Iron and Oxidative Stress in Pregnancy[1,2]

Esther Casanueva* and Fernando E. Viteri†**[3]

*Instituto Nacional de Perinatología, Mexico, DF, Mexico, †Department of Nutritional Sciences and Toxicology, University of California, Berkeley and **Children's Hospital Oakland Research Institute, Oakland (CHORI), CA 94720

ABSTRACT Pregnancy, mostly because of the mitochondria-rich placenta, is a condition that favors oxidative stress. Transitional metals, especially iron, which is particularly abundant in the placenta, are important in the production of free radicals. Protective mechanisms against free radical generation and damage increase throughout pregnancy and protect the fetus, which, however, is subjected to a degree of oxidative stress. Oxidative stress peaks by the second trimester of pregnancy, ending what appears to be a vulnerable period for fetal health and gestational progress. Conditions restricted to pregnancy, such as gestational hypertension, insulin resistance and diabetes, exhibit exaggerated indications of free radical damage. Antioxidants as well as avoidance of iron excess ameliorate maternal and early fetal damage. In rats both iron deficiency and excess result in free radical mitochondrial damage. Estimates of gestational iron requirements and of the proportion of iron absorbed from different iron supplemental doses suggest that with present supplementation schemes the intestinal mucosal cells are constantly exposed to unabsorbed iron excess and oxidative stress. Unpublished work carried out in Mexico City with nonanemic women at midpregnancy indicates that 60 mg/d of iron increases the risk of hemoconcentration, low birth weight and premature birth and produces a progressive decline in plasma copper. These risks are not observed in women supplemented with 120 mg iron once or twice per week. Studies on the influence of iron supplementation schemes on oxidative stress are needed. J. Nutr. 133: 1700S–1708S, 2003.

KEY WORDS: • *iron* • *supplementation* • *pregnancy* • *oxidative stress*

Pregnancy is a condition exhibiting increased susceptibility to oxidative stress, defined here as a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage (1,2). Pregnancy is characterized by dynamic changes in multiple body systems resulting in increased basal oxygen consumption and in changes in energy substrate use by different organs including the fetoplacental unit. From early pregnancy the human placenta influences maternal homeostasis; it is rich in mitochondria and when fully developed consumes about 1% of the basal metabolic rate of the pregnant woman (1). It is also highly vascular and is exposed to high maternal oxygen partial pressure. These characteristics explain, in part, the generation of superoxide, because about 5% of all electrons in the mitochondrial respiratory chain leak out of the mitochondria (3).

The human placenta is hemomonochorial, meaning that only one chorionic cell layer exists between maternal and fetal bloods, favoring exchanges of gases, nutrients and metabolic products. The release of oxygen from maternal hemoglobin (Hb)[4] is favored by the lower partial pressure of oxygen in the placental cellular structure and fetal circulation (rich in fetal hemoglobin), which has a greater affinity for oxygen, and by the release of fetal and placental metabolites (the placenta produces abundant lactic acid), which lower blood pH causing a displacement of the Hb dissociation curve favoring oxygen delivery (1). Initially the placenta, has a hypoxic environment. As it matures and its vascularization develops, it changes to an oxygen-rich environment and its abundant mitochondrial mass favors the production of reactive oxygen species (ROS), which increases free iron liberated from iron-sulfur clusters (4).

Nitric oxide (NO) is also locally produced by the placenta (5) and together with other reactive nitrogen species contributes to potential oxidative stress in the presence of transition metals. The placenta is also rich in macrophages favoring

[1] Manuscript prepared for the USAID-Wellcome Trust workshop on "Nutrition as a preventive strategy against adverse pregnancy outcomes," held at Merton College, Oxford, July 18–19, 2002. The proceedings of this workshop are published as a supplement to *The Journal of Nutrition*. The workshop was sponsored by the United States Agency for International Development and The Wellcome Trust, UK. USAID's support came through the cooperative agreement managed by the International Life Sciences Institute Research Foundation. Supplement guest editors were Zulfiqar A. Bhutta, Aga Khan University, Pakistan, Alan Jackson (Chair), University of Southampton, England, and Pisake Lumbiganon, Khon Kaen University, Thailand.

[2] This study was partially funded by grants from the MEXUS Program (U. of California/CONACYT Mexico), the Center for Latin American Studies, and the Agricultural Experimental Station, College of Natural Resources, U. of California at Berkeley.

[3] To whom correspondence should be addressed. E-mail: viteri@nature.berkeley.edu.

[4] Abbreviations used: CDC, Centers for Diseases Control and Prevention; Hb, hemoglobin; INACG/WHO/UNICEF, International Nutritional Anemia Consultative Group, World Health Organization and United Nations Children's Emergency Fund; NHANES, National Health and Nutrition Examination Survey; NO, nitric oxide; NTBI, non-transferrin-bound iron; ROS, reactive oxygen species; RNS, reactive nitrogen species; RCIS reactive chlorine species; SOD, superoxide dismutase; Tbars, thiobarbituric acid-reactive substances.

the local placental production of free radicals, including reactive chlorine species (RClS) in which free iron is also implicated (6). Immune functions, in general, are reduced during pregnancy possibly as a consequence of high estrogen levels (7), and clinical as well as silent infections including severity of malaria in primiparous women are more common than in the nonpregnant state (8,9).

There is no question that the pregnant state carries a risk for infection: about 18% of apparently normal pregnancies and >33% of premature deliveries present evidence of chorioamnionitis (10,11). Placental macrophages in the presence of infection are a source of NO, tumor necrosis factor-α and other cytokines that induce mitochondrial alterations and production of free radicals (10–13). Micronutrient deficiencies can reduce immunity even further (14), enhancing these alterations. Inflammatory processes are exacerbated by free intracellular iron, which induces $O_2^{\cdot -}$, $NO^{\cdot}$ hypochlorous acid and peroxynitrite formation (6). Inflammatory cytokines (interleukin-1β) initially increase free iron, possibly releasing it from ferritin or mitochondria, and then increase ferritin synthesis, all of which is regulated by iron regulatory elements and proteins (15,16). In general, iron chelation and free radical scavenging markedly reduce the inflammatory process, stressing the role of these elements in inflammation (17).

Evidence of oxidative stress and defense mechanisms in pregnancy

Thiols (mainly reduced glutathione, which can be oxidized and produce ROS in an environment rich in Fe^{3+}) have been found elevated in erythrocyte lysates (18). Superoxide dismutase (SOD) activity in erythrocytes and plasma thiol levels were found to be lower during pregnancy and ceruloplasmin levels were found to be higher during pregnancy than in nonpregnant women, suggesting an oxidative environment and stress (18,19). Other specific changes in indicators of oxidative stress and in oxidative defense mechanisms evolve as pregnancy advances: Serum levels of products of lipid peroxidation such as conjugated diene double bonds, fluorescent chromolipids and thiobarbituric acid-reactive substances (TBARS) increase in normal pregnant women, reaching their maximal concentrations in the second trimester and then decline until term, when levels similar to those in the first trimester are observed (20,21); compared with values for nonpregnant women, extracellular SOD activity has been found lower (21,22). *S*-Nitrosoalbumin and *S*-nitrosothiols, which are important circulating reservoirs of NO, increase in pregnancy and even more in preeclampsia compared with the follicular stage of the nonpregnant state (23). Ascorbic acid and copper are necessary in the plasma for these compounds to deliver NO to endothelial tissue (23), making them either reducing or oxidizing agents, vasodilators or vasoconstrictors. Their influence as antioxidants (reducing agents) on total antioxidant capacity is relatively weak (24). Plasma ascorbic acid concentration varies by intake but generally decreases in normal pregnancy, severely so in preeclampsia (25–27). These changes in the face of high NO production in normal as well as in preeclamptic placenta (5,28) and in human and experimental diabetes (29) suggest that excessive circulating total and inducible NO bound in nitriosothiols contribute to oxidative stress in pregnancy. These and other metabolites derived from free radicals are exaggerated in gestational hypertensive diseases and diabetes (30,31). TBARS in the placenta and in fetal liver are also elevated, especially early in pregnancy, those in fetal liver being significantly lower than those in the placenta (20).

Studies involving placental perfusion reveal that lipid-peroxide secretion, measured by TBARS, responds stoichiometrically with *t*-butyl hydroperoxide perfusion levels, the effluent on the fetal side containing significantly less lipid peroxides than the effluent on the maternal side. Aspirin blocks this response indicating that cyclooxygenase is involved in placental lipid peroxide production (32,33). Investigations measuring the mitochondria as a potential source of lipid peroxides also indicate that $O_2^{\cdot -}$ is the active free oxygen radical in the placenta; implicate transition metal contents in that reaction; and suggest that a significant part of the total indicators of pregnancy oxidative stress is derived from the placenta's significant mitochondrial mass, which increases with gestational age (34).

Defense mechanisms against free radical damage are also enhanced as pregnancy progresses. Placental homogenates and syncytiotrophoblastic brush border preparations from interrupted pregnancies, early, at midgestation and at term, show progressive increments in free radical scavengers such as bilirubin and glutathione as well as in the specific activities of SOD, catalase and glutathione peroxidase and reductase (20,35,36). Glutathione peroxidase in erythrocytes and platelets and extracellular SOD activity have also been found to increase progressively throughout gestation up to the third trimester, possibly as a response to increased presence of $O_2^{\cdot -}$ (21,22). In normal pregnancy the ratio of prostacyclin to thromboxane favors prostacyclin, suggesting an effective defense system against oxidative stress (37).

The role of vitamins C and E in preventing free radical damage is well known and their nutritional adequacy is important in pregnancy. Vitamin C deficiency affects placental structure and ROS and facilitates placental infection, all of which result in increased risk of premature rupture of placental membranes and premature births (10,38). The placenta is avid to absorb vitamin C so that when maternal plasma ascorbic acid concentration is low it is absorbed by active mechanisms. At higher plasma ascorbic acid concentrations it enters the placenta by passive diffusion. Curiously, ascorbate is preferentially taken up in the oxidized form (dehydroascorbic acid), which more easily passes the lipid layer, but is transformed to its reduced form at the expense of other reducing agents, which donate their electrons before it is transferred to the fetus by active transport (39). Generally, α-tocopherol contents decrease in the total placenta and in the syncytiotrophoblastic brush border membrane as pregnancy progresses but vitamin E ingestion can elevate it (40,41).

The placental environment is one of enhanced oxidative stress that induces protective mechanisms against free radicals as gestation progresses. Overall, the plasma free radical trapping and antioxidant potential are able to counteract oxidative stress in normal pregnancy through enzymatic induction and activity (i.e., catalase, SOD, glutathione peroxidase, transferase and reductase, glucose 6–phosphate dehydrogenase) as well as through nonenzymatic free radical protectors and scavengers (i.e., vitamins C and E, uric acid, protein thiols) (24,37,40,42–47). However, pregnancy is a state where this adaptation and equilibrium are easily disrupted as evidenced by the propensity toward the development of gestational hypertension and insulin resistance that in some extreme cases can lead to gestational diabetes. Gestational hypertension and diabetes are often associated. Hyperglycemia itself can favor nonenzymatic glycation, which can induce ROS formation in the presence of reactive transitional metals. It also induces reductions in serum transferrin and in the activities of CuZnSOD and of ceruloplasmin ferroxidase (6,48–50). Evidence of elevated levels of intracellular iron and ROS in insulin resistance (51)

and diabetes and the prevention of diabetic fetal anomalies by the reduction of iron and oxidative stress reinforces this concept (52–55).

The little existing data strongly suggest that the fetus is somewhat protected from placental ROS, particularly during the last few weeks of pregnancy (56). However, there is special concern about conditions that can induce ROS during the organogenesis phase. Embryos, fetuses and children of diabetic mothers have a high proportion of anomalies that have been attributed to oxidative stress, hyperglycemia-induced ROS, mitochondrial oxidation and nonenzymatically induced glycosylation together with essential fatty acid deficiencies due to peroxidation, prostaglandin alterations and possibly other undefined mechanisms (57,58). Importantly, the effects of diabetes in the embryo in mice are prevented by insulin treatment as well as by the overexpression of CuZnSOD in transgenic mice (53). Supplementation with vitamins C and E also reduces the rate of malformations in diabetic rats (54,55). Few studies have demonstrated a correlation between maternal and fetal oxidative stress, the latter based on determinations in umbilical cord blood and infant's blood up to age 3 d, particularly in premature infants (59–61).

Oxidative stress, origins and detection methods

A comprehensive review of this topic can be found in Halliwell and Gutteridge (6), from where many of the concepts and statements in this section have been summarized.

Normal mitochondrial respiration constantly leaks $O_2^{\cdot -}$, and monoamine-oxidase activity constantly produce H_2O_2 (2,62). Iron or copper in a reactive form produce free radicals (62) mainly through catalyzing the Haber-Weiss reaction generating hydroxyl radicals $OH^{\cdot}$:

$$H_2O_2 + O_2^{\cdot -} \longrightarrow O_2 + OH^{\cdot} + OH^{-}.$$

$O_2^{\cdot -}$ role in the iron or copper catalyzed Haber-Weiss reaction is the superoxide-assisted Fenton reaction:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{-} + OH^{\cdot}.$$

ROS, reactive nitrogen species (RNS), (RClS)—generically called free radicals—together with other species containing one or more unpaired electrons independent of their route of origin (radiation, excess free transition-metal ions, infection, etc.) damage cellular DNA, proteins and lipids (6,63).

Neither $O_2^{\cdot -}$ nor H_2O_2 reacts with DNA but H_2O_2 crosses the mitochondrial and plasma membranes reaching DNA, where bound transition metals produce in situ $OH^{\cdot}$, causing DNA damage. Elevated tissue level or excretion of oxidized bases such as 8–hydroxy-deoxyguanosine is one important indicator of DNA damage caused by the iron- or copper-mediated Fenton reaction. Single- and double-strand DNA breaks occur and can be detected by various assays (64).

Protein-carbonyls also result from the interaction of free radicals with amino acid residues (especially arginine, histidine, lysine and proline) oxidating sulfhydryl groups and hydroxylating tyrosine and phenylalanine. Some of these products can also be measured as markers of free radical damage (65).

Fe^{2+} or Cu^{+} reacting with unsaturated fatty acids in the presence of O_2 can initiate a lipid peroxidation cascade in biological membranes and lipoproteins by the production of highly reactive $OH^{\cdot}$. Therefore other iron-based or transition-metal-based radicals may be responsible for initiating the peroxidation of lipids and the formation of conjugated diene double bonds, fluorescent chromolipids and alkoxyl and peroxyl radicals that can propagate lipid peroxidation to final end products such as pentane, ethane, malondialdehyde, hexanal, isoprostanes etc. Iron may also favor or enhance free radical damage caused by other agents such as RNS or hyperglycemia through glycation (66,67).

Many of the aldehydes produced through lipid peroxidation can also produce DNA damage by acting as free radicals. Measurements of these products are used as indicators of lipid peroxidation in organs, plasma, breath and urine. The most commonly used assay is that which detects TBARS (68). Urinary excretion of several of these metabolites as well as ethane and pentane exhalation rates in breath attempt to measure total body lipid peroxidation (68,69); the tissue concentrations of these products reflect local damage.

Oxidative stress in pregnancy and its relation to iron

The maternal surface of the trophoblastic microvilli of the human placenta is very rich in transferrin receptors. Between the microvilli there are coated pits believed to result from receptor-mediated endocytosis, where low pH releases iron from maternal transferrin into the cytoplasm and it is transferred to the syncytiotrophoblast and into the fetus (1). As pregnancy progresses, different mechanisms enhance the transfer of iron to the placenta and the fetus (70,71). These include the thinning of the syncytiotrophoblast and increments in placental blood flow and in transferrin receptors as pregnancy progresses. In the placenta of the guinea pig, which is also hemomonochorial, the transfer of maternal iron to the syncytiotrophoblast is very fast, suggesting that non-transferrin-bound iron (NTBI) may be involved in placental transfer of iron (72). However, we are not aware of any studies directly exploring this nor whether more NTBI reaches the placenta when high daily iron supplements are ingested or in other circumstances. It is important to note that elevated NTBI has been detected shortly after the ingestion of iron supplements in plasma and in umbilical cord blood (73).

The oxidative stress and in vivo consequences caused by reactive iron species (mostly free iron) have been studied in detail. Many of the deleterious effects of hemochromatosis and other chronic iron-overload conditions, some cancers, aging, neurodegenerative diseases and atherosclerosis are attributed to or contributed by them. ROS damage is observed in and explains many of the effects secondary to temporary iron excess as in acute iron intoxication, hemolytic episodes and reperfusion injury (74).

The effects of iron excess can be generalized or local. Generalized iron excess is observed, for example, in chronic primary and transfusion iron overload. Chronic intake of supplements containing high amounts of iron has been also described as a rare cause of generalized iron overload (75,76).

Local iron excess and iron-mediated oxidative stress have been demonstrated in the intestinal mucosa, liver, spleen, bone marrow and placenta. When exposed to excess intake of iron, the intestinal mucosa retains a large proportion of it as ferritin, which ameliorates the possible effects of excessive free iron. However, it is vulnerable to oxidative damage secondary to the continuous presence of a relatively small excess of iron intake (77). Intestinal mucosal iron accumulation leading to intestinal abnormalities and injury was observed in patients receiving therapeutic iron (78). In rats receiving daily iron doses equivalent to 120 mg/d in humans, iron accumulation, mucosal necrosis, reduction of microvillus height (and even complete mucosal erosion) and evidence of oxidative stress have been demonstrated (79). The production of hydroxyl and methoxyl radicals in both the luminal and mucosal contents of the gastrointestinal tract verify the role of iron in free radical

damage. These abnormalities appeared more prominently in previously iron-deficient animals, and supplementation with α-tocopherol or a combination of α-tocopherol and ascorbic acid protected these animals (80–82).

The process of translation of mucosal iron from the intestinal cell to the liver is a second barrier to iron overload. This barrier consists of specific mechanisms at the basal cellular membrane of the intestinal mucosal cell and an excess of circulating transferrin. Under usual conditions, transferrin binds the iron leaving the cell and entering the circulation, thus avoiding the entrance of free iron. However, this mechanism appears to be overwhelmed by amounts of passively diffused iron when large iron boluses are presented to the intestine, and NTBI may reach the liver (73).

Iron-normal and iron-deficient rats were subjected to twice daily iron supplementation for 24 d providing a total daily dose 10 times their normal food iron intake (comparable to 120 mg/d doses in humans); initially the rats absorbed 10% and 21% of the supplemental iron, respectively, measured by whole-body counting. Absorption declined logarithmically to stabilize at approximately 6% by day 7 of supplementation in both groups of rats. When the rats were killed on day 24, duodenal mucosa was elevated 5–6 times, ileal mucosa 2–4 times and liver 3–4 times above the levels observed in normal, unsupplemented rats. Liver iron was already normal or slightly above normal by the supplementation day 3, after which the previously iron-deficient rats always showed higher liver iron levels that continued to increase beyond the normal levels (83). These results support the continuous absorption of a fraction of supplemental iron at a rate beyond normal (perhaps by passive diffusion mass effect) resulting in excessive liver iron levels, particularly in the previously deficient animals. This raises the question of whether previously iron-deficient pregnant women are more susceptible to oxidative stress resulting from excessive iron absorption, particularly when given daily pharmacological doses of iron (84).

A small amount of iron bound to small molecules (i.e., citrate, ATP, GTP and several phosphate esters) has been identified in the process of intestinal iron absorption and delivery, plasma transport, internalization and storage of iron to liver cells and to other iron-requiring cells. It is also present in the events involved in the cellular release of iron from stores. These iron species constitute free iron, the labile iron pool or the low-molecular-mass iron pool and are highly reactive, leading to the formation of ROS, nitric oxide synthase and RClS (85). A series of cellular mechanisms reduce the presence of free iron to a minimum, including the synthesis of ferritin, the binding of iron responsive elements to RNA and their binding to iron regulatory intracellular binding proteins and the transferrin binding of iron from intestinal cells.

Plasma free-iron is undetectable in healthy adults but is present in chronic inflammatory conditions, hemochromatosis and other forms of iron overload, soon after the ingestion of an iron bolus, and, curiously, in neonates at term or born prematurely (73,86), particularly in the latter, where higher levels of malondialdehyde and hypoxanthine also suggested elevated oxidative stress (87). The importance of the presence of free iron in blood and the mechanisms involved in these phenomena are not well understood given all the safeguards against its presence in the circulation.

Oxidative stress and damage have also been found in iron deficiency. Iron-deficient in comparison with iron-normal rats exhale more ethane and pentane per unit time and have higher levels of malondialdehyde in liver and kidney, defective liver mitochondrial functions and mitochondrial DNA damage (69,88).

In summary, both localized and generalized iron excess as well as deficiency are situations where free radical damage has been observed and can lead to functional disturbances and foster genetic alterations.

Iron needs in pregnancy and recommendations on iron supplementation

The amount of iron that would have to be absorbed to satisfy the gestational needs has been estimated to be 4–5 mg/d during the second trimester and 6–7 mg/d during the third trimester (89,90). These needs may be met in part by mobilizing available reserves of iron and a beneficial effect of pre-pregnancy iron reserves on iron status during pregnancy has been reported. Kaufer and Casanueva grouped healthy Mexican women by prepregnancy serum ferritin levels and followed them periodically throughout pregnancy and postpartum. Only women who developed anemia were subsequently supplemented with iron. Women with prepregnancy ferritin levels >20 μg/L did not have a marked decline in serum ferritin throughout the course of pregnancy. Their mean values were higher than levels indicative of iron deficiency and they did not develop anemia, in contrast to women whose prepregnancy serum ferritin was <20 μg/L (91). Most women in the developing world do not have adequate iron reserves (92). Even among U.S. women of childbearing age, the prevalence of depleted iron stores, iron deficiency and anemia are common. The National Health and Nutrition Examination Survey (NHANES) III data set revealed that ≈15% had depleted iron stores (serum ferritin <15 μg/L), ≈20% had percent saturation of transferrin <16, and ≈10% had Hb levels <120 g/L (93). The median iron intake of non-pregnant women of childbearing age was ≈11.2 mg/d and of 346 pregnant women was 15.3 ± 0.75 mg/d (mean ± SE) (94).

The average estimated iron absorption from an adequate diet with intermediate bioavailability in iron-sufficient women is ≈10%. Iron absorption from this type of diet is estimated to be ≈18% in individuals with depleted iron stores. However, data on iron absorption during pregnancy are inconsistent (92). Given these estimates of need for absorbed iron, the general inadequate iron reserves in women of childbearing age and the quality of the diet in most low socioeconomic-status women especially in developing nations, it is not surprising that food alone can barely satisfy the most conservative estimated needs for iron in the second half of pregnancy. This has led to the recommendation that pregnant women should receive a daily supplement of iron, between 60 and 120 mg/d, which to a large extent is guided by a therapeutic approach to the correction of iron deficiency anemia, using pharmacological doses. Authoritative international groups, such as the International Nutritional Anemia Consultative Group and the World Health Organization, have recommended universal supplementation from the second trimester onwards (95,96). The regimen recommended for preventing iron deficiency anemia is supplementation with 60 mg/d from as early as possible in pregnancy, but no later than the third month, and continuing for the rest of pregnancy. Further, where the prevalence of anemia is >40%, it is recommended that iron supplementation continue for 3 mo postpartum. For severe anemia in pregnancy, the recommendation is supplemental iron, 120 mg/d, for 3 mo, after which the preventive supplementation regimen continues. Compared with an estimated 20% of pregnant women in developed countries, around 55% of pregnant women in developing countries are anemic (96), and would thus qualify for continuation of supplementation with iron in the postpartum period. Given that many women present beyond the

4th month of pregnancy many would qualify to receive 120 mg iron/d.

The U.S. Institute of Medicine (97) recommends a flexible approach to supplementing pregnant women, ranging from no supplementation during the first two trimesters when serum ferritin is >20 μg/L and Hb levels are above the fifth percentile of the Centers for Diseases Control and Prevention (CDC) (98) norms to 120 mg/d at any gestational age if Hb is >90 and <109 g/L. The daily dose recommended for women at risk of iron deficiency is 30 mg/d (serum ferritin <20 μg/L and Hb within normal limits). This level of supplementation applies also to all non-anemic women consulting for the first time in the third trimester. It recommends stopping iron supplementation at delivery unless women are anemic in the third trimester.

Interpolation of the percent absorption of supplemental iron (ferrous sulfate) ingested daily on an empty stomach during the last two trimesters of pregnancy from that reported by Hahn et al. (99) results in the following for the second and third trimesters, respectively: for 30 mg/d of iron, 20% and 26%; for 60 mg/d, 11% and 14%; and for 120 mg/d, 7.5% and 8.5%. The total absorbed iron in the last 6 mo of pregnancy from supplements alone would amount to 900, 1215 and 1530 mg from doses of 30, 60 and 120 mg/d, respectively. If 1 mg food iron is absorbed daily throughout pregnancy as a consequence of depressed food iron absorption in the first trimester and during supplementation (100), an additional ≈280 mg of iron would be absorbed, increasing the total iron absorbed to 1180, 1495 and 1810 mg, thus surpassing the needs with either dose. Under these conditions, the daily unabsorbed iron remains in the intestine until excreted in the feces, amounting to ≈14 mg/d from food and ≈25, 51 and 110 mg/d from the 30-, 60- and 120-mg supplemental doses, respectively.

If moderate anemia were present, erythropoiesis rate would be able to increase initially up to three times its normal rate, diminishing as normal iron status is approached. In a 55 kg normal woman, bone marrow utilization of iron would be ≈12 mg/d and would be able to increase initially to ≈36 mg/d. Late in pregnancy, about 72% of the absorbed iron is incorporated into Hb in supplemented women (101).

During pregnancy, iron deficiency anemia of uncomplicated nutritional origin is most often mild (Hb usually >95–110 g/L). Concern has been raised about the extent to which unabsorbed luminal iron might exert adverse effects, leading to the proposition that intermittent supplementation regimens might limit the ongoing exposure of the intestinal mucosa to high luminal iron contents compared with daily iron supplementation (102,103). The efficacy and safety of weekly doses of iron in preventing iron deficiency and correcting mild anemia to safe maternal and perinatal levels has been demonstrated (102–104). The extent to which these findings can be generalized needs to be determined as weekly dosing has not been found to be more efficacious than daily dosing for improving anemia in pregnant women in all situations, and it may be that the response is not the same for all locations, depending upon variability in other background factors (105,106). However, there is increasing recognition that the current international recommendations for iron supplementation in pregnancy may be higher than necessary (106). In all this work one important point that deserves more detailed consideration is the extent to which the administration of supplemental iron might lead to a significant increase in the amount of unabsorbed iron remaining in the intestine, which would be undesirable were it to lead to a greater degree of local oxidative stress. We are concerned that if the current international recommendations are applied world wide (95), and most pregnant women receive daily iron, a high amount of unabsorbed iron will remain in the lumen of the intestine, even in those women with mild or moderate anemia, in whom iron absorption is elevated. Persistent high levels of iron in the intestinal lumen would be undesirable according to current evidence on oxidative stress.

Can excessive oral iron supplementation during pregnancy produce iron overload?

In the context of permanent whole body iron overload, excessive oral iron supplementation during pregnancy cannot produce iron overload unless the person receiving the supplements has genetic alterations in the regulation of iron absorption and metabolism. Chronic ingestion of bioavailable iron in daily doses in clear excess of the upper tolerable intake level (45 mg/d) can induce iron overload, although this condition is rare (75,76). However, temporary iron overload could be produced given the passive diffusion of soluble iron molecules, particularly if transported to the liver as NTBI. Importantly, NTBI uptake by hepatocytes is independent of the iron content of the liver, is very rapid, more efficient than the liver uptake of transferrin-bound iron, requires energy and Ca, is competitively inhibited by Zn, and is saturable (72). The finding that iron deficient supplemented rats continue to accumulate liver iron, as indicated before, could mean that NTBI plays a role in the recovery from iron deficiency. If this is the case, a greater oxidative stress would result under these conditions.

Hematological condition, iron nutrition and supplementation during gestation: when are they critical and safe?

Iron nutrition and hematological status are critical throughout pregnancy for mother, fetus, and beyond. Epidemiological evidence has shown an association between anemia before gestational weeks 24–26, and premature delivery and low birthweight (102,107–109). Moreover, mild anemia according to the CDC criteria (Hb levels between 90–110 g/L (98) after gestational week 30 has demonstrated no effect on these parameters. On the contrary, birth weight and overall health of newborns is optimal between Hb levels >90 and <130 g/L at term (110–112).

A specific association between iron deficiency anemia (low Hb and serum ferritin values) early in pregnancy and premature delivery has been found, in contrast to anemia from other causes, following the WHO (96) and CDC (98) anemia cut-off points. Women ingesting pre-pregnancy and/or gestational supplements prior to gestational week 28 containing 65 mg of iron and including folic acid, 1 mg; zinc, 25 mg; and calcium, 200 mg, had significantly fewer low and very low birth weight babies, and preterm and very preterm deliveries than unsupplemented women. Supplementation starting in the first trimester was more effective than that staring in the second trimester and adjusted odds ratios for low birth weights were even more favorable (0.14; 95% confidence limits: 0.05–0.40) when supplements were ingested both prior to pregnancy and early in pregnancy, demonstrating a favorable additive effect (113).

Perinatal maternal and fetal complications have been found to increase exponentially under extreme conditions once Hb values decrease further below 90 g/L. These values are observed almost exclusively in populations where there are chronic blood losses, malaria or other hemolytic conditions.

The other extremes, those of high Hb and ferritin levels have also a pronounced negative effect on the course and

product of pregnancy. Several studies have demonstrated negative effects associated with high Hb levels early in pregnancy, as well as in situations where Hb levels fail to show the decline secondary to physiological hemodilution after gestational week 20. The incidence of gestational hypertension, preeclampsia, eclampsia, low birth weight, and low Apgar scores increase rapidly when Hb levels surpass 130 g/L (114,115). Even Hb levels between 125 and 130 g/L at term constitute a perinatal risk that increases as Hb levels rise further. Failure of adequate plasma expansion, mostly from malnutrition, or genetic conditions have been blamed for hemoconcentration. However, Hytten and Leitch (111) acknowledge the fact that large doses of iron (100 mg/d or higher) can result in Hb concentrations as high as prior to pregnancy without exhibiting the "physiologic fall" and consider this effect as atypical. They suggest that this is a specific pharmacological effect not due to the correction of a deficiency. Lund and Donovan (114) also indicate, based on their data, that "red cell volume can be forcibly elevated by the addition of therapeutic iron compounds" in pregnancy.

Similar negative effects (very preterm delivery, infection, low birth weight and poor neonatal vitality and health) have been observed when serum ferritin levels are elevated in the third trimester. Importantly, as in the case of Hb, what appears to be critical is the increment or the lack of the expected decrease in serum ferritin concentration after gestational week 20 (116, 117). Apparently, these situations can arise from any condition that limits erythropoiesis and/or iron utilization (i.e., folate and vitamin B-12 deficiency, especially earlier in pregnancy, or infection in the course of pregnancy).

Casanueva et al. conducted a study in Mexico City and a summary is available in a non peer-reviewed publication (117). In that study, healthy, singleton pregnant women, nonanemic at gestational week 20, were randomly assigned to one of three supplementation groups up to delivery and followed every 4 wk up to 24 wk postpartum. One group received daily 60 mg iron as ferrous sulfate, 0.2 mg of folic acid and 1 μg of vitamin B-12 from week 20 to term. This group was compared with women receiving the tablets intermittently (two tablets once or twice weekly). Important outcomes were: *1*) No woman in either group had Hb $<$103 g/L (equivalent to 93 g/L at sea level at Mexico's City altitude) at any time during pregnancy or postpartum. Therefore both the daily and intermittent regimens were effective in preventing gestational and perinatal risk because of low Hb. *2*) In the daily group 61% developed Hb $\geq$135 g/L (equivalent to $\geq$125 g/L at sea level). In the intermittent group only 13% achieved such high levels. If the risk of lower reproductive performance begins at about 135 g/L at Mexico City's altitude, daily supplementation carries a greater risk than intermittent supplementation. *3*) The relative risks (RR) of mothers delivering a low-birth-weight infant ($<$2500 g) or prematurely (delivery $<$37 wk) were higher for women with Hb $>$145 g/L at weeks 20 to 28, reaching statistical significance at week 28 (for low birth weight, RR 7.1; 95% confidence limits: 1.1–45.9 and for premature births, RR 8.5; 95% confidence limits: 1.7–42.6); both risks were low after week 32 independent of Hb levels. *4*) Mean (geometric) plasma ferritin from gestational week 24 to12 wk postpartum was significantly higher in the daily group although high plasma ferritin values ($>$30 μg/L during pregnancy and $>$50 μg/L after birth) were uncommon. Oxidative stress may be involved in these results: high Hb levels can reduce placental perfusion and high ferritin levels postpartum may reflect temporary iron excess. *5*) Plasma copper level fell in the daily supplemented women during pregnancy whereas it remained stable in the intermittent group, suggesting impaired copper absorption when the intestine has a continuous high supply of iron. Plasma zinc also declined in the daily group but did not reach significance when compared with the intermittent group.

What is of particular interest to us is the rapid decline in plasma ferritin values postpartum in the daily group. Its geometric mean dropped from 35.2 μg/L at week 4 to 22.6 and 16.4 μg/L at weeks 12 and 24, respectively, while the intermittent values remained stable at 14.9, 14.2 and 13.6 μg/L, respectively. If a value of 8 mg iron in reserves is assigned for each 1 μg/L of plasma ferritin, the daily group lost an average of 101 mg iron reserves between postpartum weeks 4 and 12 (average 1.8 mg/d of iron). This amount is much larger than that which can be explained by lactation ($\approx$0.3 mg/d) plus the daily losses during this period of lactational amenorrhea, totaling a requirement of $\approx$1.1 mg/d. These women lost, on average, 50 mg iron reserves in the next 12 wk (average 0.6 mg/d above estimated requirements). Viteri et al. (118) observed a similar rapid decline of plasma ferritin after stopping 3 mo of daily iron supplementation. Possible explanations for these findings are that the high plasma ferritins after daily iron supplementation inhibit food iron absorption or that they reflect, at least partially, an inflammatory process secondary to excess iron and oxidative stress that recedes with time.

Can iron supplementation prevent as well as induce oxidative stress

The question that should be addressed is whether presently recommended iron supplementation schemes, doses and timing during pregnancy can reduce oxidative stress by correcting iron deficiency or can produce it by creating a condition of temporary iron overload. What are the benefits and the risks attributable to current practices?

Direct evidence in this regard is almost nonexistent. To our knowledge, the only publication partially addressing this issue is that of Lachili et al. (119) who gave a daily supplement of 100 mg iron plus 500 mg vitamin C to 27 healthy women in the third trimester of pregnancy and compared them with 27 healthy unsupplemented control subjects. Besides hematological levels, they measured many indicators of micronutrient nutrition and oxidative stress defense mechanisms. Initially both groups were similar but delivery occurred at weeks 37 $\pm$ 2 and 39 $\pm$ 2 in the supplemented and control groups, respectively, and Hb and serum iron levels were significantly higher in the former. Mean + 1SD Hb level was 142 g/L. The only other measurements that were different were plasma vitamin E and the ratio of vitamin E to cholesterol, which were lower, and TBARS and the ratios of TBARS to cholesterol and TBARS to vitamin E, which were elevated in the supplemented group. This work leaves many unanswered questions including the possible effects of vitamin C when consumed with iron at the doses given.

Recommendations for further research

- Insulin resistance, gestational diabetes and hypertension have been associated with high plasma ferritin and biological evidence of oxidative stress (51,115). However whether antenatal iron supplementation exacerbates or is primarily responsible for inducing these syndromes in healthy nonanemic women or in women recovering from iron deficiency or being treated with excessive iron doses still needs to be answered. Also unanswered are the mechanisms by which excessive iron supplementation in pregnancy can induce hemoconcentration.
- A thorough investigation of iron supplementation schemes in different populations, some with hemoglobinopathies, is

needed for the safe prevention and correction of iron deficiency in pregnancy and in populations at risk of iron deficiency or excess.

- Populations subjected to chronic infections that induce oxidative stress and metabolic alterations through cytokine production should also be the subject of investigations regarding the safety of different interventions for the prevention of iron deficiency. Reported increments of clinical malaria and parasite counts with daily iron supplementation in malarial regions (120) may be associated with excessive iron doses. In contrast, concurrent intermittent malaria prophylaxis and iron supplementation at recommended weekly doses appears safe (121).
- The combination of diet-based interventions, including different food fortification strategies, perinatal practices and control of infections must also be explored in different populations not only regarding their effectiveness in correcting iron deficiency and anemia but also their short- and long-term safety.

LITERATURE CITED

1. Sies, H. (1991) Oxidative stress II. Oxidants and antioxidants. Academic Press, London.
2. Page, K. R. (1993) The physiology of human placenta. pp. 164. UCL Press Limited, London.
3. Fridovich, I. (1979) Hypoxia and oxygen toxicity. Adv. Neurol. 26: 255–259.
4. Liochev, S. I. & Friedovich, I. (1997) How does superoxide dismutase protect against tumor necrosis factor: a hypothesis informed by effect of superoxide on "free" iron. Free Radic. Biol. Med. 23: 668–671.
5. Dotsch, J., Hogen, N., Nyul, Z., Hanze, J., Knerr, I., Kirschbaum, M. & Rascher, W. (2001) Increase in endothelial nitric oxide synthase and endothelin–1 mRNA expression in human placenta during gestation. Eur. J. Obstet. Gynecol. Reprod. Biol. 97: 163–167.
6. Halliwell, B. & Gutteridge, J. M. (1999) Free radicals in medicine and biology. 2nd edition. Clarendon Press, Oxford.
7. Carr, R. B. (1990) The fetal maternal placental unit. In: Principles and practice of endocrinology and metabolism (Becker, K. L., Ed.), p. 788. J. B. Lippincott, Philadelphia
8. Thong, Y. H., Steele, R. W., Vincent, M. M., Hensen, S. A. & Bellanti, J. A. (1973) Impaired in vitro cell mediated immunity to rubella virus during pregnancy. New Engl. J. Med. 28: 604–606.
9. Kochar, D. K., Thanvi, I., Joshi, A., Subhakaran, Z. Z., Aseri, S. & Kumawat, B. L. (1998) Falciparum malaria and pregnancy. Indian J. Malariol. 35: 123–130.
10. Romero, R. (2003) Intrauterine infection, premature birth and the Fetal Inflammatory Response Syndrome. J. Nutr. 133: 1668S–1673S.
11. Saji, F., Samejima, Y., Kamiura, S., Sawai, K., Shimoya, K. & Kimura, T. (2000) Cytokine production and chorioamnionitis. J. Reprod. Immunol. 47: 185–196.
12. Romero, R., Mazor, M., Manogue, K., Oyarzun, E. & Cerami, A. (1991) Human decidua a source of cachectin tumor necrosis factor. Eur. J. Obstet. Gynecol. Reprod. Biol. 41: 123–128.
13. Kirkeboen, K. A. & Strand, O. A. (1999) The role of nitric acid in sepsis-an overview. Acta Anaesthesiol. Scand. 43: 275–288.
14. Goldenberg, R. L. (2003) The plausability of micronutrient deficiency in relationship to perinatal infection. J. Nutr. 133: 1645S–1648S.
15. Piñero, D. J., Hu, J., Cook, B. M., Scaduto, R. C., Jr. & Connor, J. R. (2000) Interleukin–1 beta increases binding of the iron regulatory protein and the synthesis of ferritin by increasing the labile iron pool. Biochim. Biophys Acta 1497: 279–288.
16. Eisenstein, R. S. (2000) Iron regulatory proteins and the molecular control of mammalian iron metabolism. Annu. Rev. Nutr. 20: 627–662.
17. Yamada, T. & Grisham, M. B. (1991) Roll of neutrophil-derived oxidants in the pathogenesis of intestinal inflammation. Klin. Wochenschr. 69: 988–994.
18. Wisdom, S. J., Wilson, R., McKillop, J. H. & Walker, J. J. (1991) Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension. Am. J. Obstet. Gynecol. 165: 170–174.
19. Ilouno, L. E., Shu, E. N. & Igbokwe, G. E. (1996) Am improved technique for the assay of red blood cell superoxide dismutase (SOD) activity. Clin. Chim. Acta 247: 1–6.
20. Qanungo, S. & Mukherjea, M. (2000) Ontogenic profile of some antioxidants and lipid peroxidation in human placental and fetal tissues. Mol. Cell. Biochem. 215: 11–19.
21. Uotila, J., Tuimala, R., Aarnio, T., Pyykko, K. & Ahotupa, M. (1991) Lipid peroxidation products, selenium-dependent glutathione peroxidase and vitamin E in normal pregnancy. Eur. J. Obstet. Gynecol. Reprod. Biol. 42: 95–100.
22. Tamura, T., Olin, K. L., Goldenberg, R. L., Johnson, K. E., DuBard, M. B. & Keen, C. L. (2001) Plasma extracellular superoxide dysmutase activity in healthy pregnant women is not influenced by zinc supplementation. Biol. Trace Elem. Res. 80: 107–114.
23. Tyurin, V. A., Liu, S.-X., Tyurina, Y. Y., Sussman, N. B., Hubel, C. A., Roberts, J. M., Taylor, R. N. & Kagan, V. E. (2001) Elevated levels of S-nitrosoalbumin in preeclampsia Plasma. Clin. Res. 88: 1210–1215.
24. Kharb, S. (2000) Total free radical trapping antioxidant potential in preeclampsia. Int. J. Gynaecol. Obstet. 69: 23–26.
25. Hytten, F. E. (1980) Nutritional aspects of Human Pregnancy. In Maternal Nutrition during Pregnancy and Lactation. (Aebi. H. & Whitehead, R., eds.), pp. 66–71 & 27–38. Nestlé Foundation Publication Series. Hans Huber Publ. Bern, Switzerland.
26. Basu, T. K. & Schorah, C. J. (1982) Vitamin C in health and disease, pp. 95–100. AVI publishing Co., Westport, CT.
27. Hubel, C. A., Kagan, V. E., Kisin, E., McLaughlin, M. K. & Roberts, J. M. (1997) Increased ascorbate radical formation and ascorbate depletion in plasma from women with preeclampsia: implications for oxidative stress. Free Radic. Biol. Med. 23: 597–609.
28. Shaamash, A. H., Elsonosy, E. D., Zakhari, M. M., Radwan, S. H. & El-Dien, H. M. (2001) Placental nitric acid synthase (NOS) activity and nitric oxide (NO) production in normal pregnancy, pre-eclampsia and eclampsia. Int. J. Gynaecol. Obstet. 72: 127–133.
29. Pustovrh, C., Jawerbaum, A., Sinner, D., Pesaresi, M., Baier, M., Micote, P., Gimeno, M. & Gonzalez, E. T. (2000) Membrane-type matrix metalloproteinase–9 activity in placental tissue from patients with pre-existing and gestational diabetes mellitus. Reprod. Fertil. Dev. 12: 269–275.
30. Myatt, L., Kossenjans, W., Sahay, R., Eis, A. & Brockman, D. (2000) Oxidative stress causes vascular dysfunction in the placenta. J. Matern. Fetal Med. 9: 79–82.
31. Kossenjans, W., Eis, A., Sahay, R., Brockman, D. & Myatt, L. (2000) Role of peroxynitrate in altered fetal-placental vascular reactivity in diabetes and preeclampsia. Am. J. Physiol. Heart Circ. Physiol. 278: H1311–H1319.
32. Wang, Y. & Walsh, S. W. (1995) Aspirin inhibits both lipid peroxides and thromboxane in preeclamptic placentas. Free Radic. Biol. Med. 18: 585–591.
33. Walsh, S. W., Wang, Y. & Jesse, R. (1993) Peroxide induces vasoconstriction in the human placenta by stimulating thromboxane. Am. J. Obstet. Gynecol. 169: 1007–1012.
34. Wang, Y. & Walsh, S. W. (1998) Placental mitochondria as a source of oxidative stress in pre-eclampsia. Placenta 19: 581–586.
35. Watson, A. L., Skepper, J. N., Jauniaux, E. & Burton, G. J. (1998) Changes in concentration, localization and activity of catalase within the human placenta during early gestation. Placenta 19: 27–34.
36. Watson, A. L., Palmer, M. E., Jauniaux, E. & Burton, G. J. (1997) Variations in expression of copper/zinc superoxide dismutase in villous trophoblast of the human placenta with gestational age. Placenta 18: 295–299.
37. Wang, Y. P., Walsh, S. W., Guo, J. D. & Zhang, J. Y. (1991) Maternal levels of prostacyclin, thromboxane, vitamin E, and lipid peroxides throughout normal pregnancy. Am. J. Obstet. Gynecol. 165: 1690–1694.
38. Casanueva, E., Magaña, L., Pfeffer, F. & Baez, A. (1991) Incidence of premature rupture of membranes in pregnant women with low leukocyte levels of vitamin C. Eur. J. Clin. Nutr. 45: 401–405.
39. Choi, J. L. & Rose, R. C. (1989) Transport and metabolism of ascorbic acid in human placenta. Am. J. Physiol. 257: c110–c113.
40. Yoshioka, T., Ando, M., Taniguchi, K., Yamasaki, F. & Motoyama, H. (1990) Lipoperoxidation and antioxidant substances in the human placenta during gestation. Nippon Sanka Fujinka Gakkai Zasshi. 42: 1634–1640.
41. Chappell, L. C., Seed, P. T., Briley, A. L., Kelly, F. J., Lee, R., Hunt, B. J., Parmar, K., Bewley, S. J., Shennan, A. H., Steer, P. J. & Poston, L. (1999) Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. Lancet 354: 810–816.
42. Myatt, L. & Miodovnik, M. (1999) Prediction of preeclampsia. Semin. Perinatol. 23: 45–57.
43. Roberts, J. M., Taylor, R. N., Musci, T. J., Rodgers, G. M., Hubel, C. A. & McLaughlin, M. K. (1989) Preeclampsia: an endothelial cell disorder. Am. J. Obstet. Gynecol. 161: 1200–1204.
44. Poranen, A. K., Ekblad, U., Uotila, P. & Ahotupa, M. (1996) Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. Placenta 17: 401–405.
45. Watson, A. L., Palmer, M. E., Jauniaux, E. & Burton, G. J. (1997) Variations in expression of copper/zinc superoxide dismutase in villous trophoblast of the human placenta with gestational age. Placenta 18: 295–299.
46. Poranen, A. K., Ekblad, U., Uotila, P. & Ahotupa, M. (1998) The effect of vitamin C and E on placental lipid peroxidation and antioxidative enzymes in perfused placenta. Acta Obstet. Gynecol. Scand. 77: 372–376.
47. Wang, Y. & Walsh, S. W. (1996) Antioxidant activities and mRNA expression of superoxide dismutase, catalase, and glutathione peroxidase in normal and preeclamptic placentas. J. Soc. Gynecol. Investig. 3: 179–184.
48. Rösen, P., Nawroth, P. P., King, G., Möller, W. & Packer, L. (2001) The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a congress series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes Metab. Res. Rev. 17: 189–212.

49. Kamath, U., Rao, G., Raghothama, C., Rai, L. & Rao, P. (1998) Erythrocyte indicators of oxidative stress in gestational diabetes. Acta Paediatr. 87: 676–679.
50. Carone, D., Loverro, G., Greco, P., Capuano, F. & Selvaggi, L. (1993) Lipid peroxidation products and antioxidant enzymes in red blood cells during normal and diabetic pregnancy. Eur. J. Obstet. Gynecol. Reprod. Biol. 51: 103–109.
51. Fernandez-Real, J. M., Lopez-Bermejo, A. & Ricart, W. (2002) Perspectives in diabetes: cross talk between iron metabolism and diabetes. Diabetes 51: 2348–2354.
52. Lao, T. T., Tam, K. F. & Chan, L. Y. (2002) Third trimester iron status and pregnancy outcome in non-anemic women; pregnancy unfavourably affected by maternal iron excess. Hum. Reprod. 15: 1843–1848.
53. Hagay, Z. J., Weiss, Y., Zusman, I., Peled-Kamar, M., Reece, A., Eriksson, U. J. & 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. 173: 1036–1041.
54. Siman, C. M. & Eriksson, U. J. (1997) Vitamin C supplementation of the maternal diet reduces the rate of malformation in the rate of malformation in the offspring of diabetic rats. Diabetologia 40: 1416–1424.
55. Sivan, E., Reece, A. E., Wu, Y. K., Homko, C. J., Polansky, M. & Borenstein, M. (1996) Dietary vitamin E prophylaxis and diabetic embryopathy: Morphologic and biochemical analysis. Am. J. Obstet. Gynecol. 175: 793–799.
56. Kiely, M., Morrissey, P. A., Cogan, P. F. & Kearney, P. J. (1999) Low molecular weight plasma antioxidants and lipid peroxidation in maternal and cord blood. Eur. J. Clin. Nutr. 53: 861–864.
57. Reece, E. A., Homko, C. J. & Wu, Y. K. (1996) Multifactorial basis of the syndrome of diabetic embryopathy. Teratology 54: 171–182.
58. Eriksson, U. J. (1995) The pathogenesis of congenital malformations in diabetic pregnancy. Diabetes Metab. Rev. 11: 63–82.
59. Robles, R., Palomino, N. & Robles, A. (2001) Oxidative stress in the neonate. Early Hum. Dev. 65 (*Suppl.*), S75–S81.
60. Arikan, S., Konukoglu, D., Arikan, C., Akcay, T. & Davas, I. (2001) Lipid peroxidation and antioxidant status in maternal and cord blood. Gynecol. Obstet. Invest. 51: 145–149.
61. Phylactos, A. C., Leaf, A. A., Costeloe, K. & Crawford, M. A. (1995) Erythrocyte cupric/zinc superoxide dismutase exhibits reduced activity in preterm and low-birthweight infants at birth. Acta Paediatr. 84: 1421–1425.
62. Pierre, J. L. & Fontecave, M. (1999) Iron and activated oxygen species in biology: The basic chemistry. Biometals 12: 195–199.
63. de Zwart, L. L., Meerman, J. H. N., Commandeur, J. N. M. & Vermuelen, N. P. E. (1999) Biomarkers of free radical damage. Applications in experimental animals and humans. Free Radic. Biol. Med. 26: 202–226.
64. Aruoma, O. I. & Halliwell, B. (1995) DNA damage by free radicals: carcinogenic implications. In: The handbook of immunopharmacology; immunopharmacology of free radical species (Blake, D. & Winyard, P. G., eds.), pp. 199–214. Academic Press, New York, NY.
65. Beal, M. F. (2002) Oxidatively modified proteins in aging and disease. Free Radic. Biol. Med. 32: 796–803.
66. Morrow, J., Roberts, L. & Jackson, I. (1996) The Isoprostanes. Biochem. Pharmacol. 51: 1–9.
67. Benzie, I. F. F. (1996) Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. Int. J. Food Sci. Nutr. 47: 233–292.
68. Knutson, M. D., Handelmann, G. J. & Viteri, F. E. (2000) Methods for measuring ethane and pentane in expired air from rats and humans. Free Radic. Biol. Med. 28: 514–519.
69. Knutson, M. D., Walter, P. B., Ames, B. N. & Viteri, F. E. (2000) Daily iron supplements promote abnormal iron accumulation and lipid peroxidation in rats. J. Nutr. 130: 621–628.
70. Parkkila, S., Waheed, A., Britton, R. S., Bacon, B. R., Zhou, X. Y., Tomatsu, S., Fleming, R. & Sly, W. S. (1997) Association of the transferrin receptor in human placenta with HFE the protein defective in hereditary hemocromatosis. Proc. Natl. Acad. Sci. USA 94: 13198–13202.
71. King, B. F. (1992) Comparative studies of structure and function in mammalian placentas with special reference to maternal-fetal transfer of iron. Am. Zool. 32: 331–342.
72. Wessling-Resnick, M. (2000) Iron transport. Annu. Rev. Nutr. 20: 129–151.
73. Breuer, W., Ronson, A., Slotki, I. N., Abramov, A., Hershko, C. & Cabantchik, I. (2000) The assessment of serum nontransferrin-bound iron in chelation therapy and iron supplementation. Blood 95: 2975–2982.
74. Lauffer, R. B. (1992) Iron and human disease. CRC Press. Boca Raton, FL.
75. Hennigar, G. R., Greene, W. B., Walker, E. M. & de Saussure, C. (1979) Hemochromatosis caused by excessive vitamin, iron intake. Am. J. Pathol. 96: 611–623.
76. Green, P., Eviatar, J. M., Sirota, P. & Avidor, I. (1989) Secondary hemochromatosis due to prolonged iron ingestion. Isr. J. Med. Sci. 25: 650–651.
77. Lund, E. K., Fairweather-Tait, S. J., Warf, S. G. & Johnson, I. T. (2001) Chonic exposure to high levels of dietary iron fortification increases lipid peroxidation in the mucosa of the rat large intestine. J. Nutr. 131: 2928–2931.
78. Abraham, S. C., Yardley, J. H. & Wu, T. T. (1999) Erosive injury to the upper gastrointestinal tract in patients receiving iron medication: an underrecognized entity. Am. J. Surg. Pathol. 23: 1241–1247.
79. Srigiridhar, K., Nair, K. M., Subramanian, R. & Singotamu, L. (2001) Oral repletion of iron induces free radical mediated alterations in the gastrointestinal tract of rat. Mol. Cell. Biochem. 219: 91–98.
80. Srigiridhar, K. & Nair, K. M. (2000) Supplementation with alpha-tocopherol or a combination of alpha-tocopherol and ascorbic acid protects the gastrointestinal tract of iron-deficient rats against iron-induced oxidative damage during iron repletion. Br. J. Nutr. 84: 165–173.
81. Srigiridhar, K. & Nair, K. M. (1998) Iron-deficient intestine is more susceptible to peroxidative damage during iron supplementation in rats. Free Radic. Biol. Med. 25: 660–665.
82. Srigiridhar, K. & Nair, K. M. (1997) Protective effects of antioxidant enzymes and GSH in vivo on iron mediated lipid peroxidation in gastrointestinal tract of rat. Ind. J. Biochim. Biophys. 34: 402–405.
83. Viteri, F. E., Liu, X.-N., Martin, A. & Tolomei, K. (1995) True absorption and retention of supplemental iron is more efficient when administered every-three-days rather than daily to iron-normal and iron-deficient rats. J. Nutr. 125: 82–91.
84. Viteri, F. E. (1997) Iron supplementation for the control of iron deficiency in populations at risk. Nutr. Rev. 55: 195–209.
85. Zanninelli, G., Loreal, O., Brissot, P., Konijn, A. M., Slotki, I. N., Hider, R. C. & Ioav Cabantchik, Z. (2002) The labile iron pool of hepatocytes in chronic and acute iron overload and chelator-induced iron deprivation. J. Hepatol. 36 (*Suppl.*), 39–46.
86. Gutteridge, J. M., Mumby, S., Koizumi, M. & Taniguchi, N. (1996) Free" iron in neonatal plasma activates aconitase: evidence for biologically reactive iron. Biochem. Biophys. Res. Commun. 229: 806–809.
87. Buonocore, G., Zani, S., Perrone, S., Caciotti, B. & Bracci, R. (1998) Intraerythrocyte nonprotein-bound iron and plasma malondialdehyde in the hypoxic newborn. Free Radic. Biol. Med. 25: 766–770.
88. Walter, P. B., Knutson, M. D., Paler-Martinez, A., Lee, S., Xu, Y., Viteri, F. E. & Ames, B. N. (2002) Iron defic iency and iron excess damage mitochondria and mitochondrial DNA in rats. Proc. Natl. Acad. Sci. USA 99: 2264–2269.
89. Hallberg, L. (1992) Iron balance in pregnancy and lactation. In: Nutritional Anemias, (Fomon, S. J. & Zlotkin, S., eds.), pp. 13–28. Nestle Nutrition Workshop Series, Vol 30. Raven Press, New York, NY.
90. Viteri, F. E. (1998) A new concept in the control of iron deficiency (ID): community-based preventive supplementation (PS) of at-risk groups by weekly intake of iron supplements. Biomed. Environ. Sci. 11: 46–60.
91. Kaufer, M. & Casanueva, E. (1990) Relation of prepregnancy serum ferritin levels to hemoglobin levels throughout pregnancy. Eur. J. Clin. Nutr. 44: 709–715.
92. Viteri, F. E. (1998) Prevention of iron deficiency. In: Micronutrient Deficiencies: A Toolkit for Policymakers and Public Health Workers (Howson, C. P., Kennedy, E. & Horwitz, A., eds.), pp. 45–102. Institute of Medicine, National Academy Press, Washington, D. C.
93. McLaren, C. E., Li, K. T., Gordeuk, V. R., Hasselblad, V. & McLaren, G. D. (2001) Relationship between transferrin saturation and iron stores in the African American and US Caucasian populations: analysis of data from the third National Health and Nutrition Examination Survey. Blood 98: 2345–2351.
94. CDC / DHS. (2002) Dietary intake of macronutrients, micronutrients and other constituents: United States 1988–94. Vital Health Statistics, Series 11, No 245.
95. Stolzfus, R. J. & Dreyfuss, M. (1998) Guidelines for the use of iron supplementation to prevent and treat iron deficiency anemia. International Nutritional Anemia Consultative Group (INACG), ILSI Press, Washington, DC.
96. WHO. (2001) Iron deficiency anaemia: assessment, prevention, and control. WHO/NHD/01.3, Geneva
97. Earl, R. & Woteki, C. E. (eds.) (1994) Iron deficiency anaemia. Recommended guidelines for the prevention, detection and management among US children and women of childbearing age. National Academy Press, Washington, DC.
98. Centers for Disease Control. (1989) Criteria for anemia in children and childbearing-aged women. MMWR 38: 400–404.
99. Hahn, P. F., Carothers, E. I., Darby, W. J., Martin, M., Sheppard, C. W., Cannon, R. O., Beam, A. S., Densen, P. M., Peterson, J. C. & McClellan, G. S. (1951) Iron metabolism in early pregnancy as studied with the radioactive isotope 59Fe. Am. J. Obstet. Gynecol. 61: 477–486.
100. Roughead, Z. K. & Hunt, J. R. (2000) Adaptation in iron absorption: iron supplementation reduces nonheme-iron but not heme-iron absorption from food. Am. J. Clin. Nutr. 72: 982–989.
101. O'Brien, K. O., Zavaleta, N., Caulfield, L. E., Yang, D. X. & Abrams, S. A. (1999) Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell iron incorporation, and iron status in pregnant Peruvian women. Am. J. Clin. Nutr. 69: 509–515.
102. Viteri, F. E. (1999) Iron supplementation as a strategy for the control of iron deficiency and ferropenic anemia. Arch. Latinoamer. Nutr. 49 (*Suppl.*), S15–S22.
103. Viteri, F. E., Mendoza, C., Guiro, A., Hercberg, S. & Galan, P. (1999) Daily and weekly and reference-dose iron (Fe) absorption in Berkeley, Ca. and Dakar, Senegal. FASEB J. 13: A536.4.
104. Liu, X. N. & Liu, P. Y. (1996) The effectiveness of weekly iron supplementation regimen in improving the iron status of Chinese children and pregnant women. Biomed. Environ. Sci. 9: 341–347.
105. Beaton, G. & McCabe, G. P. (2001) Efficacy of internmittent iron supplementation in the control of iron deficiency anemia in developing countries: an analysis of experience. Micronutrient Initiative, Ottawa, Canada.

106. Ekstrom, E. C., Hyder, S. M., Chowdhury, A. M., Chowdhury, S. A., Lonnerdal, B., Habicht, J.-P. & Person, L. A. (2002) Efficacy and trial effectiveness of weekly and daily iron supplementation among pregnant women in rural Bangladesh: disentangling the issues. Am. J. Clin. Nutr. 76: 1392–1400.

107. Scholl, T. O., Hediger, M. L., Fisher, R. L. & Shearer, J. W. (1992) Anemia vs. iron deficiency: increased risk preterm delivery in a prospective study. Am. J. Clin. Nutr. 55: 985–988.

108. Scholl, T. O. & Reilly, T. (2000) Anemia, iron and pregnancy outcome. J. Nutr. 130 (*Suppl.*), 443S–447S.

109. Murphy, J. F., O'Riordan, J., Newcombe, R. J., Coles, E. C. & Pearson, J. F. (1986) Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet i: 992–995.

109. Mahomed, K. & Hytten, F. (1989) Iron and folate supplementation in pregnancy. In Effective care in pregnancy and childbirth (Chalmers, I., ed.), pp. 301–307. Oxford University Press, Oxford.

110. Hytten, F. E. & Leitch, I. (1964) The physiology of human pregnancy. p. 14. Blackwell Sci. Pub. Oxford.

111. Hytten, F. E., Leitch, I. & Baird, D. (1971) The physiology of human pregnancy. pp. 1–43. Second Edition. Blackwell Sci. Pub. Oxford.

112. Scholl, T. O., Hediger, M. L., Bendich, A., Schall, J. I., Smith, W. K. & Krueger, P. M. (1997) Use of multivitamin/mineral prenatal supplements: influence on the outcome of pregnancy. Am. J. Epidemiol. 146: 134–141.

113. Steer, P., Alam, M. A., Wadsworth, J. & Welch, A. (1995) Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. BMJ 310: 489–491.

114. Lund, C. J. & Donovan, J. C. (1967) Blood volume during pregnancy. Am. J. Obstet. Gynecol. 98: 393–397.

115. Lao, T. T., Chan, P. L. & Tam, K. F. (2001) Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess? Diabet. Med. 18: 218–223.

116. Scholl, T. O. (1998) High third-trimester ferritin concentration: associations with very preterm delivery, infection, and maternal nutritional status. Obstet. Gynecol. 92: 161–166.

117. Casanueva, E., Mares-Galindo, M., Meza, C., Schnaas, L., Gutierrez-Valenzuela, V. & Viteri, F. E. (2002) Iron supplementation in non-anaemic pregnant women. SCN News. Geneva. 25:37–38.

118. Viteri, F. E., Ali, F. & Tujague, J. (1999) Long-term weekly iron supplementation improves and sustains nonpregnant women's iron status as well or better than currently recommended short-term daily supplementation. J. Nutr. 129: 2013–2020.

119. Lachili, B., Hininger, I., Faure, H., Arnaud, J., Richard, M. J., Favier, A. & Roussel, A. M. (2001) Increased lipid peroxidation in pregnant women after iron and vitamin C supplementation. Biol. Trace Elem. Res. 83: 103–110.

120. Oppenheimer, S. J., Gibson, F. D., Macfarlane, S. B., Moody, J. B. & Hendrickse, R. G. (1984) Iron supplementation and malaria. Lancet 1: 389–390.

121. Verhoef, H., West, C. E., Nzyuko, S. M., de Vogel, S., van der Valk, R., Wanga, M. A. & Kurijstein, A. (2002) Intermittent administration of iron and sulfadoxine-pyrimethamine to control anaemia in Kenyan children: a randomized control trial. Lancet 360: 908–914.

JN THE JOURNAL OF NUTRITION