Acta Biochimica Polonica

Vol. 51 No. 2/2004

471–480

QUARTERLY

Review

The role of labile iron pool in cardiovascular diseases★

Marcin Kruszewski✉

[1]*Department of Radiobiology and Health Protection, Institute of Nuclear Chemistry and Technology, Warszawa, Poland;* [2]*Department of Experimental Hematology and Cord Blood Bank, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warszawa, Poland*

Received: 30 April, 2004; accepted: 05 May, 2004

Key words: oxidative stress, reactive oxygen species, reactive nitrogen species, atherosclerosis, ischemia, reperfusion

Although multiple factors are associated with cardiovascular pathology, there is now an impressive body of evidence that free radicals and nonradical oxidants might cause a number of cardiovascular dysfunctions. Both direct damage to cellular components and/or oxidation of extracellular biomolecules, e.g. LDL, might be involved in the aetiology of cardiovascular diseases. The key molecules in this process seem to be iron and copper ions that catalyse formation of the highly reactive hydroxyl radical. Chelation of iron ions has a beneficial effect on the processes associated with the development of atherosclerosis and formation of post-ischemic lesions. These findings are indirectly supported by the increasing body of evidence that stored body iron plays a crucial role in pathogenesis of atherosclerosis and ischemia/reperfusion injury.

There is increasing body of evidence that oxidative stress caused by an excessive exposure to reactive oxygen species (ROS), such as hydroxyl ($^{\bullet}OH$) and superoxide ($O_2^{\bullet-}$) radicals, hydrogen peroxide (H_2O_2) and to reactive nitrogen species (RNS), such as nitric ox-

★Presented as invited lecture at the 29th Congress of the Federation of European Biochemical Societies, Warsaw, Poland, 26 June–1 July 2004.

✉Corresponding author: Marcin Kruszewski, Institute of Nuclear Chemistry and Technology, Department of Radiobiology and Health Protection, Dorodna 16, 03-195, Warszawa, Poland; tel.: (48 22) 811 0736; fax: (48 22) 811 1532; e-mail: marcinkr@orange.ichtj.waw.pl

Abbreviations: DNIC, dinitrosyl iron complexes; DTM1, divalent metal transporter 1; FR, ferritin; LIP, liabile iron pool; LPL, low density lipoproteins; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase; TF, transferrin; TFR, transferrin receptor.

ide ($^{\bullet}NO$) or peroxynitrite ($ONOO^-$) has deleterious effect on the whole organism at different levels. At the cellular level, radical and nonradical by-products of the aerobic lifestyle may change the cellular redox status and, as a consequence, cellular signalling pathways. An additional consequence may be damage induction to the important biomolecules, such as DNA, proteins and lipids. The damage might be reflected at the organism level as induction of diseases, including cancer and ageing. Moreover, some products of radical damage to lipids or sugars readily react with proteins or nucleic acids, thus forming secondary products of oxidative damage, sometimes far from the initial place of origin (Stadtman & Berlett, 1997).

Although oxidative stress is an unavoidable consequence of aerobic metabolism, the majority of by-products of four electron reduction of the O_2 molecule is of a rather low reactivity. However, trace amounts of unprotected transition metal ions can catalyse the reaction of the low reactive $O_2^{\bullet-}$ and H_2O_2 that gives rise to the highly toxic hydroxyl radical.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + {}^{\bullet}OH$$

$$Fe^{3+} + O_2^{\bullet-} \rightarrow Fe^{2+} + O_2$$

$$H_2O_2 + O_2^{\bullet-} \xrightarrow{(Fe)} {}^{-}OH + {}^{\bullet}OH + O_2$$

The biological importance of formation of $^{\bullet}OH$ through the Fenton chemistry has been recently discredited due to the consumption of $O_2^{\bullet-}$ by $^{\bullet}NO$ and formation of $ONOO^-$ instead of $^{\bullet}OH$ (Koppenol, 1998; 2001). However, the hydroxyl radical is still commonly accepted as the main source of oxidative damage to the cell (Fridovich, 1998; Liochev & Fridovich, 1999; Termini, 2000).

Transition metal-driven generation of oxygen-derived free radicals is known to induce oxidation of proteins, lipids and lipoproteins, nucleic acids, carbohydrates and other cellular components. Thus, oxidative stress may play an important role in the pathological processes triggered by the presence of unshielded iron or copper ions. Studies of animals revealed an elevated level of thiobarbituric acid reactants in liver homogenates of animals injected with iron dextran (Galleano & Puntarulo, 1992). Dillard and Tappel (1979) reported also an increase in ethane and penthane, the volatile autooxidation products of unsaturated fatty acids, exhalated in rats receiving multiple iron dextran injections. Elevated mitochondrial lipid peroxidation was also observed in rats chronically exposed to ferric nitrilotriacetate (Bacon *et al.*, 1983).

LABILE IRON POOL

In most cells iron homeostasis consists of iron uptake, utilisation and storage. The process of iron uptake is carried out by transferrin receptor (TFR) and divalent metal transporter 1 (DMT1, also called DCT1; NRAMP2), whereas ferritin (FR) is an intracellular iron-sequestering protein. Since uptake and storage of iron is carried out by different proteins, there is a pool of accessible iron ions, called labile iron pool (LIP) that constitutes crossroads of metabolic pathways of iron-containing compounds. The term "labile iron pool" was proposed in 1946 by Greenberg and Wintrobe (1946) and reintroduced by Jacobs (1977) as "a transient iron pool". More recently, the term "chelatable iron" has been introduced, as most methodological approaches for detection of this pool of iron ions are based on the use of metal chelators.

LIP is defined as a low-molecular-weight pool of weakly chelated iron that rapidly passes through the cell. It likely consists of both forms of ionic iron (Fe^{2+} and Fe^{3+}) associated with a variety of ligands with low affinity for iron ions. LIP represents only a minor fraction of the total cellular iron (3–5%). It has been proposed that iron is complexed by

diverse low-molecular weight chelators, such as citrate and other organic ions, phosphate, carbohydrates and carboxylates, nucleotides and nucleosides, polypeptides and phospholipids (Kakhlon & Cabantchik, 2002; Petrat *et al.*, 2002; Kruszewski, 2003); however, the actual nature of the intracellular ligands participating in LIP formation remains obscure.

In spite of the variety of methods for LIP quantification (for reviews see: Kakhlon & Cabantchik, 2002; Petrat *et al.*, 2002; Kruszewski, 2003) little is known of its intracellular distribution. The use of laser scanning microscopy enabled the determination of the concentration and distribution of chelatable iron in intracellular compartments. Using this technique Petrat *et al.* (2001) determined in rat hepatocytes an average cellular iron concentration of 5.0 ± 2.0 μM. Interestingly, they found that hepatocyte nucleus contained 6.6 ± 2.9 μM of chelatable iron. In rat liver endothelial cells, the nuclear LIP concentration was even higher (11.8 ± 3.9 μM). Although the amount of total nuclear iron in hepatocytes was estimated to be similar to that of the cytoplasm (Lai *et al.*, 1996), the surprisingly high content of redox-active iron in the nucleus presents a hazard of DNA damage. In the same set of study the authors found that intramitochondrial chelatable iron concentration in rat hepatocytes was at least 4.8 ± 2.3 μM (see for discussion Petrat *et al.*, 2001), and 9.2 ± 2.7 μM in the mitochondria of rat liver endothelial cells. Also found was a small subpopulation of endosomes/lysosomes containing a high amount of chelatable iron (15.8 ± 4.1 μM), likely those degrading iron-containing proteins.

Despite the mysterious nature of LIP, its source is better defined than that of its ligands. A continuous demand for iron available for synthesis of iron-containing proteins forces a permanent iron flux from the extracellular milieu (environment) to the cytoplasm. Hence, iron uptake is to be one of the major sources of LIP. In most mammalian cells, iron uptake is carried out in two different ways, depending on the form of iron to be taken up and cell type (reviewed in Andrews, 2000). Figure 1 briefly summarises the iron delivery pathways and their impact on the LIP formation. Transferrin bound iron (TBI) is taken up *via* receptor-depended endocytosis. Transferrin (TF) binds Fe^{3+} iron ions present in blood serum, and in turn, is bound by TFR present on the cell surface. The complex is internalised and bound iron ions are released in the endosomes and transported to the cytoplasm. The transferrin bound iron and TF/TFR mediated iron delivery pathway play a crucial role in the metabolism of erythroid precursors and hepatocytes. Its disruption leads to early embrionic death, as has been shown in TFR knocked-out mice (Levy *et al.*, 1999).

The non-transferrin bound iron enters cells *via* the DTM1-mediated pathway. DMT1 is a low affinity divalent metal cation transporter with a broad substrate specifity (Gunshin *et al.*, 1997). Mutation in DMT1 protein causes severe iron deficiency anaemia due to the reduced intestinal iron absorption (Fleming *et al.*, 1997; 1998) and defective iron uptake by erythroid cells (Edwards *et al.*, 1978). Exposure to non-transferrin iron is a common way for elevating LIP level in mammalian cells (Breuer *et al.*, 1995).

Apart from iron uptake, a potential source of LIP are intracellular iron-containing proteins. The best known is FR, an iron storage protein that is capable of storing 4500 Fe^{3+} ions per molecule. FR plays a dual role in LIP homeostasis. In iron rich conditions it acts as an iron sequestering protein, protecting cells against iron toxicity. Overexpression of the heavy subunit of FR decreases the LIP level (Picard *et al.*, 1998) and decreases the H_2O_2 induced DNA damage (Cozzi *et al.*, 2000). At low iron conditions it acts as a source of iron ions necessary for iron containing protein synthesis, however, the mechanism of iron release from ferritin remains obscure (Lipinski & Drapier, 1998). Incubation of erythroid precursor cells with iron-loaded FR caused an in-

crease in cellular LIP (Meyron-Holtz *et al.*, 1999). Konijn *et al.* (1999) have also shown that restoration of LIP in LIP-depleted K562 cells is accompanied by a decrease in the amount of intracellular FR.

The ubiquitous occurrence of heme proteins in organisms makes these proteins another potential source of LIP. Unlike TF- and FR-bound iron, iron bound in the heme prosthetic group enters the Fenton reaction resulting in generation of the highly toxic hydroxyl radical (Alayash *et al.*, 2001). To prevent the toxic action of heme iron in oxidative stress conditions heme oxygenase (HO) is induced (Applegate *et al.*, 1991). HO, which exists in an inducible (HO-1) and a constitutive (HO-2) form, oxidises heme to CO, biliverdin, and ferrous iron. Indeed, Kvam *et al.* (2000) found that HO activity causes a transient hypersensitivity to the oxidative effect of ultraviolet A radiation that depends on release of iron from heme. It has also been found that induction of HO-1 in response to hemin administration causes an increase in iron ferritin levels and ferritin content in rat brain (Gonzales *et al.*, 2002).

The least known in LIP formation is the role of non-heme iron proteins. Of the major interest from the point of view of LIP formation are 4Fe-4S clusters. The activity of many 4Fe-4S proteins is sensitive to oxidative stress. Hydrogen peroxide rapidly reacts with 4Fe-4S clusters of mammalian cytosolic aconitase, converting the enzyme into the inactive 3Fe-4S form (Brazzolotto *et al.*, 1999), and the released iron ion probably enters the LIP. An EPR study revealed that a similar mechanism was involved in nitric oxide induced inhibition of aconitase activity (Kennedy *et al.*, 1997). Prolonged incubation of recombinant human cytosolic aconitase (Soum & Drapier, 2003) or of human fibroblasts (Castro *et al.*, 1998) with nitric oxide or peroxynitrite promotes complete disruption of the 4Fe-4S cluster and induction of the protein's RNA binding activity. Inhibition of aconitase enzymatic activity and induction of it's RNA binding activity by nitric oxide is accompanied by an increase in the LIP level in mouse lymphoma cells (Kruszewski *et al.*, 2002). Disassembly of the 4Fe-4S cluster of mitochondrial or cytosolic aconitase by H_2O_2

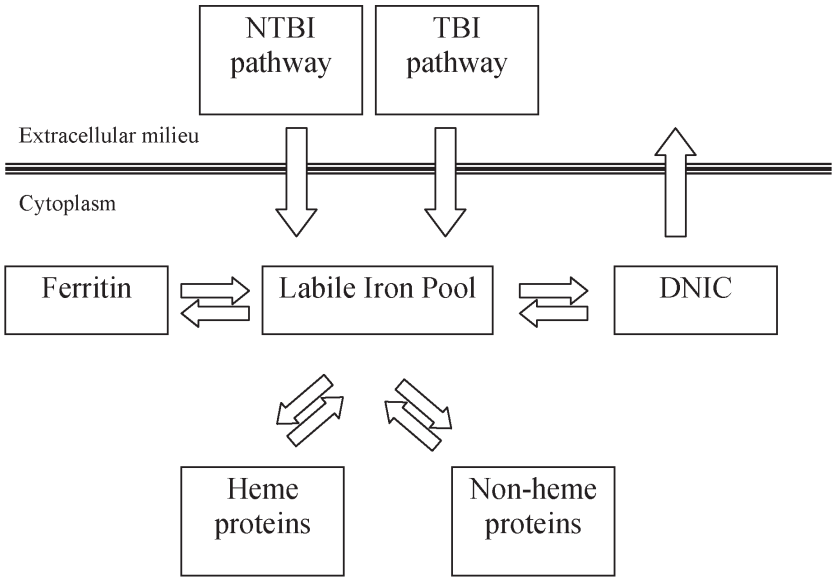

Figure 1. Origins and fate of the cellular LIP.

This general diagram summarises the potential sources and utilisation of cellular LIP. NITB, non-transferrin bound iron; TBI, transferrin bound iron; DNIC, dinitrosyl iron complexes.

and $O_2^{\bullet-}$ was also reported (Gardner, 1997; Bulteau *et al.*, 2003).

Little is known about the mechanism of iron export from the cell. A divalent iron ion exporter, ferroportin 1, has been found on the basolateral membrane of enterocytes. Exported Fe^{2+} ions are subsequently oxidised to the Fe^{3+} form by hephastine, a ferrooxidase that probably co-operates with ferroportin 1 or by plasma ceruloplasmin and it is then bound by transferrin. Ferroportin 1 is expressed in liver, spleen and kidney (Donovan *et al.*, 2000) and is likely involved in the release of iron from the cells that need to export it. The mechanism of iron export from other cell types is unclear. It has been proposed that iron ions might leave cells as a complex with low molecular weight thiols and/or nitric oxide. Mononuclear dinitrosyl iron complexes (DNIC) are formed when ferrous iron and $^{\bullet}$NO react with low molecular weight thiols, amino acids, peptides or proteins (McDonald *et al.*, 1965; Woolum *et al.*, 1968). Although $^{\bullet}$NO has a high affinity for the metal centers of metalloproteins (Henry *et al.*, 1993; Vanin & Kleschyov, 1999) and has been found to bind the iron-sulphur center of mitochondrial aconitase (McDonald *et al.*, 1965; Drapier, 1997), LIP might be involved in formation of DNIC complexes of $^{\bullet}$NO with low molecular weight thiols, such as glutathione or cysteine (Vanin & Kleschyov, 1999). DNIC and RSNO complexes were shown to coexist at equilibrium in systems that include $^{\bullet}$NO, thiols and LIP (Vanin *et al.*, 1997). DNIC complexes with glutathione and cysteine have been considered as a transmembrane NO transporter, and can serve as physiological regulatory factors, especially in immune and cardiovascular systems (Ueno & Yoshimura, 2000).

THE ROLE OF LIP IN ATHEROSCLEROSIS

Atherosclerosis is one of the major causes of coronary heart disease, and is responsible for about 40% of deaths in U.S.A., Europe and Japan. Both environmental and genetic factors are involved in the ethiology of atherosclerosis. The major risk factors are age, sex, familiar predisposition, hyperlipidemia, smoking, obesity, diabetes and infections (Knight, 1999). Several hypotheses have been proposed to explain the process, among those at least two are relevant to oxidative stress. The most prevalent hypothesis concerning the mechanism of atherosclerosis aetiology is the "inflammation theory" (Ross, 1999) that describes atherosclerosis as proliferation of smooth muscle cells in response to different insults to the arterial wall. There is a large body of evidence that production of ROS and RNS occurs at the site of inflammation and contributes to the cellular damage (Salvemini *et al.*, 1996; Cuzzocrea *et al.*, 1997). Production of ROS and RNS during the inflammation process can induce oxidation of low density lipoproteins (LDL) that have been proposed as a major factor inducing plaque formation (Knight, 1999). In laboratory animals and in humans it has been demonstrated that increased oxidative stress results in modification of LDL (Steinberg, 1993). Moreover, in clinical studies increased plasma level of lipid peroxides has been demonstrated in patients with peripheral atherosclerosis (Traverso, 2001). This could be completely inhibited by chelation of iron and copper ions (Steinbrecher *et al.*, 1984); the observation points to the crucial role of Fenton reaction products in the induction of LDL peroxidation. In an *in vitro* study, LIP catalysed free radical-mediated oxidation of LDL that occurred in endothelial cells, smooth muscle cells and macrophages. An iron chelator, defepriprone, prevented the oxidation of LDL and protected human umbilical vein epithelial cells from the cytotoxicity of oxidised LDL (Matthews *et al.*, 1997). Moreover, addition of LDL to cell cultures results in elevation of intracellular free iron level; this suggests a direct relationship between LDL and iron metabolism (Van Lenten *et al.*, 1995). Further evidence of the role of

iron ions in atherosclerosis pathology was recently provided by Gackowski *et al.* (2001). The authors found a higher level of LIP in lymphocytes of atherosclerotic patients as compared with those of matched control.

The discovery that the cells of atherosclerotic plaque derive from a single smooth muscle cell led to the proposal of an alternative hypothesis – the "monoclonal hypothesis". This hypothesis assumes that atherosclerosis begins as a mutation of a single cell that proliferates into an atherosclerotic plaque. A hypothesis similar in principle to this one has been proposed for tumor induction (Trosko & Chang, 1980). Recent investigations suggest that oxidative base damage may play a crucial role in pathogenesis of the disease (De Flora *et al.*, 1997; Traverso, 2001; Lee & Blair, 2001; Martinet *et al.*, 2002). An elevated level of 8-hydroxy-2′-deoxyguanosine (8-oxoGua) has been found in lymphocytes of atherosclerotic patients (Gackowski *et al.*, 2001) and in all cell types of the plaque, including macrophages, smooth muscle cells, and endothelial cells (Martinet *et al.*, 2002). The elevated level of 8-oxoGua in lymphocytes of atherosclerotic patients showed a good correlation with the LIP level (Gackowski *et al.*, 2002). On the other hand, availability of iron ions is crucial for cell proliferation. Porreca *et al.* (1994) found a significant antiproliferative effect of the iron chelator deferoxamine on smooth muscle cells both *in vitro* and *in vivo*.

Dysfunction of endothelium likely contributes to the pathogenesis of cardiovascular disease. Duffy *et al.* (2001) reported that patients with coronary artery disease have impaired endothelium-dependent vasodilatation. This response is restored by infusion of an iron chelator. The authors hypothesise that deferoxamine also affects NO-mediated vasodilatation; this is consistent with the assumed role of DNIC in NO-mediated processes.

THE ROLE OF LIP IN ISCHEMIA/REPERFUSION INJURY

Atherosclerosis is a common cause of the restriction or abolition of blood supply, known as ischemia. Due to the decline in oxygen availability, to continue ATP regeneration, cells have to switch from aerobic to anaerobic metabolic pathways. As a result of decreased efficacy of ATP production, cellular ATP stores become depleted, as ATP is converted to ADP and subsequently to AMP and uric acid. Ischemic cells and tissues cannot survive without restitution of blood flow and oxygen supply. Although ischemia ultimately evokes cell death, a sudden reconstitution of blood flow, i.e. reperfusion, also induces severe cell and tissue injury. It has been shown in the heart that during the reperfusion phase $O_2^{\bullet-}$ is produced that rapidly reacts with $^{\bullet}NO$ and forms $ONOO^-$ (Matheis *et al.*, 1992). Alternatively, dismutation of $O_2^{\bullet-}$ (spontaneous or SOD-dependent) leads to formation of H_2O_2, which, in turn, gives rise to the highly reactive hydroxyl radical. In a recent study of global ischemia in rat heart, early reperfusion resulted in 600% increase in myocardial H_2O_2 content (Slezak *et al.*, 1995). Both $ONOO^-$ and H_2O_2 are known to release iron ions from iron-sulphur proteins thus increasing intracellular LIP that can be liberated from the cells, either in the form of DNIC or as a result of disintegration of necrotic cells. Indeed, substantial mobilization of copper and iron in the coronary flow immediately following prolonged cardiac ischemia was observed. In the first coronary flow fraction of reperfusion after 35 min of ischemia, the level of copper and iron was 8- to 9-fold higher than the pre-ischemic value (Chevion *et al.*, 1993). Further, in a randomised study of ischemia/reperfusion in dogs, Reddy *et al.* (1989) demonstrated that administration of

a potent iron chelator, deferoxamine, reduced the extent of myocyte necrosis, presumably due to the lesser availability of Fenton reaction catalysts.

SUMMARY

In addition to traditional risk factors, such as hypertension, hyperlipidaemia, diabetes and cigarette smoking, there is increasing body of evidence that iron metabolism may also influence cardiovascular disease. Reduction of the total body iron through phlebotomy may play a role in the treatment or prevention of atherosclerosis, whereas the rise in body iron stores after adolescence in men or menopause in women is linked to the pathogenesis of the disease. Establishing the relationship between LIP and body iron stores would help to understand the role of LIP in the aetiology of cardiovascular diseases. Prospective studies are necessary to clarify the impact of iron stores and LIP on overall and cardiovascular morbidity and mortality.

REFERENCES

Alayash AI, Patel RP, Cashon RE. (2001) Redox reactions of hemoglobin and myoglobin: biological and toxicological implications. *Antioxid Redox Signal.*; **3**: 313–27.

Andrews NC. (2000) Iron homeostasis: insights from genetics and animal models. *Nat Rev Genet.*; **1**: 208–17.

Applegate LA, Luscher P, Tyrrell RM. (1991) Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. *Cancer Res.*; **51**: 974–8.

Bacon BR, Tavill AS, Brittenham GM, Park CH, Recknagel RO. (1983) Hepatic lipid peroxidation *in vivo* in rats with chronic iron overload. *J Clin Invest.*; **71**: 429–39.

Brazzolotto X, Gaillard J, Pantopoulos K, Hentze MW, Moulis JM. (1999) Human cytoplasmic aconitase (iron regulatory protein 1) is converted into its [3Fe-4S] form by hydrogen peroxide *in vitro* but is not activated for iron-responsive element binding. *J Biol Chem.*; **274**: 21625–30.

Breuer W, Epsztejn S, Millgram P, Cabantchik IZ. (1995) Transport of iron and other transition metals into cells as revealed by a fluorescent probe. *Am J Physiol.*; **268**: C1354–61.

Bulteau AL, Ikeda-Saito M, Szweda LI. (2003) Redox-dependent modulation of aconitase activity in intact mitochondria. *Biochemistry.*; **42**: 14846–55.

Castro LA, Robalinho RL, Cayota A, Meneghini R, Radi R. (1998) Nitric oxide and peroxynitrite-dependent aconitase inactivation and iron-regulatory protein-1 activation in mammalian fibroblasts. *Arch Biochem Biophys.*; **359**: 215–24.

Chevion M, Jiang Y, Har-El R, Berenshtein E, Uretzky G, Kitrossky N. (1993) Copper and iron are mobilized following myocardial ischemia: possible predictive criteria for tissue injury. *Proc Natl Acad Sci USA.*; **90**: 1102–6.

Cozzi A, Corsi B, Levi S, Santambrogio P, Albertini A, Arosio P. (2000) Overexpression of wild type and mutated human ferritin H-chain in HeLa cells: *in vivo* role of ferritin ferroxidase activity. *J Biol Chem.*; **275**: 25122–9.

Cuzzocrea S, Zingarelli B, Costantino G, Szabo A, Salzman AL, Caputi AP, Szabo C. (1997) Beneficial effects of 3-aminobenzamide, an inhibitor of poly (ADP-ribose) synthetase in a rat model of splanchnic artery occlusion and reperfusion. *Br J Pharmacol.*; **121**: 1065–74.

De Flora S, Izzotti A, Walsh D, Degan P, Petrilli GL, Lewtas J. (1997) Molecular epidemiology of atherosclerosis. *FASEB J.*; **11**: 1021–31.

Dillard C, Tappel A. (1979) Volatile hydrocarbon and carbonyl products of lipid peroxidation: A comparison of penthane, ethane, hexanal and acetone as *in vivo* indices. *Lipids.*; **14**: 969–95.

Donovan A, Brownlie A, Zhou Y, Shepard J, Pratt SJ, Moynihan J, Paw BH, Drejer A, Barut B, Zapata A, Law TC, Brugnara C, Lux SE, Pinkus GS, Pinkus JL, Kingsley PD, Palis J, Fleming MD, Andrews NC, Zon LI. (2000) Positional cloning of zebrafish ferroportin1 identifies a conserved vertebrate iron exporter. *Nature.*; **403**: 776–81.

Drapier JC. (1997) Interplay between NO and [Fe-S] clusters: relevance to biological systems. *Methods.*; **11**: 319–29.

Duffy SJ, Biegelsen ES, Holbrook M, Russell JD, Gokce N, Keaney JF Jr, Vita JA. (2001) Iron chelation improves endothelial function in patients with coronary artery disease. *Circulation.*; **103**: 2799–804.

Edwards JA, Garrick LM, Hoke JE. (1978) Defective iron uptake and globin synthesis by erythroid cells in the anemia of the Belgrade laboratory rat. *Blood.*; **51**: 347–57.

Fleming MD, Trenor CC, Su MA, Foernzler D, Beier DR, Dietrich WF, Andrews NC. (1997) Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet.*; **16**: 383–6.

Fleming MD, Romano MA, Su MA, Garrick LM, Garrick MD, Andrews NC. (1998) Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc Natl Acad Sci USA.*; **95**: 1148–53.

Fridovich I. (1998) Oxygen toxicity: a radical explanation. *J Exp Biol.*; **201** (Pt 8): 1203–9.

Gackowski D, Kruszewski M, Jawien A, Ciecierski M, Olinski R. (2001) Further evidence that oxidative stress may be a risk factor responsible for the development of atherosclerosis. *Free Radic Biol Med.*; **31**: 542–7.

Gackowski D, Kruszewski M, Bartlomiejczyk T, Jawien A, Ciecierski M, Olinski R. (2002) The level of 8-oxo-7,8-dihydro-2′-deoxyguanosine is positively correlated with the size of the labile iron pool in human lymphocytes. *J Biol Inorg Chem.*; **7**: 548–50.

Galleano M, Puntarulo S. (1992) Hepatic chemiluminescence and lipid peroxidation in mild iron overload. *Toxicology.*; **76**: 27–38.

Gardner PR. (1997) Superoxide-driven aconitase FE-S center cycling. *Bioscience Rep.*; **17**: 33–42.

Gonzales S, Erario MA, Tomaro ML. (2002) Heme oxygenase-1 induction and dependent increase in ferritin. A protective antioxidant stratagem in hemin-treated rat brain. *Dev Neurosci.*; **24**: 161–8.

Greenberg GR, Wintrobe MM. (1946) A labile iron pool. *J Biol Chem.*; **165**: 397–8.

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA. (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature.*; **388**: 482–8.

Henry Y, Lepoivre M, Drapier JC, Ducrocq C, Boucher JL, Guissani A. (1993) EPR characterization of molecular targets for NO in mammalian cells and organelles. *FASEB J.*; **7**: 1124–34.

Jacobs A. (1977) An intracellular transit iron pool. *Ciba Found Symp.*; **51**: 91–106.

Kakhlon O, Cabantchik ZI. (2002) The labile iron pool: characterization, measurement, and participation in cellular processes. *Free Radic Biol Med.*; **33**: 1037–46.

Kennedy MC, Antholine WE, Beinert H. (1997) An EPR investigation of the products of the reaction of cytosolic and mitochondrial aconitases with nitric oxide. *J Biol Chem.*; **272**: 20340–7.

Knight JA. (1999) *Free Radicals, Antioxidants, Aging and Disease.* AACC Press, Washington, DC.

Konijn AM, Glickstein H, Vaisman B, Meyron-Holtz EG, Slotki IN, Cabantchik ZI. (1999) The cellular labile iron pool and intracellular ferritin in K562 cells. *Blood.*; **94**: 2128–34.

Koppenol WH. (1998) The basic chemistry of nitrogen monoxide and peroxynitrite. *Free Radic Biol Med.*; **25**: 385–91.

Koppenol WH. (2001) The Haber–Weiss cycle — 70 years later. *Redox Rep.*; **6**: 229–34.

Kruszewski M. (2003) Labile iron pool: the main determinant of cellular response to oxidative stress. *Mutat Res.*; **531**: 81–92.

Kruszewski M, Starzyński R, Drapier JC, Smuda E, Lipinski P. (2002) Modulation of IRP1 by NO: an unexpected correlation between RNA-binding activity of IRP1 and labile iron pool. *Free Radic Biol Med.*; **33**: (Suppl. 2) S378.

Kvam E, Hejmadi V, Ryter S, Pourzand C, Tyrrell RM. (2000) Heme oxygenase activity causes transient hypersensitivity to oxidative ultraviolet A radiation that depends on release of iron from heme. *Free Radic Biol Med.*; **28**: 1191–6.

Lai CC, Huang WH, Klevay LM, Gunning WT, III, Chiu TH. (1996) Antioxidant enzyme gene transcription in copper-deficient rat liver. *Free Radic Biol Med.*; **21**: 233–40.

Lee SH, Blair I A. (2001) Oxidative DNA damage and cardiovascular disease. *Trends Cardiovasc Med.*; **11**: 148–55.

Levy JE, Jin O, Fujiwara Y, Kuo F, Andrews NC. (1999) Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nat Genet.*; **21**: 396–9.

Liochev SI, Fridovich I. (1999) The relative importance of $HO^{\cdot}$ and $ONOO^{-}$ in mediating the toxicity of $O_2^{\bullet-}$. *Free Radic Biol Med.*; **26**: 777–8.

Lipinski P, Drapier JC. (1998) Interplay between ferritin metabolism, reactive oxygen species and nitric oxide. *J Biol Inorg Chem.*; **2**: 559–66.

Martinet W, Knaapen MW, De Meyer GR, Herman AG, Kockx MM. (2002) Elevated levels of oxidative DNA damage and DNA repair enzymes in human atherosclerotic plaques. *Circulation.*; **106**: 927–32.

Matheis G, Sherman MP, Buckberg GD, Haybron DM, Young HH, Ignarro LJ. (1992) Role of L-arginine-nitric oxide pathway in myocardial reoxygenation injury. *Am J Physiol.*; **262**: H616–H620.

Matthews AJ, Vercellotti GM, Menchaca HJ, Bloch PH, Michalek VN, Marker PH, Murar J, Buchwald H. (1997) Iron and atherosclerosis: inhibition by the iron chelator deferiprone (L1). *J Surg Res.*; **73**: 35–40.

McDonald C, Phillips W, Mower H. (1965) An electron spin resonance study of some complexes of iron, nitric oxide, and anionic ligands. *J Am Chem Soc.*; **87**: 3319–26.

Meyron-Holtz EG, Vaisman B, Cabantchik ZI, Fibach E, Rouault TA, Hershko C, Konijn A M. (1999) Regulation of intracellular iron metabolism in human erythroid precursors by internalized extracellular ferritin. *Blood.*; **94**: 3205–11.

Petrat F, De Groot H, Rauen U. (2001) Subcellular distribution of chelatable iron: a laser scanning microscopic study in isolated hepatocytes and liver endothelial cells. *Biochem J.*; **356**: 61–9.

Petrat F, De Groot H, Sustmann R, Rauen U. (2002) The chelatable iron pool in living cells: a methodically defined quantity. *Biol Chem.*; **383**: 489–502.

Picard V, Epsztejn S, Santambrogio P, Cabantchik ZI, Beaumont C. (1998) Role of ferritin in the control of the labile iron pool in murine erythroleukemia cells. *J Biol Chem.*; **273**: 15382–6.

Porreca E, Ucchino S, Di Febbo C, Di Bartolomeo N, Angelucci D, Napolitano AM, Mezzetti A, Cuccurullo F. (1994) Antiproliferative effect of desferrioxamine on vascular smooth muscle cells *in vitro* and *in vivo*. *Arterioscler Thromb.*; **14**: 299–304.

Reddy BR, Kloner RA, Przyklenk K. (1989) Early treatment with deferoxamine limits myocardial ischemic/reperfusion injury. *Free Radic Biol Med.*; **7**: 45–52.

Ross R. (1999) Atherosclerosis — an inflammatory disease. *N Engl J Med.*; **340**: 115–26.

Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT. (1996) Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema. *Eur J Pharmacol.*; **303**: 217–20.

Slezak J, Tribulova N, Pristacova J, Uhrik B, Thomas T, Khaper N, Kaul N, Singal PK. (1995) Hydrogen peroxide changes in ischemic and reperfused heart. Cytochemistry and biochemical and X-ray microanalysis. *Am J Pathol.*; **147**: 772–81.

Soum E, Drapier JC. (2003) Nitric oxide and peroxynitrite promote complete disruption of the [4Fe-4S] cluster of recombinant human iron regulatory protein 1. *J Biol Inorg Chem.*; **8**: 226–32.

Stadtman ER, Berlett BS. (1997) Reactive oxygen-mediated protein oxidation in aging and disease. *Chem Res Toxicol.*; **10**: 485–94.

Steinberg D. (1993) Modified forms of low-density lipoprotein and atherosclerosis. *J Intern Med.*; **233**: 227–32.

Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL, Steinberg D. (1984) Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. *Proc Natl Acad Sci USA.*; **81**: 3883–7.

Termini J. (2000) Hydroperoxide-induced DNA damage and mutations. *Mutat Res.*; **450**: 107–24.

Traverso N. (2001) Oxidative elements in the pathogenesis of atherosclerosis. *Ital Heart J.*; **2** (Suppl 3): 37S–9S.

Trosko JE, Chang CC. (1980) An integrative hypothesis linking cancer, diabetes and atherosclerosis: the role of mutations and epigenetic changes. *Med Hypotheses.*; **6**: 455–68.

Ueno T, Yoshimura T. (2000) The physiological activity and *in vivo* distribution of dinitrosyl dithiolato iron complex. *Jpn J Pharmacol.*; **82**: 95–101.

Van Lenten BJ, Prieve J, Navab M, Hama S, Lusis AJ, Fogelman AM. (1995) Lipid-induced changes in intracellular iron homeostasis *in vitro* and *in vivo*. *J Clin Invest.*; **95**: 2104–10.

Vanin AF, Malenkova IV, Serezhenkov VA. (1997) Iron catalyzes both decomposition and synthesis of *S*-nitrosothiols: optical and electron paramagnetic resonance studies. *Nitric Oxide.*; **1**: 191–203.

Vanin A, Kleschyov A. (1999) EPR detection and biological implications of nitrosyl iron complexes. In *Nitric Oxide in Transplant Rejection and anti-tumor Defence*. Lukiewicz S, Zweier JL, eds, pp 49–82. Kluwer Academic Publishing, Boston.

Woolum JC, Tiezzi E, Commoner B. (1968) Electron spin resonane of iron-nitric oxide complexes with amino acids, peptides and proteins. *Biochim Biophys Acta.*; **160**: 311–20.