradable and composed of polymerized ferritin residues) accumulate in some lysosomes, there is an increase in iron content. This increases the lysosomes' sensitivity to ROS.

The central nervous system contains considerable amounts of iron although the regional distribution of the metal differs, thus conferring varying sensitivity to oxidative stress (Zecca et al., 2004; Hall et al., 2010). Normally, the cytosolic iron is regulated and maintained at a low level adequate to ensure synthesis of essential iron containing molecules and yet to prevent free or of redox-active iron at low levels to prevent damage. Although both ferritin and Tf have high affinity for iron at neutral pH, the metal dissociates easily from the two proteins at pH below 6.0, which may occur in injured areas.

Whenever there is a requirement for iron, it is taken up by the cell from its surroundings. However, in non-dividing cells, iron that is available is determined primarily by turnover

and reutilization. Cells are unable to get rid of the intralysosomal iron bound to lipofuscin. Thus, over a prolonged period, iron accumulates despite regulation of iron uptake, which is especially seen in long-lived postmitotic cells like neurons. Histologic and magnetic resonance imaging studies indicate that there is increased iron level in the gray matter in PD and AD as well as other neurological disorders. Abnormal iron deposits have been observed in neurons and oligodendrocytes of patients with multiple sclerosis, with increased in the ferric iron content in the caudate and putamen. Magnetic resonance imaging studies on humans indicate a possible relationship between damage to gray matter and iron deposition in patients with multiple sclerosis (Stankewicz et al., 2007). Thus, as these cells age and especially as the end of their lifespan approaches, they become sensitive to oxidative stress. When there is enhanced autophagy, such as when there is repair following injury coexisting with oxidative stress, there is more rapid formation of lipofuscin.

Hemoglobin is another source of catalytically active iron, a situation that may be encountered in hemorrhage. The iron released from the Hb is likely responsible for the Hb-mediated oxidative stress. In conditions of hypoxia or ischemia, due to brain injury for example in children with anoxia or in animal models of stroke, iron deposition has been reported in basal ganglia, thalamus, periventricular and subcortical white matter. Insufficient oxygen levels increase lactic acid locally which reduces the ability of Tf to bind iron and simultaneously result in release of the iron bound to ferritin (Stankewicz et al, 2007).

In addition to directly affecting the brain adversely, oxidative stress may cause damage through overexpression of inducible nitric oxide synthase (iNOS) that in turn would augment the production of nitric oxide. Nitric oxide reacts with superoxide anion and produces the highly reactive peroxynitrite, resulting in oxidation and aggregation of proteins. Consequently there can be conformational changes in the proteins exposing hydrophobic residues which may result in loss of structural or functional activity, accumulation of oxidized proteins in the cytoplasm as aggregates e.g tau and amyloid-β aggregates as seen in AD. Mitochondrial dysfunction and damage from intracellularly produced ROS may lead to age-related neurodegenerative disease. In mice, mitochondrial deficiency of superoxide dismutase was associated with hyperphosphorylation of tau and exacerbation of amyloid burden (Melov et al., 2007).

Increased intensity of oxidative stress results in peroxidation of the lysosomal contents and the membrane, rendering the lysosome leaky. This phenomenon may occur even after only brief exposure to oxidative stress. Lysosomes contain a fairly high amount of redox active iron originating from degraded iron-containing proteins. The low pH in the lysosomes favours iron catalysed oxidative reactions. Kurz et al., (2004) stated that ferritin needs to undergo autophagocytosis and degradation before the iron the released and incorporated into ferritin for storage or in other iron-containing proteins. Each molecule of ferritin can store about 4500 atoms of iron. As a result, even if there is a relatively small number of autophagocytosed ferritin molecules, there can be substantial reduction of lysosomal iron. Autophagy occurs continually, with the degraded lysosomal ferritin being replaced (Kurz et al., 2008). Kurz et al., (2004) stated that when the lysosome ruptures, it releases hydrolytic enzymes, The rupture also induces coordinated aopoptotic cell death. Besides release of lytic enzymes, iron is also released into the cytosol. This iron may reach the nucleus where hydroxyl radicals may be generated if the oxidative stress continues.

It is not only excess iron and consequent oxidative stress that can induce lysosomal damage. Iron depletion has been found to damage lysosomes although it has been suggested that the mechanism may be a consequence of other apopotogenic factors (Kurz et al., 2008). Iron has been implicated in dysfunction of the central nervous system. Neurodegenerative diseases such as PD, AD, amyotrophic lateral sclerosis, Huntington's disease, Friederich's ataxia and aceruloplasminemia have been linked to iron (Achcar et al., 2011) Sites of neuronal death in the brain have been observed to be sites where iron accumulates. In cases with multiple sclerosis, redox active iron but not total iron content of cerebrospinal fluid was significantly higher.

Besides this, monoamine oxidase (MAO) has also been linked to oxidative stress (Zecca et al., 2004; Mandel et al., 2005). Activity of MAO in both humans and animals is influenced by iron levels (Symes et al., 1969; Youdim et al., 1975). MAO, a flavo-protein located on the outer mitochondrial membrane, can exist in two forms A and B-the B form activity is greater in basal ganglia. In rodents, MAO-A is present in the extraneuronal compartment and within the dopaminergic terminals, it plays a role in the metabolism of intraneuronal and released dopamine. In the process of its role in oxidative deamination of primary, secondary and tertiary amines to the corresponding aldehyde and free amine, H_2O_2 is generated (Moussa, Youdhim and Bakhle, 2006). H_2O_2 is inactivated in the brain by glutathione peroxidase. However, if brain glutathione oxidase levels are low, H_2O_2 can accumulate and participate in the Fenton reaction whereby iron as the ferrous ion, generates hydroxyl radicals that are highly active free radicals.

Both monoamine oxidase activity and brain iron concentration increase with age, thus increasing the potential for generation of hydroxyl radicals and oxidative stress with age. Mandel et al., (2005) reported increased activity of MAO in patients with PD and AD. In PD, samples of substantia nigra from PD patients were found to be deficient in aldehyde dehydrogenase . This could lead to accumulation of neural and toxic aldehydes derived from dopamine by MAO (Moussa, Youdhim and Bakhle, 2006. Ansari et al (2008) reported that oxidative stress results in oxidation of synaptic proteins and has adverse effects on synaptic plasticity, connection. Impaired mitochondrial transport to synapses contributes to neuronal degeneration and death. Loss of synapsin-I and PSD-95 which influence neuronal function and survival was caused by oxidative stress.

5.2 Parkinson's disease

PD is a progressive neurodegenerative disorder. There is degeneration of nigrostratial dopaminergic neurons in the substantia nigra, resulting in depletion of dopamine. Consequently there is loss of motor functions, formation of intracytoplasmic inclusions (Lewy bodies) and Lewy neuritic inclusions (LNs) in the surviving dopaminergic neurons. Dopamine is oxidatively deamined to its aldehyde-3,4-dihydroxyphenylacetaldehyde by MAO and then to 3,4-dihydroxyphenylacetic acid by aldehyde dehydrogenase. 4-HNE and malondialdehyde, products of lipid peroxidation are potent inhibitors of mitochondrial dehydrogenase but do not inhibit MAO. Even low levels of oxidative stress yield the aldehyde. The aldehyde has been shown to be toxic towards dopaminergic cells via oxidative stress and other mechanisms. Thus, cells located close to dopamine neurons such as astrocytes and microglia may well be exposed to high concentrations of the aldehyde during or following

oxidative stress. The aldehyde may target and modify proteins important for dopamine neuron homeostasis including α-synuclein (α-syn) (Jinsmaa et al, 2009).

LNs are derived from fibrillar aggregates of α-syn belonging to the synuclein family. α-syn is largely expressed at presynaptic terminals specially in some parts of the brain like neocortex, hippocampus, striatum, thalamus and cerebellum. The central hydrophobic peptide in α-syn has been implicated in Alzheimer's disease and is an important component of amyloid plaques. Oligomers of α- syn through crosslinking of di-tyrosine may occur through nitration, an oxidative mechanism. Nitrated α-syn is not processed well by proteases and has been detected in brains of persons with PD. If α-syn aggregates, there is decrease in the soluble α-syn and it ultimately permits large amounts of dopamine to enter the cell, consequently increasing the potential for generating ROS.

Further iron associated proteins and receptors have been reported to be upregulated in the striatum and substantia nigra of patients with PD. Regan et al. (2008) reported that ferritin levels in the central nervous system increased with normal aging. However, in patients with PD, there was little increase in the substantia nigra, although there was iron accumulation, suggesting that less sequestration into ferritin may make the brain vulnerable to toxic effects of iron and oxidative stress. These authors observed an inverse relationship between cell death in mixed cortical cultures and ferritin expression.

Neuromelanin is a pigment that is protective against oxidative stress because it can bind and concentrate transition metals like iron. In normal brain, only 50% of this pigment is saturated with iron, such that it can still chelate iron in the neurons if there is an onslaught of the mineral. However, in PD, when brain iron concentration is increased, the pigment may become saturated and iron would be bound to low affinity sites, so that some amount of the bound iron is redox-active and potentially toxic(Gaeta and Hider, 2005).

Brain tissues of patients with Parkinson's have been found to contain substantial amounts of iron in the substantia nigra (a 25% to 100% increase) compared to healthy age-matched controls. α-syn can interact with cations and this binding in turn catalyzes protein aggregation (Stankewicz et al., 2008). Metal-induced aggregation is attributable to the oxidation by redox metals. Increase in the redox active metal, like iron results in oxidation and therefore in the degeneration of dopaminergic neurons in the substantia nigra. Besides aggregation/oligomerization of α-syn, other effects of oxidation can be mitochondrial dysfunction, cytotoxicity and increase in cytosolic free Ca^{2+} resulting in cell death. Redox elements like iron also accelerate the enzymatic and non-enzymatic catabolism of dopamine. High iron concentrations in the substantia nigra can catalyze conversion of hydrogen peroxide during catabolism of dopamine with the production of highly reactive hydroxyl radicals.

Oxidative stress can further exacerbate the situation by removal of iron from ferritin, Hb and cytochrome c peroxides, iron sulfur protein by the action of ONOO-, which in turn will increase the iron levels. Iron also catalyses conversion of excess dopamine to neuromelanin. This is an insoluble pigment that has been found to accumulate in aged dopaminergi neurons. Neuromelanin sequesters redox ions that have affinity for ferric ions. If it is bound to an excess of ferric ions, neuromelanin functions like a prooxidant, reduces the ferric to ferrous and increases the amount of iron available to react with H_2O_2 (Olivares et al., 2009). Iron not only induces oxidative stress but damages proteins, membranes, and nucleic acids and eventually leads dopaminergic neuron death in the substantia nigra (Logroscino et al., 2008).

Powers et al (2009) studied dietary intakes of 266 men and 154 women who were newly diagnosed patients with idiopathic PD. They were compared with a control group consisting of 351 men and 209 women. In men, the highest level of iron intake was associated with increased risk of PD. A combination of low saturated fat intake with high iron was linked to moderate risk in both sexes, with the association being stronger for men. The authors suggested that cholesterol is a protective factor for PD, possibly through the relationship between cholesterol and coenzyme Q10 which is an antioxidant and may reduce oxidative stress generated by dopamine metabolism.

Brains of patients with PD have been observed to have increased amounts of nonheme iron compared to controls but ferritin and Tf content were low. Logroscino and coworkers (2008) stated that this may indicate that there is altered regulation of synthesis of proteins including ferritin that are involved in iron metabolism. The low ferritin levels in the tissues may reflect inadequate neuronal response to the excess amount of nonheme iron entering the brain. Alternatively in PD there may be disturbance in the mechanism for iron transport through the neuronal membrane. Lactoferrin activity was found to be significantly higher in patients'mesencephalon where loss of dopamine is more severe. Gorell et al., (1999) reported that in autosomal recessive juvenile Parkinsonism associated with mutations in the Parkin gene, iron levels were increased in the substantia nigra(Moussa, Youdhim and Bakhle, 2006).

5.3 Alzheimer's disease

In selected regions of the brain, there are deposits of the amyloid precursor protein, amyloid-β (Aβ) deposits in plaques and vessel walls and neuritic plaques (Liu et al., 2010). Amyloid precursor protein (APP) is the precursor of the neurotoxic Ab-amyloid. It contains an IRE in its mRNA which allows cellular levels of iron to control translation (Moussa, Youdhim and Bakhle, 2006). Gaeta and Hider (2005) reported that when the Aβ deposits accumulate sufficiently for oxidative stress to occur; it induces its own production, starting a vicious cycle. Also, there are structural lesions due to collapse of the neuronal cytoskeleton and accumulation of hyperphosphorylated and polyubiquinaed microtubule –associated proteins with formation of neurofibrillary tangles. Cerebral atrophy progressively becomes worse with loss of nerve fibres and cells and there is disconnection of the synaptic circuitry. There is activation of prodeath genes and signaling pathways;. Mitochondrial dysfunction occurs, energy metabolism is impaired and chronic oxidative stress is typical along with DNA damage (De la Monte and Wands 2008).

Aβ has multiple binding sites for iron and has greater affinity for Fe(II) than does transferrin. When Aβ binds iron, ferric becomes more soluble and thus the Fe(III) present in complexes now becomes available to cellular reductants. This can disrupt iron homeostasis and perhaps is why iron is present along with Aβ aggregates in senile plaques. Further, Aβ may accumulate iron from the LIP. The implications of this in generating oxidative stress need further study (Jiang et al., 2009). In animal models increased iron worsened the course of increased risk of developing the disease. Data from postmortem pathology studies indicate iron deposition in neurons, neurofibrillary tangles and plaques of patients with AD. *In vivo* and *in vitro* studies show that amyloid protein aggregation was more and amyloid-induced neuronal injury in human neuroblastoma cell line M1783 was worsened by iron (Stankewicz, 2007). Clinical trials with iron chelators showed favourable results in terms of decreased beta amyloid levels, improved cognition and living skills of patients lend support

to results of reduced toxicity in *in vitro* studies on neurons treated with iron chelator and then to beta amyloid (Stankewicz et al., 2007).

6. Iron and liver

Liver is the site of important metabolic pathways including detoxification of ammonia, alcohol and xenobiotics. It serves as a storage organ and acts as a filter by removing bacteria and debris through phagocytic action of Kupffer cells. Liver faces the onslaught of iron and is vulnerable to iron overload. Iron absorbed from food is first transported to the liver. Hepatocytes express TfR as well as its homolog, TfRII, both mediating the uptake of Tf-bound iron. Unlike TfRI, TfRII lacks IRE and is not regulated reciprocally in response to iron levels, thereby allowing iron uptake by hepatocytes. Further, there are reports that hepatocytes can import iron by involving routes not involving TfRs. NTBI is absorbed primarily by liver via SLC39A zinc transporter–ZIP14 (Takami and Sakaida, 2011). Import of iron by more than one route, can contribute to iron loading in hepatocytes, thereby increasing their potential for iron-induced oxidative stress. Iron or iron-induced oxidative stress have also been found to activate cell signaling cascades triggering apoptosis and necrosis pathway via NF-κB and AP-1 pathways respectively. NF-κB promotes the synthesis and release of cytotoxic, proinflammatory and fibrogenic factors such as tumor necrosis factor (TNFα), IL-6 and MIP-1 that alter Kuppfer cells and hepatocyte function, and trigger hepatic stellar cell activation. In hepatic stellar cells, AP-1 transcription factors are involved in the regulation of procollagen (I). In addition, AP-1 and NF-κB-dependent gene products modulate hepatocyte death induced by oxidative stress (Zuwała-Jagiełło et al., 2011).

Oxidative stress leads to formation of glycoxidation products, including advanced glycation end products (AGEs) — among them Nε-(carboxymethyl) lysine and advanced oxidation protein products (AOPPs). Both AGEs and AOPPs trigger the inflammatory response via interaction with receptors for advanced glycation end products (RAGE). RAGE is a signal transduction receptor that binds both AGEs and AOPPs and by causing activation of NF-κB. *In vitro* experiments have shown that AGEs enhance transcription of genes for pro-inflammatory cytokines such as IL-6 and TNF-α. They may increase C-reactive protein (CRP) production. CRP is primarily produced byhepatocytes, and its chief inductor is the pro-inflammatory cytokine IL6. Glycosylated and oxidized proteins indirectly up-regulate CRP expression in hepatocytes by stimulating monocytes to produce IL-6 (Harrison-Findik, 2007). Further iron catalyzes the formation of NO from peroxynitrite and can modulate gene expression in cells leading to altered cell functions (Zuwała-Jagiełło et al., 2011).

Lipid peroxidation products such as 4-HNE and malondialdehyde can react with DNA bases and the ε-NH2 group of lysine and histidine residues. Acetaldehyde which is a product of ethanol oxidation, increases the binding of MDA and its own binding to proteins in a synergistic manner, resulting in the generation of new hybrid adducts i.e. MDA-acetaldehyde adducts. These adducts may play a role in the development and progression of liver fibrosis as they have been shown to stimulate the secretion of several cytokines and chemokines by liver endothelial cells (Zuwała-Jagiełło et al., 2011).

Oxidative stress is implicated in a number of pathological disorders. It is of importance that oxidative stress and associated liver disorders can influence iron metabolism and thereby have tremendous implications for further generation of ROS and exacerbation of disease.

Hepatic iron overload is common in many liver diseases where iron is a risk factor in disease progression (Hou et al., 2009). Hemochromatosis is a well-defined syndrome characterized by normal iron-driven erythropoiesis and toxic accumulation of iron in parenchymal cells of vital organs that can be caused by mutations in any gene that limits iron entry into the blood.

6.1 Hepatitis C virus infection

Hepatitis C virus (HCV) infection affects nearly 2% of the human population and is a major cause of liver disease worldwide. In majority of persons who suffered HCV infection, chronic state can be established which is often accompanied by alterations in liver function culminating in cirrhosis or hepatocellular carcinoma in 20% of infected individuals (Price and Kowdley, 2009). Total iron stores were reported to be elevated in patients with chronic hepatitis C compared to those with chronic hepatitis B, although in both sets of patients, the degree of necroinflammation did not differ (Muller et al., 2009). HCV infection more than hepatitis B infection has been reported to be associated with iron deposition, increased hepatic iron concentration, iron overload, increased serum Tf saturation, and serum ferritin. Franchini et al.,(2008) reported that chronic HCV infection is the main cause of hyperferritinemia.

Elevation in iron indices was reported by Girelli et al., (2009) to be correlated with progression of liver disease. Iron is mostly deposited in Kupffer cells and portal macrophages and interferon therapy reduced hepatic iron. The authors stated that hepatic iron overload is a consequence of hepatocyte necrosis which releases ferritin from hepatocytes to be subsequently taken up by macrophages. Alternatively, these authors suggested that increased iron absorption may contribute to increased iron stores. Further, hepcidin levels are decreased which in turn increase the expression of FPN, thereby increasing iron transport in the duodenum, release by macrophages and accumulation of iron in the liver(Muller et al., 2009). Price and Kowdley (2009) reported that increased levels of TfRII on the hepatocyte membrane may increase iron uptake in chronic HCV infection.

Excess iron which causes ROS generation, leads to lipid peroxidation, protein and DNA damage. The excess iron and lipid peroxidation products, such as 4-HNE can activate hepatic stellate cells as well as cause their proliferation and increase the synthesis of smooth muscle actin and collagen, all of which can contribute to hepatic fibrogenesis (Tanaka et al., 2008). Increased 4-HNE levels upregulate procollagen and inhibits the expression of metalloproteinase – 1 gene. The latter plays an important role in degrading collagen. Thus, oxidative stress contributes to hepatic fibrosis (Gomez et al., 2010)

Results of in vitro studies indicate that iron deposition in hepatocytes enhances HCV replication thereby aggravating the viral infection. Further, hepatocytic DNA damage results in accumulation of 8-hydroxy-2′-deoxyguanosine, which has been implicated in hepatocellular carcinoma. Muller et al., (2009) suggested that reducing body iron by phlebotomy or by consumption of a low iron diet may help to retard disease progression and risk of hepatoceullar carcinoma (Tanaka et al., 2008).

6.2 Alcoholic liver disease (ALD)

Alcohol related liver disease contribute substantially to disability and mortality, globally. Chronic alcohol consumption results in fatty liver, alcoholic hepatitis and cirrhosis and

manifests itself in ALD (Phillipe et al., 2007). Ethanol oxidation increases the NADH/ NAD^+ ratio, which leads to increased fatty acid synthesis, inhibits β-oxidation and the tricarboxylic acid cycle and increases the accumulation of triacylglycerols. Chronic alcohol consumption inhibits AMP kinase and activates sterol regulatory element binding protein-1, which increase lipogenesis resulting in hepatic steatosis(Mantena et al., 2008). Harrison-Findik(2009) suggested that mitochondrial DNA damage and ribosomal defects may inhibit mitochondrial protein synthesis and compromise oxidative phosphorylation in chronic alcohol consumption. Mitochondrial dysfunction has been reported to possibly play an important role in development of non-alcoholic fatty liver disease (NAFLD) and non alcoholic steatohepatitis (NASH).

Hou et al., (2009) reported that ingestion of even mild to moderate amounts of alcohol can increase iron overload, with the iron being localized in Kupffer cells and hepatocytes. On the other hand, alcohol metabolism to form acetaldehyde generates ROS. Thus iron and alcohol together contribute to and aggravate liver disease (Harrison-Findik, 2007). Although the mechanisms underlying iron accumulation are not well understood, it has been reported that alcohol-induced oxidative stress in hepatocytes downregulates hepcidin expression in liver, by altering transcription factor C/EBPα activity, thus dysregulating iron homeostasis (Hou et al., 2009; Harrison-Findik, 2009). To make matters worse, hepcidin expression is not responsive to body iron levels in alcohol consumption (Hou et al., 2009). H_2O_2 increases the expression of TfRI leading to iron accumulation and thus may support ALD progression (Girelli et al., 2009).

6.3 Nonalcoholic fatty liver disease

NAFLD is an important cause of liver damage ranging from fatty liver to nonalcoholic steatohepatitis (NASH). Simple steatosis is usually considered benign, but the development of NASH is recognized as a precursor of more severe liver disease, and in some cases, cirrhosis and hepatocellular carcinoma (Machado et al., 2009). Machado et al., (2009) reported that pathogenesis of NASH can be explained by the "two-hit" hypothesis, (a) insulin resistance and the resultant steatosis being the "first hit". Hyperinsulinemia, increased peripheral lipolysis and reduced β- oxidation lead to fat accumulation (b) the "second hit"- increased ROS/RNS. Once steatosis sets in, the liver progresses to more severe pathologic states when it is exposed to the second hit or other environmental stressors. Iron can increase the potential magnitude of the "second hit". Genetic risk factors also influence the susceptibility and severity of fatty liver disease.

Further, Fujita et al.,(2009) reported that NASH patients had elevated levels of hepatic 8-oxodG which is is a DNA base-modified product generated by hydroxyl radicals. The level of this product was reduced by iron reduction therapy. These authors also reported that in steatosis, saturation of the β-oxidation pathway with excess free fatty acids leads to further generation of H_2O_2, and other ROS. Also, serum iron, transferring saturation and ferritin levels were higher in NASH patients, which could be attributed to iron overload.

The first hit i.e. insulin resistance has also been reported to be linked to iron overload, through mobilization of peripheral fat to the liver and development of hyperinsulinemia. Hyperinsulinemia and peripheral lipolysis promote hepatic uptake of fatty acids as well as fatty acid synthesis, resulting in accumulation of fat and fatty liver (Mantena et al., 2008). A

syndrome of "insulin resistance-associated iron overload" has proposed where in the presence of unexplained hepatic iron overload at least one component of the insulin resistance may be present. Insulin resistance also seemed to be closely linked to total body iron stores in the general population. Circulating prohepcidin was reported to be associated with parameters of glucose and iron metabolism, in subjects with altered glucose tolerance (Fujita et al., 2009). The researchers suggested that failure to increase synthesis of prohepcidin which can decrease iron absorption, could contribute to iron-induced disorders of glucose metabolism. Further support comes from reports that iron depletion can improve insulin sensitivity. ROS (induced by iron overload) can interfere with insulin signaling by inhibiting translocation of glucose transporter 4 to the plasma membrane, thus impairing insulin uptake. In insulin resistance, glycation of proteins including Tf occurs, which decreases its ability to bind ferrous iron, and increasing the free iron pool and amplifying oxidative stress (Fernandez-Real et al., 2009). Oxidative stress itself induces insulin resistance, thus creating a vicious cycle.

7. Pros and cons of high iron intakes

Normally in a well-nourished individual, the antioxidant defense mechanisms should protect against ROS injury, however in case of undernutrition, including micronutrient deficits, the antioxidant system would be compromised. In developing countries, anemic children, adolescents and women who receive iron supplements are also malnourished and chronically consume poor quality diets. During the life cycle, the individual is more vulnerable at certain life stages either because of physiological immaturity or senescence or due to alterations in physiology and metabolism as encountered in pregnancy. Thus infants, pregnant women and the elderly may be at higher risk, especially if their nutritional status is compromised by either deficiency or excess of any one nutrient.

Iron supplementation is widely adopted since iron deficiency anemia is a global health problem. However, in public health systems, the issue of iron overload and its possible adverse consequences need to be paid attention in terms of well-designed studies and may need to be monitored closely, if deleterious effects are observed. There are reports in the literature on the effect of iron on the gastrointestinal tract, risk of cancer etc but these are not taken into account in public health programs. In animal models, iron-induced increase in oxidative damage was associated with gastric inflammation, induction of gastric ulcers and increase in colorectal cancer (Seril et al., 2002; Naito et al., 1995). In patients with Crohn's disease treatment with 120 mg ferrous sulphate per day for 7 days increased clinical symptoms of disease activity (Erichsen et al., 2003). There is a dire need to examine the effects of iron deficiency, supplementation and their interaction on several parameters of oxidative stress, besides Hb which is routinely used as an indicator of iron deficiency anemia. In pregnancy, iron supplements are routinely recommended and there needs to be a consideration of the possible effects on ROS generation and pregnancy complications. Per se, the role of ROS in pregnancy in well-nourished and iron deficient women warrants attention. Fortification of foods with iron is adopted as an important public health measure to combat iron deficiency. When persons consume iron fortified foods, along with supplements, intakes could well be in excess of the recommended dietary allowances (RDA), which may pose risk for iron overload. Fisher and Naughton (2004) reported that daily intake of iron was 1874% of the Korean RDA for those who used supplements as

compared to 62% of the RDA for non-users. The potential for oxidative stress could be high, if vitamin C is co-ingested, especially through supplements and fortified foods. High concentration of iron taken as bolus dose along with reducing substances like vitamin C can have deleterious effects. Multivitamin/ multimineral complexes are most common supplements administered. Unlike minerals in food where they are part of bioorganic substances, minerals in supplements and in fortified foods are in inorganic forms. In this form, minerals are capable of catalyzing free radicals generation (Rabovsky et al., 2010). Hence there is a need to reformulate nutritional supplements to obtain the benefits while simultaneously minimizing the risks.

A number of diseases have been shown to be associated with the intracellular accumulation of iron especially within mitochondria and lysosomes making them more susceptible to oxidative stress. Even a modest increase in the iron concentration in the substantia nigra may increase oxidative stress and neurodegeneration (Logroscino et al., 2008). Logroscino et al., (2008) stated that brain iron is responsive to peripheral iron status, hence brain iron status can be modulated by the iron content of the diet, both restriction and supplementation. The iron that accumulates in the brain is mostly nonheme iron. Case control studies and a prospective study in the US on suggest that dietary iron intake may be associated with higher risk of Parkinson's disease. The prospective study on a cohort of 47,406 men and 76,947 women indicated that risk was greater for persons whose vitamin C intakes were lower. The risk for Parkinson's disease was higher among those whose diets contained large amounts of non-heme iron primarily derived from fortified grains and cereals. Men were found to have some risk with the use of supplemental iron. The investigators did not find an association with heme iron (Logroscino et al., 1998; Gorell et al., 1999; Powers et al., 2003).

Besides this, with the growth of nanotechnology industry metallic nanoparticles are being increasingly used for variety of purposes including food products and medicines. Nanoparticles are able to cross semipermeable membrane via transcellular pathway. Since these particles are small in size and have a large surface area they will be highly reactive which in turn will increase their ability to produce ROS. Pujalté et al., (2011) have reported that metallic nanoparticles, such as, zinc oxide and cadmium sulphite exerted cytotoxic effects on glomerular and tubular renal cells. These effects were correlated with metal size and metal solubility. In an *in vitro* study, Apopa et al., (2009) have demonstrated that iron-nanoparticles induced an increase in endothelial cell permeability through the production of ROS.

In summary, both iron deficiency and excess are risk factors for oxidative stress. The emphasis should be on well-balanced diets to meet the RDA of all essential nutrients as well as inclusion of foods rich in phytochemicals that provide protection against oxidative stress.

8. Acknowledgement

The authors are extremely grateful to Ms. Mitravinda Savanur and Ms. Neha Lohia for technical support.

9. References

Achcar, F.; Camadro, JM. & Mestivier, D. (2011) A Boolean Probabilistic Model of Metabolic Adaptation to xygen in Relation to Iron Homeostasis and Oxidative Stress. *BMC Systems Biology,* 2011, Vol.5, pp.51—69.

Agarwal, A.; Gupta, S. & Sharma, RK. (2005) Role of Oxidative Stress in Female Reproduction. *Reprod Biol Endocrinol,* Vol.3, pp.28-48.

Aitken, RJ. & Roman, SD. (2008) Antioxidant Systems and Oxidative Stress in the Testes. *Oxid Med Cell Longev,* Vol.1, No.1, (Oct-Dec) pp.15–24.

Ansari, MA.; Roberts, KN. & Scheff, SW. (2008) Oxidative Stress and Modification of Synaptic Proteins in Hippocampus After Traumatic Brain Injury. *Free Radic Biol Med*, Vol.45, No.4, pp.443–452.

Apopa, PL.; Qian, Y.; Shao, R.; Guo, NL.; Schwegler-Berry, D.; Pacurari, M.; Porter, D.; Shi, X.; Vallyathan, V.; Castranova V. & Flynn, DC. (2009) Iron oxide nanoparticles induce human microvascular endothelial cell permeability through reactive oxygen species production and microtubule remodeling. *Particle and Fibre Toxicology, Vol.*6, pp.1-14.

Aune, R.; Kersti, Z.; Klaar, U.; Karro, H, Tiiu K & Zillmer M (2004) Ferrous Iron Administration During Pregnancy and Adaptational Oxidative Stress (Pilot Study). *Medicina (Kaunas),* Vol.40, No.6, pp.547-552.

Aydin, S.; Benian, A.; Madazli, R.; Uludag, S.; Uzun, H. & Kaya, S. (2004) Plasma Malondialdehyde, Superoxide Dismutase, sE-Selectin, Fibronectin, Endothelin-1 and Nitric Oxide Levels in Women with Preeclampsia. *Eur J Obstet Gynecol Reprod Biol.* Vol.113, pp.21-25.

Baker, MA. & Aitken, RJ. (2005) Reactive oxygen species in spermatozoa: methods for monitoring and significance for the origins of genetic disease and infertility. *Reprod Biol Endocrinol,* Vol.3, pp.67-75.

Barbeito, AG.; Garringer, HJ.; Baraibar, MA.; Gao, X.; Arredondo, M.; Marco Núñez, T.; Smith, MA.; Ghetti, B. & Vidal, R. (2009) Abnormal Iron Metabolism and Oxidative Stress in Mice Expressing a Mutant Form of the Ferritin Light Polypeptide Gene. *J Neurochem,* Vol.109, No.4, pp.1067–1078.

Barbeito, AG.; Levade, T.; Delisle, MB.; Ghetti, B. & Vidal, R. (2010) Abnormal Iron Metabolism in Fibroblasts From A Patient with the Neurodegenerative Disease Hereditary Ferritinopathy. *Mol Neurodegeneration,* Vol.5, pp.50-59.

Brabin, BJ.; Premji, Z. & Verhoeff, F. (2001) An Analysis of Anemia and Child Mortality. *J Nutr,* Vol.131, pp.636S-648S.

Branco, MR.; Marinho, HS.; Cyrne L. & Antunes F. (2004) Decrease of H_2O_2 Plasma Membrane Permeability during Adaptation to H_2O_2 in *Sacchromyces cerevisiae. J Biol Chem*, Vol.279, No.8, pp.6501-6506.

Briley-Saebo, KC.; Cho, YS. & Tsimikas, S. (2011) Imaging of Oxidation-Specific Epitopes in Atherosclerosis and Macrophage-Rich Vulnerable Plaques. *Curr Cardiovasc Imaging Rep,* Vol.4, pp.4–16.

Burton, GJ. (2009) Oxygen, The Janus Gas; Its Effects on Human Placental Development and Function. *J Anat, Vol.*215, pp.27–35.

Burton, GJ. & Hung, TH. (2003) Hypoxia-Reoxygenation; A Potential Source of Placental Oxidative Stress in Normal Pregnancy and Preeclampsia. *Fetal Mat Med Rev, Vol.*14, pp.97–117.

Cairo, G. & Recalcati, S. (2007) Iron-Regulatory Proteins: Molecular Biology and Pathophysiological Implications. *Expert Rev Mol Med,* Vol.9, No.33, pp.1-13.

Callens, C.; Coulons, S.; Naudin, J.; Radford-Weiss, I. et al. (2007) Targeting Iron Homeostasis Induces Cellular Differentiation and Synergizes With Differentiating Agents in Acute Myeloid Leukemia. *J Exp Med,* Vol.207, No.4, pp.731-750.

Casanueva, E. & Viteri, FE. (2003) Iron and Oxidative Stress in Pregnancy. *J Nutr,* Vol.133, pp.1700S-1708S.

Centlow, M.; Carninci, P.; Nemeth, K.; Mezey, E.; Brownstein, M. & Hansson, SR. (2008) Placental Expression Profiling in Preeclampsia: Local Overproduction of Hemoglobin May Drive Pathological Changes. *Fertil Steril,* Vol.90, No.5, pp.1834–1843.

Cindrova-Davies, T.; Spasic-Boskovic, O.; Jauniaux, E.; Charnock-Jones, DS. & Burton, GJ. (2007a) Nuclear Factor-κB, p38, and Stress-Activated Protein Kinase Mitogen-Activated Protein Kinase Signaling Pathways Regulate Proinflammatory Cytokines and Apoptosis in Human Placental Explants in Response to Oxidative Stress Effects of Antioxidant Vitamins. *Am J Path*, Vol.170, No.5, pp.1511-1519.

Cindrova-Davies, T.; Yung, HW.; Johns, J.; Spasic-Boskovic, O.; Korolchuk, S.; Jauniaux, E.; Burton, GJ. & Charnock-Jones, DS. (2007b) Oxidative Stress, Gene Expression, and Protein Changes Induced in The Human Placenta During Labor. *Am J Path,* Vol.171, No.4, pp.1168-1179.

Clark, JF.; Loftspring, M.; Wurster, WL.; Beiler, S.; Beiler, C.; Wagner, KR. & Pyne-Geithman, GJ. (2008) Bilirubin Oxidation Products, Oxidative Stress, and Intracerebral Hemorrhage. *Acta Neurochir Suppl*, Vol.105, pp.7–12.

Crampton, N.; Kodiha, M.; Shrivastava, S.; Umar, R.; & Stochaj, U. (2009) Oxidative Stress Inhibits Nuclear Protein Export by Multiple Mechanisms That Target FG Nucleoporins and Crm1. *Mol Biol Cell*, Vol.20, pp.5106-5116.

Crist, BL.; Alekel, DL.; Ritland, LM.; Hanson, LN.; Genschel, V. & Reddy, MB. (2009) Association of Oxidative Stress, Iron and Centralized Fat Mass in Healthy Postmenopausal Women. *J Women's Hlth*, Vol.18, No.6, pp.795-801.

Cutler, RG.; Kelly, J.; Storie, K.; Pedersen, WA.; Tammara, A.; Hatanpaa, K.; Troncoso, J. & Mattson, MP. (2004) Involvement of Oxidative Stress-Induced Abnormalities in Ceramide and Cholesterol Metabolism in Brain Aging and Alzheimer's Disease. *Proc Natl Acad Sci*, Vol.101, No.7, pp.2070-2075.

D'Amelio, P.; Cristofaro, MA.; Tamone, C.; Morra, E.; Di Bella, S.; Isaia, G.; Grimaldi, A.; Gennero, L.; Gariboldi, A.; Ponzetto, A.; Pescarmona, GP. & Isaia, GC. (2008) Role of Iron Metabolism and Oxidative Damage in Postmenopausal Bone Loss. *Bone*, Vol.43, pp.1010–1015.

De la Monte, S. & Wands, JR. (2008) Alzheimer's Disease is Type 3 Diabetes—Evidence Reviewed. *J Diabetes Sci Technol*, Vol.2, No.6, pp.1106-1113.

de la Rosa, LC.; Moshage, H. & Nieto, N. (2008) Hepatocyte Oxidant Stress and Alcoholic Liver Disease. *Rev ESP Enferm Dig*, Vol.100, No.3, pp.156-163.

Deb, S.; Johnson, EE.; Robalinho-Teixeira, RL. & Wessling-Resnick, M. (2009) Modulation of Intracellular Iron Levels by Oxidative Stress Implicates A Novel Role For Iron in Signal Transduction. *Biometals*, Vol.22, No.5, pp.855–862.

Deck, KM.; Vasanthakumar, A.; Anderson, SA.; Goforth, JB.; Kennedy, MC.; Anatholine, WE. & Eisentein, RS. (2009) Evidence that Phosphorylation of Iron Regulatory Protein 1 at Serine 138 Destabilizes the [4fe-4s] Cluster in Cytosolic Aconitase by Enhancing 4fe-3fe Cycling. *J Biol Chem*, Vol.284, No.19, pp.12701–12709.

Doreswamy, K. & Muralidhara. (2005) Genotoxic Consequences Associated with Oxidative Damage in Testis of Mice Subjected to Iron Intoxication. *Toxicology, Vol.*206, No.1, pp.169-78.

Eaton, JW. & Qian, M. (2002) Molecular Bases of Cellular Iron Toxicity. *Free Radic Biol Med*, Vol.32, pp.833-840.

Erichsen, K.; Hausken, T.; Ulvik, RJ.; Svardal, RJ.; Berstad, A. & Berge, RK. (2003) Ferrous Fumarate Deteriorated Plasma Antioxidant Status in Patients with Crohn Disease. *Scand J Gastroenterol,Vol.*38, pp.543-548.

Fardy, CA. & Silverman, M. (1995) Antioxidants in Neonatal Lung Disease. *Arch Dis Child Fetal Neonatal*, Vol.73, No.2, pp.F112–F117.

Fernández-Real, JM.; Equitani, F.; Moreno, JM.; Manco, M.; Ortega, F. & Ricart, W. (2009) Study of circulating prohepcidin in association with insulin sensitivity and changing iron stores. *J Clin Endocrinol Metab*, Vol.94, No.3, pp.982- 988.

Fisher, AE. & Naughton, DP. (2004) Iron Supplements: The Quick Fix with Long Term Consequences. *Nutr J*, Vol.3, No.2, pp.1-5.

Franchini, M.; Targher, G.; Capra, F.; Montagnana, M. & Lippi, G. (2008) The Effect of Iron Depletion on Chronic Hepatitis C Virus Infection. *Hepatol Int*, Vol.2, No.3, pp.335–340.

Fujii, J.; Iuchi, Y.; Okada, F.; Watson, AL.; Skepper, JN.; Jauniaux, E. & Burton, GJ. (1998) Susceptibility of Human Placental Syncytiotrophoblastic Mitochondria to Oxygen-Mediated Damage in Relation to Gestational Age. *J Clin Endocrinol Metab*, Vol.83, pp.1697–1705.

Fujita, N.; Miyachi, H.; Tanaka, H.; Takeo, M.; Nakagawa, N.; Kobayashi, Y.; Iwasa, M.; Watanabe, S. & Takei, Y. (2009) Iron Overload is Associated with Hepatic Oxidative Damage to DNA in Nonalcoholic Steatohepatitis. *Cancer Epidemiol Biomarkers Prev*, Vol.18, No.2, pp.424-32.

Gaeta, A. & Hilder, RC. (2005) The Crucial Role of Metal Ions in Neurodegeneration: The Basis for a Promising Therapeutic Strategy. *Br J Pharmacol*, Vol.146, pp.1041–1059.

Gandley, RE.; Rohland, J.; Zhou, Y.; Shibata, E.; Harger, GF.; Rajakumar, A.; Kagan, VE.; Markovic, N. & Hubel, CA. (2008) Increased Myeloperoxidase in the Placenta and Circulation of Women with preeclampsia. *Hypertension*, Vol.52, No.2, pp.387–393.

Gao, J.; Ehen, J.; Kramer, N.; Tsukamoto, H.; Zhang, A. & Enns, C. (2009) Interaction of Hereditary Hemochromatosis Protein HFE with Transferrin Receptor 2 is Required for Transferrrin –Induced Hepcidin Expression. *Cell Metab*, Vol.9, pp.217-227.

Gao, X.; Hu, X.; Qian, L.; Yang, S.; Zhang, W.; Zhang, D.; Wu, X.; Fraser, A.; Wilson, B.; Flood, PM.; Block, M. & Hong, JS. (2008). Formyl-Methionyl-Leucyl-Phenylalanine–Induced Dopaminergic Neurotoxicity via Microglial Activation: A Mediator between Peripheral Infection And Neurodegeneration? *Environ Hlth Pers*, Vol.116, No.5, pp.593-598.

Gautier, CA.; Kitada, T. & Shen, J. (2008). Loss of PINK1 Causes Mitochondrial Functional Defects And Increased Senitivity to Oxidative Stress. *Proc Natl Acad Sci*, Vol.15 No.32, pp.11364-11369.

George, JL.; Mok, S.; Moses, D.; Wilkins, S.; Bush, AI.; Cherny, RA. & Finkelstein, DI. (2009). Targeting The Progression Of Parkinson's Disease. *Curr Neuropharmacol,* Vol.27, pp.9-36.

Girelli, D.; Pasino, M.; Goodnough, JB.; Nemeth, E.; Guido, M.; Castagna, A.; Busti, F.; Campostrini, N.; Martinelli, N.; Vantini, I.; Corrocher, R.; Ganz, & Fattovich, G. (2009). Reduced Serum Hepcidin Levels In Patients With Chronic Hepatitis C. *J Hepatol*, Vol.51, No.5, pp.845–852.

Goforth, JB.; Anderson, SA.; Nizzi, CP. & Eisenstein, RS. (2010). Multiple Determinants within Iron-Responsive Elements Dictate Iron Regulatory Protein Binding and Regulatory Hierarchy. *RNA*, Vol.16 pp. 154-169.

Gomez, EV.; Perez, YM.; Sanchez, H.; Forment, GR.; Soler, EA.; Bertot, LC.; Garcia, AY.; Vazquez, MRA.; Fabian, LG. (2010). Antioxidant and Immunomodulatory Effects of Viusid in Patients with Chronic Hepatitis C. *World J Gastroenterol*, Vol.16 No.21, pp.2638-2647.

Gorell, JM.; Johnson, CC.; Rybicki, BA.; Peterson, EL.; Kortsha, GX.; Borwn, GG. & Richardson, RJ. (1999). Occupational Exposure to Manganese, Copper, Lead, Iron, Mercury and Zinc and the Risk of Parkinson's Disease. *Neurotoxicology*, Vol.20, No.2-3, pp.239–247.

Guller, S. (2009). Role of The Syncytium in Placenta-Mediated Complications of Preeclampsia. *Thromb Res*, Vol.124, No.4, pp.389–392.

Gupta, P.; Narang, M.; Banerjee, BD. & Basu, M. (2004). Oxidative Stress In Term Small For Gestational Age Neonates Born To Undernourished Mothers: A Case Control Study. *BMC Pediatrics*, Vol.4, No14, doi:10.1186/1471-2431-4-14

Haas, JD. & Brownlie, T. (2001). Iron Deficiency and Reduced Work Capacity. A Critical Review of Research to Determine a Causal Relationship. *J Nutr*, Vol.131, pp.676S-688S.

Hagay, ZJ.; Weiss, Y.; Zusman, I.; Peled-Kamar, M.; Reece, EA.; Eriksson, UJ. & Groner, Y. (1995). Prevention of Diabetes Associated Embryopathy by Overexpression of the Free Radical Scavenger Copper Zinc Superoxide Dismutase in Transgenic Mouse Embryos. *Am J Obstet Gynecol*, Vol.173, pp.1036–1041.

Hall, ED.; Vaishnav, RA. & Mustafa, AG. (2010). Antioxidant Therapies for Traumatic Brain Injury. *Neurotherapeutics*, Vol.7, No.1, pp.51-67.

Harrison-Findik, DD. (2007). Role of Alcohol in the Regulation of Iron Metabolism. *World J Gastroenterol*, Vol.13, No.37, pp.4925-4930.

Harrison-Findik, DD. (2009). Is The Iron Regulatory Hormone Hepcidin A Risk Factor For Alcoholic Liver Disease? *World J Gastroenterol*, Vol.15, No.10, pp.1186-1193.

He, X.; Hahn, P.; Lacovelli, J.; Wong, R.; King, C.; Bhisitkul, R.; Massaro-Giordana, M. & Duraief, J. (2007). Iron Homeostasis and Toxicity in Retinal Degeneration . *Prog Retin Eye Res,* Vol.26, No.6, pp.649-673.

Hintze, KJ. & Theil, EC. (2005). DNA and mRNA Elements with Complementary Responses to Hemin, Antioxidant Inducers, and Iron Control Ferritin-L Expression. *Proc Natl Acad Sci*, Vol.102, No.42, pp.15048-15052.

Hofe, T.; Marzetti, E.; Xu, J.; Seo, AY.; Gulec, S.; Knutson, MD.; Leeuwenburgh, & Dupont-Versteegden, EE. (2008). Increased RNA Oxidative Damage And Iron Content In Skeletal Muscle With Aging And Disuse Atrophy. *Exp Gerontol*, 43(6):563-570.

Holgado-Madruga, M. & Wong, AJ. (2003). Gab1 is an Integrator of Cell Death Versus Cell Survival Signals in Oxidative Stress. *Mol Cell Biol*, 23(12): 4471-4484

Hou, WH.; Rossi, L.; Shan, Y.; Zheng, JY.; Lambrecht, RW.; Bonkovsky, HL. (2009). Iron Increases HMOX1 and Decreases Hepatitis C Viral Expression in HCV-Expressing Cells. *World J Gastroenterol*, Vol.15, No.36, pp.4499-510.

Hsieh, Y.; Chang, CC. & Lin, CS. (2006). Seminal Malondialdehyde Concentration But Not Glutathione Peroxidase Activity Is Negatively Correlated With Seminal Concentration and Motility. *Int J Biol Sci*, Vol.2, No.1, pp.23-29.

Huang X (2008) Does Iron Have A Role in Breast Cancer? *Lancet Oncol*, Vol.9, No.8, pp.803–807.

Ianotti, L.; Tielsch, J.; Black, MM. & Black, R. (2006). Iron Supplement in Early Childhood: Health Benefits and Risks. *Am J Clin Nutr*, Vol.84, pp.1261-1276.

Jaillard, T .; Roger, M .; Galinier, A .; Guillou, P .; Benani, A.; Leloup, C.; Casteilla, L .; Penicaud, L. & Lorsignol, A. (2009) Hypothalamic Reactive Oxygen Species are Required for Insulin-Induced Food Intake Inhibition an NADPH Oxidase–Dependent Mechanism. *Diabetes*, Vol.58, pp.1544-1549.

Jana, A. & Pahan, K. (2007). Oxidative Stress Kills Human Primary Oligodendrocytes via Neutral Sphingomyelinase: Implications for Multiple Sclerosis. *J Neuroimmune Pharmacol*, Vol.2, No.2, pp.184–193.

Jauniaux, E.; Watson, AL.; Hempstock, J.; Bao, YP.; Skepper, JN. & Burton, GJ. (2000). Onset of Maternal Arterial Blood Flow and Placental Oxidative Stress: A Possible Factor In Human Early Pregnancy Failure. *Am J Pathol*, Vol.157, No.6, pp.2111-2121.

Jauniaux, E.; Hempstock, J.; Greenwold, N. & Burton, GJ. (2003). Trophoblastic Oxidative Stress in Relation to Temporal and Regional Differences in Maternal Placental Blood Flow in Normal And Abnormal Early Pregnancies. *Am J Pathol*, Vol.162, No. 1, pp.115-125.

Jian, J.; Pelle, E. & Huang, X. (2009). Iron and Menopause: Does Increased Iron Affect the Health of Postmenopausal Women? *Antioxid Redox Signal*, Vol.11, No.12, pp.2939–2943.

Jiang, D.; Li, X.; Williams, R.; Patel, S.; Men, L.; Wang, Y. & Zhou, F. (2009). Ternary Complexes Of Iron, Amyloid-B And Nitrilotriacetic Acid: Binding Affinities, Redox Properties, And Relevance To Iron-Induced Oxidative Stress In Alzheimer's Disease. *Biochemistry*, Vol.48, No.33, pp. 7939–7947.

Jinsmaa, Y.; Florang, VR.; Rees, JN.; Anderson, DG.; Strack, S. & Doorn, JA. (2009). Products of Oxidative Stress Inhibit Aldehyde Oxidation and Reduction Pathways in Dopamine Catabolism Yielding Elevated Levels of a Reactive Intermediate . C*hem Res Toxicol*, Vol.22, No.5, pp.835–841.

Jones, DP. (2006). Extracellular Redox State: Refining the Definition of Oxidative Stress in Aging. Rejuvenation Res Vol.9, No.2, pp.169-181.(Abstr)

Manusco, C. (2004). Heme Oxygenase and Its Products in the Nervous System. *Antioxd Redox Signal*, Vol.6, No.5, pp.878-887.(Abstr)

Kaczmarek, M.; Cachau, RE.; Topol, IA.; Kasprzak, KS.; Ghio, A.; Salnikow, K. (2009). Metal Ions-Stimulated Iron Oxidation in Hydroxylases Facilitates Stabilization of HIF-1a Protein. *Toxicol Sci*, Vol.107, No.2, pp.394-403.

Kapralov, A.; Vlasova, II.; Feng, W.; Maeda, A.; Walson, K.; Tyurin, VA.; Huang, Z.; Aneja, RK.; Carcillo, J.; Bayır, H. & Kagan, VE. (2009). Peroxidase Activity of Hemoglobin·Haptoglobin Complexes.Covalent Aggregation and Oxidative Stress in Plasma and Macrophages *J Biol Chem*, Vol.284, No.44, pp.30395-30407.

Kastman, EK.; Willette, AA.; Coe, CL.; Bendlin, BB.; Kosmatka, KJ.; McLaren, DG.; Xu, G.; Canu, E.; Field, AS., Alexander, AL.; Voytko, ML.; Beasley, TM.; Colman, RJ.; Weindruch, R. & Johnson, SC. (2010). A Calorie-Restricted Diet Decreases Brain Iron Accumulation and Preserves Motor Performance in Old Rhesus Monkeys. *J Neurosci*, Vol.30, No.23, pp.7940–7947.

Kautz, L., Meynard, D.; Monnier, A.; Darnaud, V. & Baunet, R. (2008). Iron Regulates Phosphorylation of Smad1/5/8 and Gene Expression of BMP6, Smad 7, Id1 And Atoh 8 in Mouse Liver. *Blood,* Vol.112, pp.1503-1509.

Kell, DB. (2009). Iron Behaving Badly: Inappropriate Iron Chelation as a Major Contributor to the Etiology of Vascular and Other Progressive Inflammatory and Degenerative Diseases. *BMC Med Genomics*, Vol.2, No.2, pp.1-79.

Knutson, MD. (2010). Iron-Sensing Proteins that Regulate Hepcidin and Enteric Iron Absorption. *Ann Rev Nutr,* 30:149-171.

Kohgo, Y.; Ikuta, K.; Ohtake, T.; Torimoto, Y. & Kalo, J. (2008). Body Iron Metabolism and Pathophysiology Of Iron Overload. *Int J Hematol*, Vol.88, No.1, pp.7-15.

Kurz, T.; Leake, A.; von Zglinick, T.; & Brunk, UT. (2004). Relocalized Redox-Active Lysosomal Iron Is An Important Mediator Of Oxidative-Stress-Induced DNA Damage. *Biochem J*, Vol.378, pp.1039-1045

Kurz, T.; Terman, A.; Gustafsson, B. & Brunk, UT. (2008). Lysosomes in Iron Metabolism, Ageing and Apoptosis. *Histochem Cell Biol*, Vol.129, pp.389-406.

Liu, G.; Mena, P.; Kudob, W.; Perry, G. & Smith, MA. (2009). Nanoparticle -Chelator Conjugates as Inhibitors of Amyloid-B Aggregation and Neurotoxicity: A Novel Therapeutic Approach for Alzheimer Disease . *Neurosci Lett*, Vol.455, No.3, pp.187–190.

Logroscino, G.; Gao, X.; Chen, H.; Wing, A. & Ascherio, A. (2008). Dietary Iron Intake and Risk Of Parkinson's Disease. *Am J Epidemiol,* Vol.168, pp.1381-1388.

Logroscino, G.; Marder, K.; Graziano, J.; Freyer, G.; Slakovich, V.; Lojacono, N.; Cote, L. & Mayeux, R. (1998) Dietary Iron, Animal Fats, And Risk Of Parkinson's Disease. *Mov Disord*, 13(suppl 1):13–16.

Lucesoli, F.; Caligiuri, M.; Roberti, MF.; Perazzo, J.C. & Fraga, C.G. (1999) Dose-Dependent Increase Of Oxidative Damage in the Testes of Rats Subjected to Acute Iron Overload. *Toxicology*, Vol.132, No. 2-3, pp.179-186.

Lushchak, VI. (2011) Adaptive Response to Oxidative Stress: Bacteria, Fungi, Plants & Animals. *Comp Biochem Physiol C Toxicol Pharmacol,* Vol.153, No.2, pp.175-190.

References and further reading may be available for this article. To view references and further reading you must purchase this article.

Machado, MV.; Ravasco, P.; Martins, A.; Almeida, MR.; Camilo, ME. & Cortez-Pinto, H. (2009). Iron Homeostasis And H63D Mutations in Alcoholics with and without Liver Disease. *World J Gastroenterol*, Vol.15, No.1, pp.106–111.

Mackenzie, EL.; Iwasaki, K. & Tsuji, Y. (2008). Intracellular Iron Transport and Storage: From Molecular Mechanisms To Health Implications. *Antioxid Redox Signal*, Vol.10, No.6, pp.998-1016.

Mandel, S.; Weinreb, O.; Amit, T. & Youdim, MB. (2005). Mechanism Of Neuroprotective Action of the Anti-Parkinson Drug Rasagiline and its Derivatives. *Brain Res Brain Res Rev*, Vol.48, pp.379–387.

Mantena, SK.; King, AL.; Andringa, KK.; Eccleston, HB. & Bailey, SM. (2008). Mitochondrial Dysfunction And Oxidative Stress In The Pathogenesis of Alcohol and Obesity-Induced Fatty Liver Diseases. *Free Radic Biol Med*, Vol.44, No.7, pp.1259-1272.

Martinez-Outschoorn, UE.; Balliet, RM.; Rivadeneira, DB.; Chiavarina, B.; Pavlides, S.; Wang, C.; Whitaker-Menezes, D.; Daumer, KM.; Lin, Z.; Witkiewicz, AK.; Flomenberg, N.; Howell, A.; Pestell, RG.; Knudsen, ES.; Sotgia, F. & Lisanti, MP. (2010). Oxidative Stress in Cancer Associated Fibroblasts Drives Tumor-Stroma Co-Evolution: A New Paradigm For Understanding Tumor Metabolism, the Field Effect and Genomic Instability in Cancer Cells. *Cell Cycle*, Vol.9, No.16, pp.3256-3276.

Melov, S.; Adlard, PA.; Morten, K.; Johnson, F.; Golden, TR.; Hinerfeld, D.; Schilling, B.; Mavros, C.; Masters, CL.; Volitakis, I.; Li, QX.; Laughton, K.; Hubbard, A.; Cherny, RA.; Gibson, B. & Bush, AI. (2007). Mitochondrial Oxidative Stress Causes Hyperphosphorylation of Tau. PLoS ONE 2(6): e536. doi:10.1371/ journal.pone.0000536 mice subjected to iron intoxication. *Toxicology*, Vol.206, pp.169-78.

Mikhail, MS.; Anyaegbunam, A.; Garfinkel, D.; Palan, PR.; Basu, J.; Romney, SL. (1994). Preeclampsia And Antioxidant Nutrients: Decreased Plasma Levels Of Reduced Ascorbic Acid, Alpha-Tocopherol, And Beta-Carotene In Women With Preeclampsia. *Am J Obstet Gynecol*, Vol.171, pp.150-157.

Mills, E.; Dong, XP.; Wang, F.; Xu, H. (2010) Mechanisms Of Brain Iron Transport: Insight Into Neurodegeneration And CNS Disorders. *Future Med Chem*, Vol.2, No.1, pp.51-64.

Moslemi, MK. & Tavanbakhsh, S. (2011). Selenium–Vitamin E Supplementation In Infertile Men: Effects On Semen Parametersand Pregnancy Rate. *Int J Gen Med* Vol.4, pp.99–104.

Moussa, B.H.; Youdim. & Bakhle, YS. (2006). Monoamine oxidase: Isoforms and inhibitors in Parkinson's disease and depressive illness. *Br J Pharmacol* Vol.147, pp.S287-S 296.

Muckenthaler, M.; Galy, B. & Hentze, M. (2008). Systemic Homeostasis and IRE/IRP Regulatory Network. *Ann Rev Nutr, Vol.*28, pp.197-213.

Mueller, S.; Millonig, G.; & Seitz, HK. (2009). Alcoholic Liver Disease and Hepatitis C: A Frequently Underestimated Combination. *World J Gastroenterol.* Vol.15, No.28, pp.3462-3471.

Mulder, DJ.; van Haelst, PL.; Wobbes, MH.; Gans, RO.; Ziljstra, F.; May, JF.; Amit, AJ.; Teravaert, AWC. & van Doormal, JJ. (2007) The Effect of Aggressive versus Conventional Lipid-Lowering Therapy on Markers of Inflammatory and Oxidative Stress. *Cardiovasc Drugs Ther*, Vol.21, pp.91-97.

Myatt, L. *(*2010). Reactive Oxygen and Nitrogen Species and Functional Adaptation of the Placenta. *Placenta*, Vol.31(Suppl), pp.S66–S69.

Nagababu, E.; Gulyani, S.; Earley, C J.; Cutler, RG.; Mattson, MP. & Rifkind, JM. (2008). Iron-Deficiency Anemia Enhances Red Blood Cell Oxidative Stress. *Free Radic Res*, Vol.42, No.9, pp.824–829.

Naito, Y.; Yoshikawa, T.; Yoneta, T.; Yagi, N.; Matsuyama, K.; Arai, M.; Tanigawa, T.; Kondo, M. (2009) A New Gastric-Ulcer Model in Rats Produced by Ferrous Iron and Ascorbic-Acid Injection. *Digestion, Vol.* 56, pp.472-478.

Nemeth, E. & Ganz, T. (2009) The Role of Hepcidin in Iron Metabolism *Acta Hematol* Vol.12, pp.78-86.

Nicol, CJ.; Zielenski, J.; Tsui, LC. & Wells, PG. (2000). An Embryoprotective Role for Glucose-6-Phosphate Dehydrogenase in Developmental Oxidative Stress and Chemical Teratogenesis. *FASEB J,* Vol.14, pp.111– 127.

Nuhn, P.; Kunzlim, BM.; Hennig, R.; Mitkus, T.; Ramanauskas, T.; Nobiling, R.; Meuer, SC.; Friess, H. & Berberat PO. (2009). Heme Oxygenase-1 and Its Metabolites Affect Pancreatic Tumor Growth in Vivo. *Mol Cancer*, 8:37

Olivares, D.; Huang, X.; Branden, L.; Grieg, NH. & Rogers, JT. (2009). Physiological and pathological role of alpha-synuclein in parkinson's disease through iron mediated oxidative stress:; the role of a putative iron-responsive element. *Int.J. Mol. Sci*. 10: 1226-1260.

Outten, FW. & Theil, EC. (2009). Iron-Based Redox Switches in Biology. *Antioxid Redox Signal* Vol.11, No.5, pp. 1029-1046.

Perez, LR. & Franz, KJ. (2010). Minding Metals: Tailoring Multifunctional Chelating Agents for Neurodegenerative Disease. *Dalton Trans*, Vol.39, No.9, pp.2177–2187.

Philippe, MA.; Ruddell, RG.; Ramm, GA. (2007). Role of Iron in Hepatic Fibrosis: One Piece in The Puzzle. *World J Gastroenterol*, Vol.13, No.35, pp.4746-4754.

Picard, E.; Fontaine, I.; Jonet, L.; Guillou, F.; Behar-Cohen, F.; Courtois, Y. & Jeanny, JC. (2008). The Protective Role of Transferrin in Müller Glial Cells after Iron Induced Toxicity. *Mol Vis*, Vol.14, pp.928-941.

Pivotraiko, VN.; Stone, SL.; Roth, KA. & Shacka, JJ. (2009). Oxidative Stress and Autophagy in the Regulation of Lysosome-Dependent Neuron Death. *Antioxid Redox Signal,* Vol.11, No.3, pp.481-496.

Powers , KM.; Smith – Weller, T.; Franklin, G.; Longstreth, WT.; Swanson, PD. & Checkoway, H. (2009). Dietary Fats, Cholesterol and Iron as Risk Factors for Parkinson's Disease. *Parkinsonism Relat Disord*, Vol.15, No.1, pp.47–52.

Powers, KM.; Smith-Weller, T.; Franklin, GM.; Longstreth, WT.; Swanson, PD. & Checkoway, H.(2003). Parkinson's Disease Risks Associated with Dietary Iron, Manganese, and Other Nutrient Intakes. *Neurology*, Vol.60, No.11, pp.1761–1766.

Pujalté, I.; Passagne, I.; Brouillaud, B.; Tréguer, M.; Durand, E.; Ohayon-Courtès, C. & L'Azou, B. (2011) Cytotoxicity and Oxidative Stress Induced by Different Metallic Nanoparticles on Human Kidney Cells. *Particle and Fibre Toxicology, Vol.*8, pp.10-26.

Prater, MR.; Laudermilch, CL.; Liang, C. & Holladay, SD. (2008). Placental Oxidative Stress Alters Expression of Murine Osteogenic Genes and Impairs Fetal Skeletal Formation. *Placenta* Vol.29, No.9, pp.802–808.

Price, L. & Kowdley, KV. (2009). The Role of Iron in the Pathophysiology and Treatment of Chronic Hepatitis C. *Can J Gastroenterol*, 23(12): 822–82

Pringle, KG.; Kind, KL.; Ferruzzi-Perri, AN.; Thompson, JG. & Roberts, CT. (2010). Beyond Oxygen: Complex Regulation and Activity of Hypoxia Inducible Factors in Pregnancy. *Hum Reprod Update*, Vol.16, No.4, pp.415–431.

Rabovsky, AB.; Komarov, AM.; Ivie, JS. & Buettner, GR. (2010) Minimization of Free Radical Damage by Metal Catalysis of Multivitamin/Multimineral Supplements. *Nutrition Journal*, Vol.9, pp.61-68.

Ramakrishnan, U. (2002). Prevalence of Micronutrient Malnutrition Worldwide. *Nutr Rev*, Vol.60, pp.546-62.

Regan, R.; Li, Z.; Chen, ZM.; Zhang, X. & Chen-Roetling, J.(2008). Iron Regulatory Proteins Increase Neuronal Vulnerability to Hydrogen Peroxide. *Biochem Biophys Res Commun*, Vol.375, No.1, pp.6–10.

Roualt, T. & Cooperman, S. (2006). Brain Iron Metabolism. *Semin Pediatr Neurol*, Vol.13, pp.142-148.

Ruder, EH.; Terry, J.; Hartman, J.; Blumberg, J.; & Goldman, MB. (2008). Oxidative Stress and Antioxidants: Exposure and Impact on Female Fertility. *Hum Reprod Update*, Vol.14, No.4, pp.345–357.

Ruder, E H.; Hartman, TJ. & Goldman, MB. (2009). Impact of Oxidative Stress on Female Fertility. *Curr Opin Obstet Gynecol*, Vol.21, No.3, pp.219–222.

Rutherford, JC. & Bird, AJ. (2004). Metal-Responsive Transcription Factors that Regulate Iron, Zinc and Copper Homeostasis in Eukaryotic Cells. *Eukaryotic Cell*, Vol.3, No.1, pp.1-13.

Ryter, SW. & Choi, AMK. (2009). Heme Oxygenase-1/Carbon Monoxide from Metabolism to Molecular Therapy. *Am J Respir Cell Mol Biol*, Vol.41, pp.251–260

Sanchez-Ortiz, E .; Hahm , BK.; Armstrong, DL. & Rossie, S. (2009). Protein Phosphatase 5 Protects Neurons Against Amyloid B Toxicity. *J Neurochem*, Vol.111, No.2, pp.391–402.

Seril, DN.; Liao, J.; Ho, KL.; Yang, CS. & Yang, GY. (2002) Dietary Iron Supplementation Enhances Dss-Induced Colitis and Associated Carcinoma Development in Mice. *Dig. Dis Sci, Vol.*47, pp.1266-1278.

Shi, H.; Bencze, KZ.; Stemmlet, TL. & Philpott, S. (2008). A Cytosolic Iron Chaperone that Delivers Iron to Ferritin. *Science*, Vol.30,320 No.5880, pp.1207-1210.

Siedlak, SL.; Casadesus, G.; Webber, KM.; Pappolla, MA.; Atwood, CS.; Smith, MA. & Perry, G. (2009). Chronic Antioxidant Therapy Reduces Oxidative Stress in a Mouse Model of Alzheimer's Disease. *Free Radic Res*, Vol.43, No.2, pp.156–164.

Slonima, DK.; Koidec, K.; Johnson, KL.; Tantravahid, U.; Cowanc, JM.; Jarrah, Z. & Bianchi, DW. (2009). Functional Genomic Analysis of Amniotic Fluid Cell-Free Mrna Suggests that Oxidative Stress is Significant in Down Syndrome Fetuses . *Proc Natl Acad Sci*, Vol.106, No.23, pp. 9425–9429.

Smith, MA.; Zhua, X.; Tabatonb, M.; Liuc, G.; McKeel, DW.; Cohen, ML.; Wang, X.; Siedlak, SL.; Hayashi, T.; Nakamura, M.; Nunomura, A. & Perry, G. (2010). Increased Iron and Free Radical Generation in Preclinical Alzheimer Disease and Mild Cognitive Impairment. *J Alzheimers Dis*, Vol.19, No.1, pp.363–372.

Sompol, P.; Ittarat, W.; Tangpong, J.; Chen, Y.; Doubinskaia, I.; Batinic-Haberle, I.; Abdul, HM.; Butterfield, DA. & St. Clair, DK. (2008). A Neuronal Model of Alzheimer's Disease: An Insight into the Mechanisms of Oxidative Stress-Mediated Mitochondrial Injury. *Neuroscience,* Vol.153, No.1, pp.120-30.

Stankiewicz, J.; Panter, SS.; Neema, M.; Arora, A.; Batt, C. & Bakshi, R. (2007) Iron in Chronic Brain Disorders: Imaging and Neurotherapeutic Implications. *Neurotherapeutics*, Vol.4, No.3, pp.371–386.

Szczepanska, M.; Kozlik, J.; Skrzypczak, J. & Mikolajczyk, M. (2003). Oxidative Stress may be a Piece in the Endometriosis Puzzle. *Fertil Steril*, Vol.79, pp.1288-1293.

Takagi, Y.; Nikaido, T.; Toki, T.; Kita, N.; Kanai, M.; Ashida, T.; Ohira, S. & Konishi, I. (2004). Levels of Oxidative Stress and Redox-Related Molecules in the Placenta in Preeclampsia and Fetal Growth Restriction. *Virchows Arch*, Vol.444, pp.49-55.

Takami, T. & Sakaida, I. (2011). Iron Regulation by Hepatocytes and Free Radicals. *J Clin Biochem Nutr*, Vol.48, No.2, pp.103- 106.

Tanaka, H.; Fujita, N.; Sugimoto, R.; Urawa, N.; Horiike, S.; Kobayashi, Y.; Iwasa, M.; Ma, N.; Kawanishi, S.; Watanabe, S.; Kaito, M. &Takei, Y. (2008). Hepatic Oxidative DNA Damage is Associated with Increased Risk for Hepatocellular Carcinoma in Chronic Hepatitis C. *Br J Cancer*, Vol.98, No.3, pp.580–586.

Thiel, C. & Goss, DJ. (2009) Living with iron (and oxygen): questions and answers about iron homeostasis. *Chem Rev*, Vol.109, No.10, pp.4568-4579.

Tunc, O. & Tremellen, K J. (2009). Oxidative DNA Damage Impairs Global Sperm DNA Methylation in Infertile Men. *Assist Reprod Genet,* Vol.26, pp.537–544.

Uchiyama, A.;, Kim, JS.; Kon, K.; Jaeschke, H.; Ikejima, K.; Watanabe, S. & Lemasters, JJ. (2008). Translocation of Iron from Lysosomes into Mitochondrria is a Key Event during Oxidative Stress-Induced Hepatocellular Injury. *Hepatology*, Vol.48, No.5, pp.1644-1654.

Vegeto, E.; Belcredito, S.; Etteri, S.; Ghisletti, S.; Brusadellli, A.; Meda, C.; Krust, A.; Dupont, S.; Clana, P.; Chambon, P. & Maggi, A. (2003). Estrogen Receptor-_ Mediates the Brain Antiinflammatory Activity of Estradiol. *PNAS,* Vol.100, No.16, pp.9614-9619.

Vyas, K. (2005). Iron And Zinc Status, Oxidative Stress and Pregnancy Outcome. Unpublished M.Sc. dissertation submitted to S.N.D.T. Women's University, Mumbai, India.

Wang, J. & Pantopoulos, K. (2011). Regulation of Cellular Iron Metabolism, *Biochem J*, Vol.434, pp.365-381.

Wang, X. & Michaelis, EK. (2010). Selective Neuronal Vulnerability to Oxidative Stress in the Brain. *Frontiers in Aging NeuroSci*, doi: 10.3389/fnagi.2010.00012.

Wessling-Resnick, M. (2010). Iron Homeostasis and Inflammatory Response. *Ann Rev Nutr*, Vol.30, pp.105-122.

White-Gilbertson, S.; Kasman, L.; McKillop, J.; Tirodkar, T.; Lu, P. & Voelkel-Johnson, C. (2009). Oxidative Stress Sensitizes Bladder Cancer Cells to Trailmediated Apoptosis by Downregulating Anti-Apoptotic Proteins. *J Urol*, Vol.182, No.3, pp.1178–1185.

Wise, T.; Lunstra, DD.; Rohrer, A. & Ford, JJ. (2003) Relationships of Testicular Iron and Ferritin Concentrations with Testicular Weight and Sperm Production in Boars. J Animal Sci, Vol.81, No. 2, pp.503-511.

Wu, M.; Bian, Q.; Liu, Y.; Fernandes, AF.; Taylor, A.; Pereira, P. & Shang, F. (2009). Sustained Oxidative Stress Inhibits Nf-Kb Activation Partially via Inactivating the Proteasome. *Free Radic Biol Med*, Vol.46, No.1, pp.62–69.

Zecca, L.; Stroppolo, A.; Gatti, A.; Tampellini, D.; Toscani, M.; Gallorini, MMH.; Giaveri, G.; Arosio, P.; Santambrogio, P.; Fariello, RG.; Karatekin, E.; Kleinman, MH.; Turro, N.; Hornykiewicz, O. & Zucca, FA. (2004). The Role of Iron and Copper Molecules in the Neuronal Vulnerability of Locus Coeruleus and Substantia Nigra during Aging. *Proc Natl Acad Sci*, Vol.101, No.26, pp.9843–9848.

Zecca, L.; Youdim, MB.; Riederer, P.; Connor, JR. & Crichton, RR. (2004). Iron, Brain Ageing and Neurodegenerative Disorders. *Nat. Rev. Neurosci*, Vol.5 , pp.863–873.

Zhang, A. & Enns, C. (2009). Iron Homeostasis: Recently Identified Proteins Provide Insight Into Novel Control Mechanisms. *J Biol Chem*, Vol.284, No.2, pp.711-715.

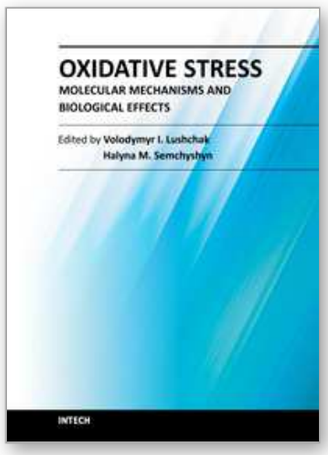
Zhang, J.; Masciocchi, M.; Lewis, D.; Sun, W.; Liu, A. & Wang, Y. (2008). Placental Anti-Oxidant Gene Polymorphisms, Enzyme Activity, and Oxidative Stress In Preeclampsia. *Placenta*, Vol.29, No.5, pp.439–443.

Zhaoa, H.; Wonga, RJ.; Kalish, FS.; Nayak, NR. & Stevenson DK. (2009). Effect of Heme Oxygenase-1 Deficiency on Placental Development. *Placenta*, Vol.30, No.10, pp.861–868.

Zini, A.; San Gabriel, M. & Baazeem, AA. (2009). Antioxidants and Sperm DNA Damage: A Clinical Perspective. *J Assist Reprod Genet,* Vol.26, pp 427–432.

Zribi, N.; Chakroun, NF.; Elleuch, H.; Ben Abdallah, F.; Ben Hamida, AS.; Gargouri, J.; Fakhfakh, F. & Keskes, LA. (2011). Sperm DNA Fragmentation and Oxidation are Independent of Malondialdheyde. *Reprod Biol Endocrinol*, Vol.9. pp 47-54.

Zuwała-Jagiełło, J.; Pazgan-Simon, M.; Krzysztof, S. & Warwas, M. (2011). Advanced Oxidation Protein Products and Inflammatory Markers in Liver Cirrhosis: A Comparison between Alcohol-Related and HCV-Related Cirrhosis, *Acta Biochim Pol.* Vol.58 No.1, pp. 59-65.



Oxidative Stress - Molecular Mechanisms and Biological Effects
Edited by Dr. Volodymyr Lushchak

ISBN 978-953-51-0554-1
Hard cover, 362 pages
Publisher InTech
Published online 25, April, 2012
Published in print edition April, 2012

Since the discovery of free radicals in biological systems researchers have been highly interested in their interaction with biological molecules. Denoted in 1980, and due to fruitful results and ideas, oxidative stress is now appreciated by both basic and applied scientists as an enhanced steady state level of reactive oxygen species with wide range of biological effects. This book covers a wide range of aspects and issues related to the field of oxidative stress. The association between generation and elimination of reactive species and effects of oxidative stress are also addressed, as well as summaries of recent works on the signaling role of reactive species in eukaryotic organisms. The readers will gain an overview of our current understanding of homeostasis of reactive species and cellular processes they are involved in, as well as useful resources for further reading.

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