

## Vitamin C-Driven Free Radical Generation from Iron<sup>1,2</sup>

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**ABSTRACT** Circulating free iron is lethal. Humans have two circulating iron binding proteins to soak up free iron to prevent it from generating toxic quantities of free radicals. These proteins are transferrin, a high-affinity, low-capacity protein (2 atoms of iron per molecule of transferrin) for which there are receptors on the surface of every iron-requiring cell; and ferritin, a lower-affinity, high-capacity protein (maximum of 4500 atoms of iron per molecule of ferritin) for which there are receptors only on the surface of iron-storage cells such as RE (reticulo-endothelial) cells. Iron is trapped inside the ferritin protein shell as harmless Fe<sub>3</sub>. When there is a high serum level of reduced ascorbic acid, it drives through the pores of the ferritin protein shell to the inside surface, where it converts the Fe<sub>3</sub> to catalytic Fe<sub>2</sub>, which then leaks out of the pores of the ferritin protein shell and generates billions of free radicals. In normal individuals, per milliliter of serum, there are approximately 300,000 molecules of transferrin per molecule of ferritin. Ferritin protein is an acute phase reactant that sharply rises in the presence of inflammation of any kind, whereas transferrin is a reverse acute phase reactant that falls in the presence of inflammation of any kind. *J. Nutr.* 126: 1213S-1220S, 1996.

### INDEXING KEY WORDS:

- vitamin C • iron • free radicals
- ferritin protein • ferritin iron • transferrin

Iron is the major generator of free radicals. In moderate quantities, free radicals are necessary for cell survival; in large quantities they are cytotoxic (Herbert et al. 1994, Olson 1996).

Iron participation in electron transfer processes, enzymatic reactions and oxygen transport makes it essential for life and health. Because free iron can be lethal, two types of protein, one with high affinity (the transferrins) and the other with high capacity (ferritin) for iron have evolved to keep it bound while transported and stored (Harrison et al. 1990).

Figure 1 (Cross et al. 1987) shows the pathways for generation of catalytic iron. The powerful oxidizing

properties of a solution of ferrous salt and hydrogen peroxide were recognized by Fenton a century ago (Fenton 1894). Haber and Weiss (1934) recognized that free radical intermediates drove the Fenton reaction.

The catalytic free radical intermediates formed in the reduction of oxygen to water include superoxide, hydrogen peroxide and, most catalytic of all, hydroxyl radicals (Ames 1983, Aisen et al. 1990, Gutteridge and Halliwell 1990). Most of the <sup>•</sup>OH generated in vivo comes from iron-dependent reduction of H<sub>2</sub>O<sub>2</sub> (Aisen et al. 1990, Gutteridge and Halliwell 1990).

Figure 2 shows the stages of iron status (Herbert et al. 1995). Everyone should be tested for iron status because the yield of discovering a need for restoration to normal balance is ~18%. About 12% of Americans are in positive iron balance and ~6% are in negative iron balance (Herbert 1992). This 6% is almost all infants, children up to the age of 4 y, children at the onset of puberty and fertile females, particularly when pregnant (Herbert 1992, Yip 1994).

About half of the 6% are iron *deficient*; the other half are usually only iron *depleted* without deficiency (Herbert 1992) (Fig. 2), although some have early deficiency with subtle liabilities (Baynes 1994). Of the average adult American male's total body iron stores of ~4 g, 2.5 are in circulating hemoglobin, ~0.5 in various iron-containing (particularly respiratory) enzymes (Cammack et al. 1990) and ~1 in body iron stores (Herbert 1987).

It may be that ~20% of Americans have a gene for iron overload, because Gordeuk et al. (1992a) noted that ~20% of adult American males have a mean trans-

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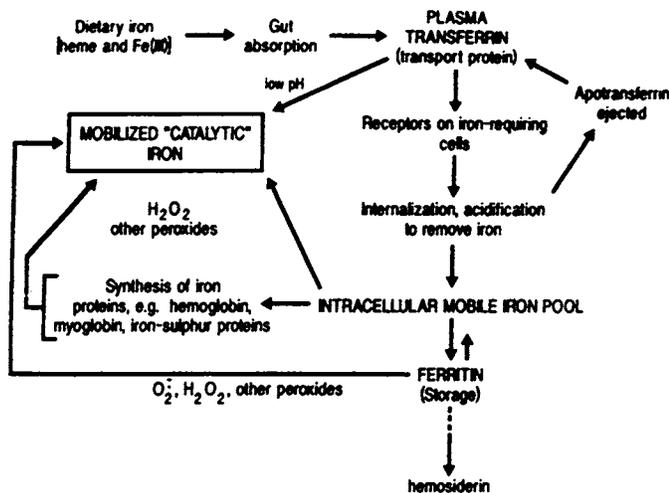


FIGURE 1 Pathways to catalytic iron (from Cross et al. 1987).

ferrin saturation of 41% compared with 26% for the other 80% of adult American males.

The significance of iron excess has been markedly underestimated (Conrad et al. 1994). Confounding clinical diagnosis is the fact that both deficiency and excess can produce both anemia and that tired and run-down feeling (Herbert 1991). Both iron supplementation

(Kadiiska et al. 1995) and iron from excessive meat eating (Conrad et al. 1994) produce excess free radicals.

Vitamin C-driven free radical generation from iron

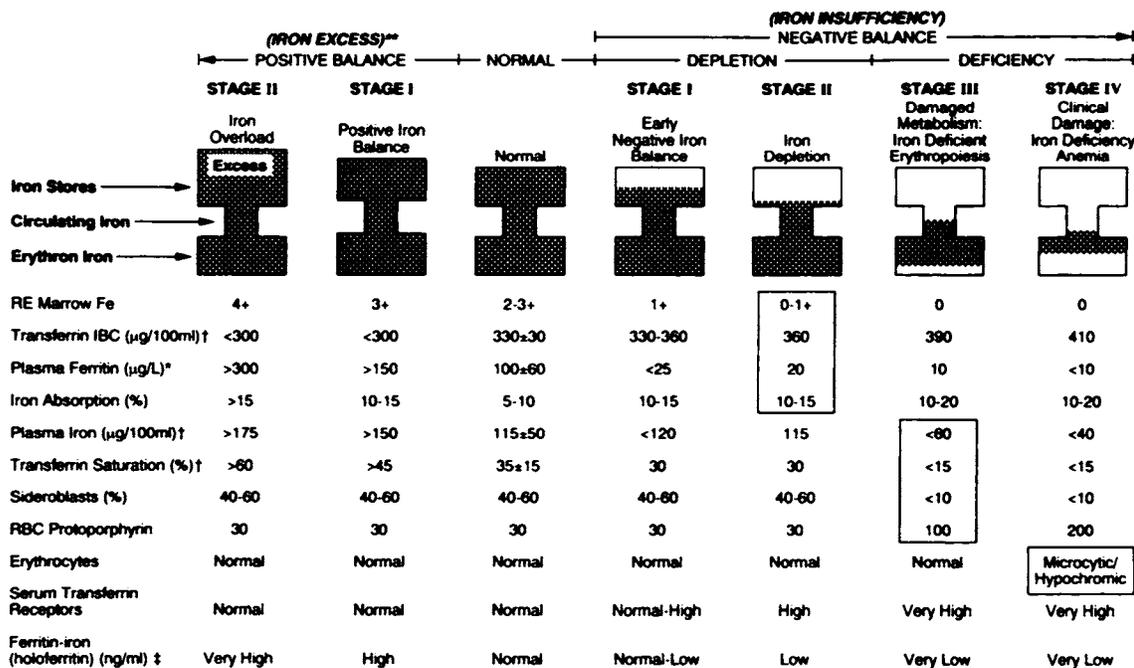
When ferrous iron reduces H<sub>2</sub>O<sub>2</sub> to generate <sup>•</sup>OH, it becomes ferric iron. Vitamin C (ascorbic acid) converts ferric iron back to ferrous, itself becoming oxidized ascorbic acid, thus allowing another cycle of <sup>•</sup>OH generation from renewed ferrous iron (Aisen et al. 1990). Supplements of vitamin C provide a constant supply of new reduced ascorbic acid, thus turning a sole cycle of iron-dependent <sup>•</sup>OH generation in situations of localized iron overload into a series of cycles, i.e., ascorbate-driven repetitive free radical generation by iron (Aisen et al. 1990, Herbert et al. 1994).

Heme oxidation is also ascorbate driven: another endogenous source of catalytic free iron is the iron released when the heme ring is opened by heme oxygenase (Abraham et al. 1988); induction of heme oxygenase is itself a cytoprotective response to destroy heme proteins (hemoglobin and myoglobin) that when released into the extracellular space can instigate tissue toxicity (Balla et al. 1992, Balla et al. 1993, Nath et al. 1992, Scott and Wagner 1992).

Like iron, vitamin C is a double-edged sword, essen-

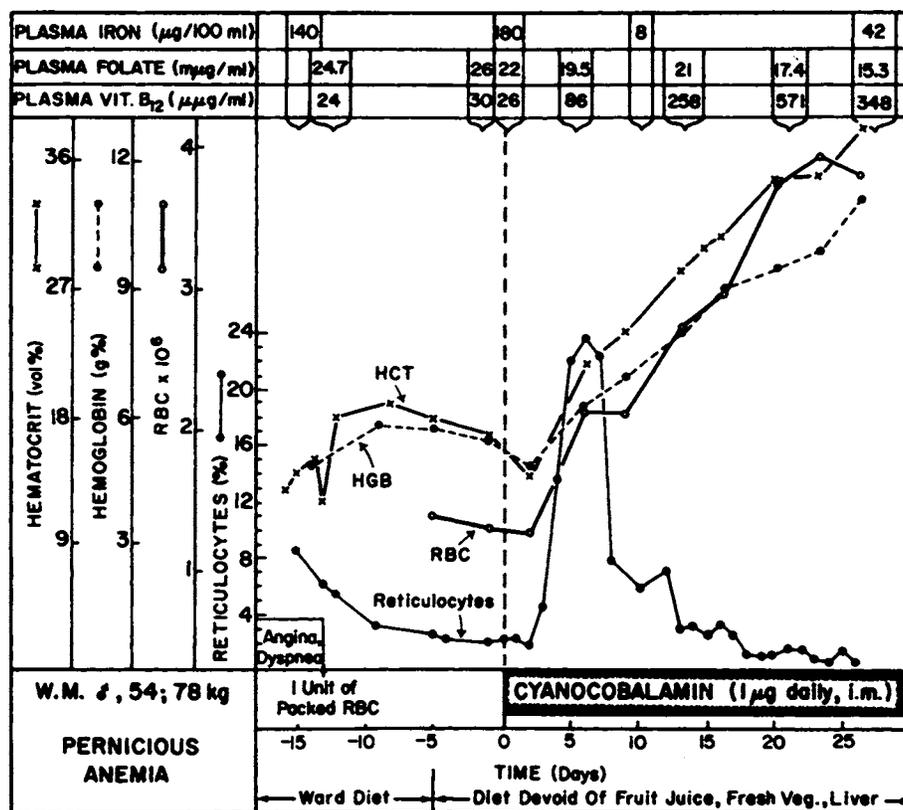
Sequential Stages of Iron Status

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\* Inflammation produces elevated ferritin, because ferritin protein is an acute phase reactant.  
 † Inflammation reduces transferrin (and the plasma iron on it), because transferrin is a reverse acute phase reactant.  
 ‡ Ferritin-iron is unaffected by inflammation, so it is reliable when ferritin, transferrin, and plasma iron are not.  
 \*\*Randall Lauffer of Harvard and Joe McCord of Univ. Colorado-Denver hold that any storage iron is excessive because of its potential to promote excessive free radical generation. See V. Herbert et al. *Stem Cells* 1994; 12: 289-303. Most free radical injury is iron-related.  
 Dallman (pediatrician) definition of negative balance: less absorbed than excreted.  
 Herbert (Internist) definition of negative balance: less absorbed than needed.

FIGURE 2 Staging iron status (© Herbert 1995).



**FIGURE 3** Release of iron from stores onto transferrin, i.e., onto total iron binding capacity by 1 mol of vitamin C daily, in a patient with scurvy and folic acid deficiency-induced megaloblastic anemia and high iron stores (Herbert 1963).

tial for health and antioxidant in physiological amounts, but prooxidant and cancer promoting in pharmacological amounts (Borg and Schaich 1989, Herbert et al. 1994). Whether the antioxidant or prooxidant actions of ascorbate will predominate depends on the bodily system and the concentration within it of iron, ascorbate and other reactants (Aisen et al. 1990, Borg and Schaich 1989, Herbert et al. 1994).

When a macrophage ingests three or more red cells, it dies (Kondo et al. 1988). If it also ingests vitamin C, ingesting only one or two red cells will kill it (Kondo et al. 1988), demonstrating the lethality of ascorbate-driven heme oxidation. The Kupffer cell is a major site for restoring iron from senescent erythrocytes to the circulation, and ascorbate enhances that iron release. Thus, ascorbate could well be a major promoter of hepatic failure and death in iron overload (Herbert et al. 1994, McLaran et al. 1982).

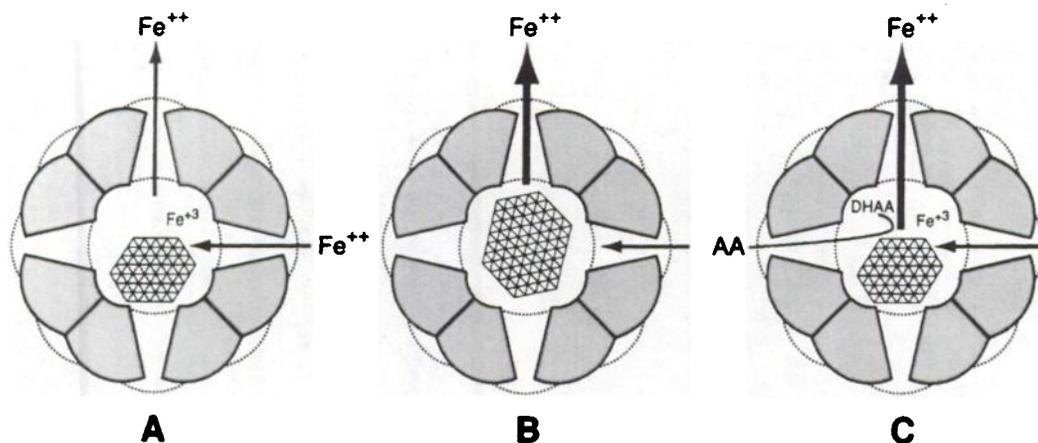
We agree with Lauffer (1991), who wrote:

I also worry about the effects of excess vitamin C on people with even moderately high iron levels. Since iron may contribute to major killers such as heart disease and cancer, vitamin C could accelerate this destructive process by increasing iron absorption and exacerbating iron-induced tissue damage. The concern that undiagnosed hemochromatosis victims could be harmed by vitamin C was one reason the 1980–1985 RDA Committee of the National Academy of Sciences

tried to decrease the RDA for this vitamin from 60 milligrams a day to 40 milligrams for men and 30 for women (Herbert 1991). . . "pro-vitamin C" NAS staff members . . . rewrote these recommendations and . . . issued the . . . RDA for vitamin C set back at its original (Ed: high) value. (See also: Marshall 1990).

Over 10% of nonblacks and up to 30% of blacks have a gene for iron overload. Vitamin C supplements are more likely to harm than help them. Vitamin C enhances iron absorption, releases catalytic iron from ferritin and drives the cycle of repetitive reduction of ferric to ferrous iron. Because ~12% of Americans are in positive iron balance and ~6% are in negative iron balance (Herbert 1992, Yip 1994), vitamin C supplements can be expected to harm twice as many people as they help.

In 1963, we reported that vitamin C releases iron from body stores (Herbert 1963) (Fig. 3) (Herbert et al. 1981). The vitamin C we gave to our patient with scurvy plus megaloblastic anemia released so much iron from the stores into the blood that it saturated the transferrin iron-binding protein (Fig. 3). This occurred because the bolus of reduced ascorbic acid in the bloodstream went through the pores of the ferritin protein shell (Fig. 4), converting harmless ferritin protein-bound Fe<sub>3</sub> to harmful free Fe<sub>2</sub>, which was soaked up by transferrin as soon as it leaked out of the ferritin shell.



**FIGURE 4** How ascorbic acid (AA) releases catalytic  $Fe_2$  to generate free radicals from harmless  $Fe_3$  stored in the ferritin protein shell (Herbert et al. 1995).

### Iron, vitamin C and cancer

National Health and Nutrition Examination Survey I (NHANES) data indicate high body iron stores are associated with increased cancer risk (Stevens et al. 1988). Catalytic iron free radical generation mutates DNA and promotes cancer. Contrary to the claims that vitamin C protects against such mutations, we found (Shaw et al. 1994) that it does not when iron is also present.

Both alcohol or vitamin C enhance iron absorption, as does a hemochromatosis (H) gene. They do more harm than the gene, because they also enhance release of dangerous excess catalytic iron from harmless iron stores (Herbert et al. 1994, Lauffer 1991, Lauffer 1992).

Alcohol, in its normal catabolism, releases catalytic iron (Herbert et al. 1981, Herbert et al. 1994). So much catalytic iron is released from ferritin by vitamin C (Aisen et al. 1990, Herbert et al. 1994) that thalassemic patients with heavy iron overload have died from a single dose of vitamin C releasing so much catalytic iron that a fatal cardiac arrhythmia was produced (Herbert et al. 1994).

Iron deficiency slows the progression of malignancy (Hann et al. 1988, Bergeron et al. 1985). Catalytic iron free radical generation mutates DNA and promotes cancer (Ames 1993, Borg and Schaich 1989, Shaw and Jayatilleke 1990).

As we suggested (Herbert et al. 1994), where vitamin C appears to act against cancer, it may be that it is acting in its role of preserving folic acid against oxidative destruction. It may be that it is the folic acid that suppresses the development of cancer, by methylating DNA; methylation of DNA stops cell proliferation (Ran et al. 1993, Herbert et al. 1994).

An aim in cancer chemotherapy is to selectively kill cancer cells without killing host cells; much research is doing this using iron as the killer (Donfrancesco et al. 1993, Herbert et al. 1994, Voest 1993, Weinberg 1990).

Because vitamin C drives repetitive generation of

destructive free radicals in the presence of high iron levels, future cancer therapies may focus on destruction of iron-containing tumor cells by activating that iron. They may be even more effective if oral or parenteral vitamin C is added (Herbert et al. 1994).

### Iron, vitamin C and heart disease

Antioxidant vitamin C supplements were recently found worthless against heart disease (Rimm et al. 1993, Steinberg 1993), and evidence that they were damaging to the heart was presented in other studies (Herbert et al. 1994, Herbert et al. 1994a, Lauffer 1991).

The group of Chaim Hershko (Hershko et al. 1992, Hershko 1994), among others (Herbert et al. 1994), has shown that the cardiotoxicity of anthracycline antineoplastic drugs is due to free hydroxyl radical formation through the iron-driven Haber-Weiss reaction, which vitamin C supplements can make lethal (Halliwell 1994, Hershko et al. 1992, Hershko 1994, Herbert et al. 1994).

Vitamin C supplements can cause rapid progression to death of the otherwise more slowly progressive congestive cardiomyopathy of hemochromatosis (McLaran et al. 1982). Three young athletes so died (Herbert 1993, Herbert et al. 1994). No one thought to assess their iron status before letting them take vitamin C supplements.

The study by Salonen et al. (1992) reported that when one divides patients with high LDL cholesterol into two groups, those with elevated serum ferritins in the range  $>200 \mu\text{mol/L}$ , due to the moderately increased body iron stores of heterozygous hemochromatosis, have more than twice the coronary artery disease risk of those with serum ferritins in the range of  $100 \mu\text{mol/L}$ .

The concept that high iron promotes heart attacks was first proposed in 1981 by J. L. Sullivan (1992). Steinberg's group showed that high LDL cholesterol per se is relatively harmless, but oxidized LDL is damaging to the coronary artery (Steinberg 1993).

We found in vitro (Herbert et al. 1994a) that iron-catalyzed lipid peroxidation (which converts harmless LDL cholesterol to coronary artery-damaging oxidized LDL) was enhanced from a mean of 6.4 nmol of malondialdehyde produced per hour per milligram protein to 17.6 when we added 10  $\mu$ mol vitamin C (the plasma level when an adult absorbs 0.1 g of vitamin C) and to 104.0 when we added 100  $\mu$ mol vitamin C (the plasma level when an adult absorbs 1 g of vitamin C).

This confirms the report by O'Connell et al. (1985) that ferritin in the presence of ascorbate leads to lipid peroxidation and the report by O'Connell et al. (1986) that ferritin in the presence of ascorbate generates free radicals.

Heterozygous hemochromatosis afflicts ~12% of American whites and perhaps up to 30% of American blacks (Gordeuk et al. 1992a, Gordeuk et al. 1992b). They have *moderately* elevated body iron, manifested by a high serum ferritin iron. The millions of them with high LDL cholesterol are at risk from either iron supplements or supplements of vitamin C, which enhance both iron absorption and iron toxicity. Salonen is now setting up ferritin iron assay in Finland.

#### ***Lethality of vitamin C supplements in the presence of iron overload***

Hematologists who study iron overload due to transfusion therapy of patients with thalassemia or sickle cell disease have long known (Herbert 1963, Herbert et al. 1981) that vitamin C can mobilize such an enormous amount of iron from their high body iron stores to overwhelm the iron-binding capacity of iron-binding proteins, with the resultant free iron producing death within minutes to hours from iron-induced cardiac failure. By releasing from high iron stores more free iron than iron-binding proteins can bind, vitamin C supplements can kill persons with iron overload (Halliwell 1994, Herbert 1993).

Because of the potential lethality of vitamin C supplements in persons with iron overload, the 1992 *Management Protocol for the Treatment of Thalassemia Patients* of the International Thalassemia Federation, states:

Iron-loaded patients usually become vitamin C deficient, probably because iron oxidizes the vitamin. When this is the case, administration of vitamin C increases excretion of iron in response to Desferal. (Ed. Note: Desferal is the iron-chelating agent, desferrioxamine). Vitamin C increases the availability of iron, and so may increase its toxicity if large doses are taken without simultaneous Desferal infusion. Therefore the following precautions are recommended: a. Start treatment with vitamin C only after an initial month of treatment with Desferal. b. Give vitamin C supplements only if the patient is receiving Desferal regularly. c. Do not exceed a daily dose of 200 mg. The minimum effective dose of vitamin C is about 2–5 mg/kg (N.

DiPalma, A. Piga, unpublished data). In general, 50 mg suffice for children under 10 years of age and 100 mg for older children. Vitamin C should be given only on days when Desferal is taken, ideally when the pump is set up.

The protocol is available from the Cooley's Anemia Foundation, Box CEP, 105 East 22nd Street, New York, NY 10010.

#### ***Genetics of iron***

Our genetic nutrition pyramid (Simopoulos et al. 1993) (Fig. 5), created by putting a genetic base and appropriate sidebars on the U.S. government's nutrition pyramid of the five basic food groups, converts the government's pyramid, usable for the nonexistent theoretical average American, into one usable by each individual American. The genetic blueprint of each individual determines whether that individual should eat more or less than the theoretical average American should eat daily from each of the five food groups.

For example, those with a gene for increased iron absorption should be vegetarians (Herbert 1991, Simopoulos et al. 1993) because the average iron absorbability by normal people averages only 3% from plant foods but 5 times as much (15%) from animal foods (Herbert 1987, Herbert 1992).

Homozygous hemochromatosis afflicts one in every 200–300 Caucasians (Powell et al. 1994), and secondary iron overload afflicts many millions more (Pippard 1994).

#### ***Protections against catalytic iron***

The intracellular generation of apoferritin is a cytoprotective antioxidant strategem of endothelial cells (Balla et al. 1992, Balla et al. 1993).

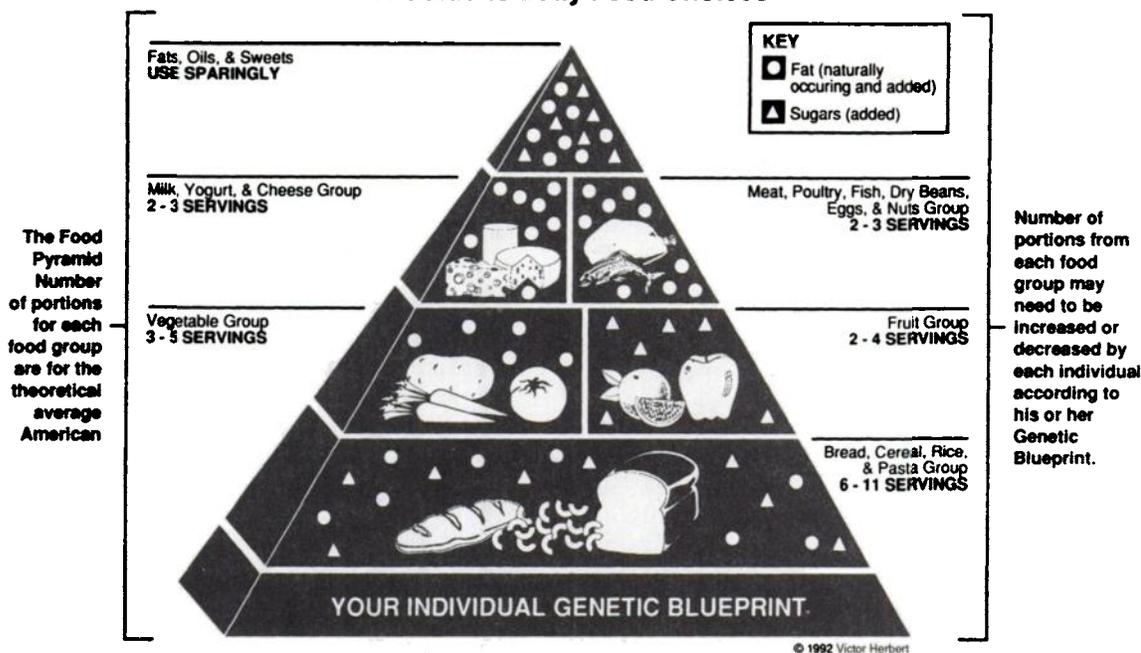
Serum ferritin was first measured in 1972 (Worwood 1994). The ferritin protein molecule is a tetrahedron (Harrison et al. 1990), whose purpose is to soak up and render harmless catalytic iron ( $\text{Fe}_2$ ). Catalytic iron is released by any inflamed cell or tissue. Ferritin protein is an acute phase reactant protein, i.e., a protein generated in response to iron released at any site of inflammation. Each single molecule of ferritin is capable of binding up to 4500 atoms of iron.

#### ***Ferritin protein and ferritin iron***

Although the literature almost exclusively uses the word ferritin, what is generated within cells in response to an iron challenge is *apoferritin*, almost free of iron, which then binds iron that would otherwise be cell damaging. One ferritin molecule is capable of binding up to 4500 atoms of iron.

Serum iron measurements do not measure the small amount of iron on ferritin (nmol/L) but only the large

## Food Guide Pyramid A Guide to Daily Food Choices



**FIGURE 5** Genetic Nutrition Food Guide Pyramid (© Herbert 1992) updating the 1992 USDA Food Guide Pyramid by adding a base consisting of "your genetic blueprint" and sidebars noting that the food pyramid number of portions are for the nonexistent theoretical average American and therefore may need to be increased or decreased by each individual according to his or her unique genetic blueprint.

amount of iron on transferrin, plus low molecular weight iron (Borg and Schaich 1989) such as might have been absorbed from iron succinate citrate (Aisen et al. 1990).

We can assume that a serum ferritin of 200 nmol/L accompanied by a low serum iron and iron-binding capacity, is largely apoferritin with almost no iron in it and that the elevated ferritin protein must be present as an acute phase reactant due to inflammation. Conversely, if a serum ferritin of 200 nmol/L is accompanied by a high serum iron and iron-binding capacity, then we can assume those 200 nmol/L of serum ferritin contain a great deal of iron.

Except when there is acute serum ferritin elevation in response to inflammation, the serum ferritin level so closely mirrors body iron stores that multiplying the serum ferritin in nmol/L by 10 gives the body iron stores in  $\mu\text{mol/L}$  (Bothwell et al. 1979, Herbert et al. 1987, Herbert 1992).

Leif Hallberg's group has recently shown in Swedish teenage girls that when they get the flu, their acute phase reactant serum ferritin protein remains elevated for 6–8 wk (Hallberg, L., personal communication). He is setting up ferritin iron assay.

There is very little iron in each new molecule of ferritin because apoferritin is generated in response to any inflammation. Being a strong iron attractant, over time ferritin gradually builds up its iron content as it soaks up available iron.

Our U.S. standards say that in men, normal ferritin is 50–400 (Herbert 1992). That is wrong (Herbert 1992). It was based on measuring the range of serum ferritin in a large number of so-called normal people and finding it to be 50–400. However, up to half of normal people at any instant in time, such as during flu season, may have or may be recovering from, one or another inflammation. Any inflammatory process produces elevated serum apoferritin generated by the cells affected by inflammation.

One milliliter of serum ferritin from people with normal iron levels normally contains 10–35 nmol/L of iron (Herbert et al. 1995). Ferritin-iron ranged in normals from 10–35 nmol/L. Those in negative iron balance had values  $\leq 10$  nmol. Thirty of 36 in positive iron balance had values  $>35$  nmol/L, in contrast to 11 of 19 with inflammation. Seventeen of 22 with overload had values  $>100$  nmol/L; only 1 of 19 with inflammation had such a value. Ferritin-iron in ferritin protein was  $>15\%$  by weight in 14 of 22 with iron overload, but in 0 of 19 with inflammation. We concluded that percent saturation of serum ferritin with iron is the most reliable serum test for the whole range of human body iron status, unconfounded by inflammation (Herbert et al. 1995).

### The bottom line

Both oxidants and antioxidants are needed in the biochemical economy of human cells. We need to in-

hale oxygen because it is the fundamental oxidant we use; without it we would die. Cells walk a balance between essential oxidant and essential antioxidant processes. The concept of oxidant-antioxidant balance is succinctly delineated by McCord (1992).

Representing vitamins and other phytochemicals as antioxidant in either labeling or advertising tells only the antioxidant side and deceives by omitting the pro-oxidant side. To protect the public, the FDA and FTC should forbid using the word antioxidant to describe vitamins and other phytochemicals in labeling or advertising.

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