It has been known since the Framingham study that women have a lower risk for cardiovascular disease than do men. Furthermore, at the time of menopause, this protection disappears and a woman's risk for cardiovascular disease (CVD) steadily increases. It was also well known that pre-menopausal women lose iron during monthly menses and thus have lower body iron stores than men or post-menopausal women. So, in 1981, Sullivan attempted to reconcile these facts by postulating the iron hypothesis (1) . Simply stated, high stores of iron predispose one to atherosclerotic changes and increase one's risk of CVD. Although this does not appear to be the only mechanism by which pre-menopausal women are protected (ie. estrogen) (2), this hypothesis sparked considerable interest in the connection of heart disease with high stores of iron.

Chemistry of Iron (pro-oxidant properties)

Free ferric iron (Fe3+) can participate in a variety of redox reactions. In particular, the Haber-Weiss reaction, which produces a hydroxyl radical from hydrogen peroxide (O2- + H2O2 -> O2 + OH- + OH ), can be catalyzed by iron (3) (Fig. 1).

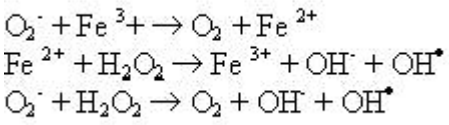


Fig. 1 Haber-Weiss reaction
(Adapted from Meyers)

The significance of this reaction is that iron helps to form hydroxyl radicals, which turn out to be one of the most potent biological oxidizing agents. Thus, free iron will have pro-oxidant properties.

Physiology of Iron (storage and usage)

Almost no free iron (and only free iron can catalyze reactions) exists in the normal person. Instead, our iron is bound by proteins like ferritin, transferrin, globin and the various cytochromes. Transferrin transports the iron through the circulation after it has been absorbed through the intestinal epithelium. Once the iron reaches its target tissue, it dissociates and is immediately bound by ferritin for storage or by globins/cytochromes for usage. Interestingly, molecules like superoxide (O2-) can cause iron to be released from proteins like ferritin (3). Once in its free form, iron can then catalyze reactions involving superoxide itself (see above). Thus, tissues that have high concentrations of iron-storing proteins will be able to liberate large amounts of iron and incite oxidative chain-reactions.

Furthermore, as cells naturally die, they release their contents into the extracellular space. Since this space is continuous with the blood plasma, one can get a good picture of intracellular iron stores by looking at the plasma levels of ferritin. In fact, serum ferritin

levels are the only accurate measures of the body's iron content (4). Measuring serum iron, serum transferrin or iron intake are not as accurate because: 1) transferrin is a transporter which has the iron for only a brief moment; 2) iron can only stay "free" transiently until it is bound by another protein; 3) dietary absorption of iron can change in response to iron status.

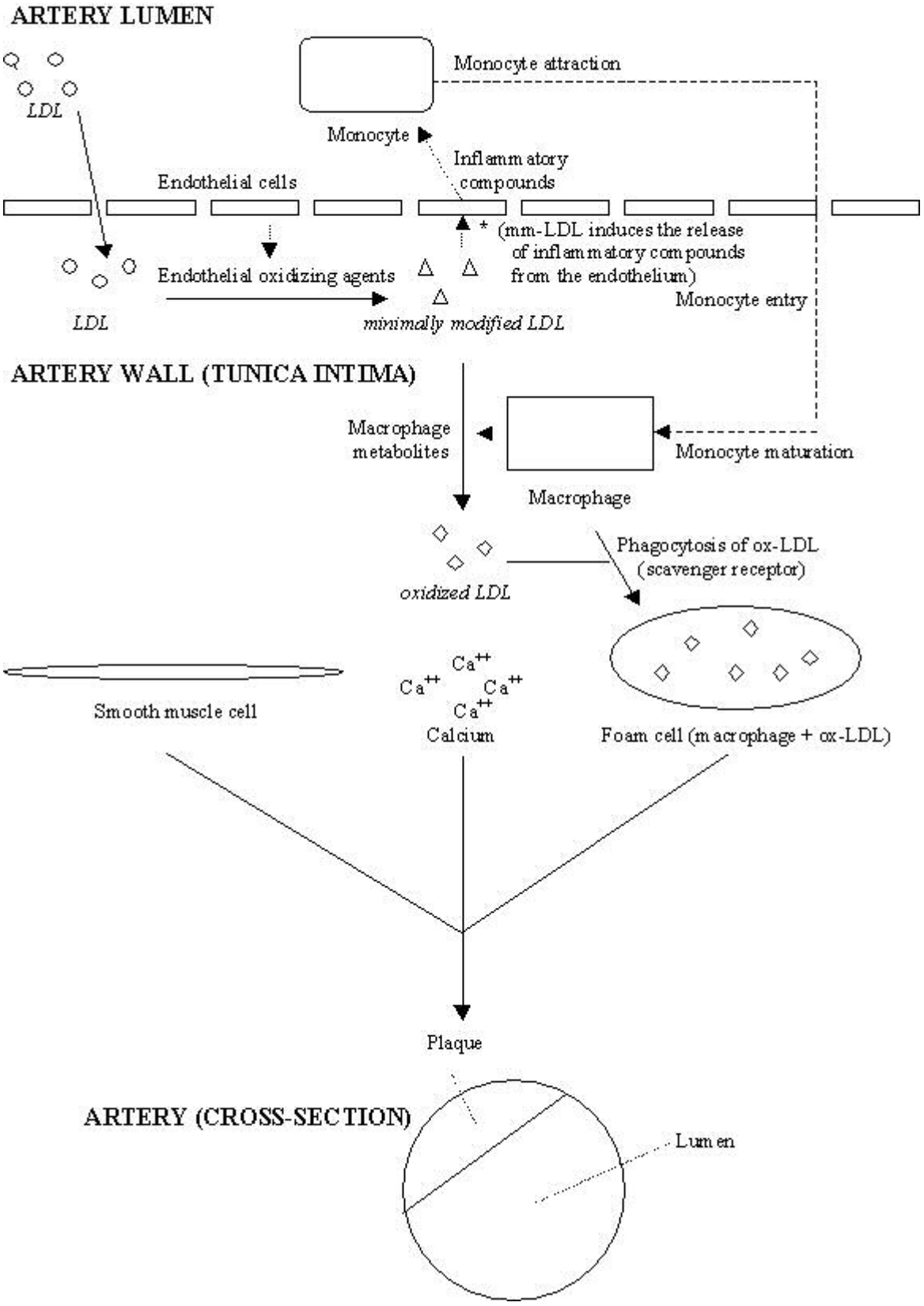
Atherosclerosis and Oxidized LDL

Atherosclerosis leads the developed world as a cause of morbidity and mortality(5). Simply put, plaques develop in the artery wall which diminish blood flow leading to conditions like angina pectoris, stroke and gangrene. If the plaque ruptures, the blood coagulates to seal off the damaged wall. Unfortunately, the clot can dislodge and completely occlude the downstream vessel causing life-threatening infarctions of the tissues (ie. acute myocardial infarction) (6).

The "plaque" described above is a complex entity, characterized mainly by fat, inflammatory cells, calcification and connective tissue. The mechanism of plaque formation (atherogenesis) is not completely understood, although a great deal is known about the process (7):

**FIG 2. Atherogenesis**

Adapted from Berliner, J.A. *et al.* 1995. Atherosclerosis: Basic Mechanisms. *Circulation.* **91**: 2488-96.



Low-density lipoprotein (LDL) is the body's transporter of dietary fat. Thus, high fat intake leads to high plasma levels of LDL. When concentrated in the blood, LDL begins to cross the endothelium of arteries and accumulate within the artery wall. Once there, the LDL becomes slightly oxidized to form minimally modified LDL (mm-LDL). This step is crucial since it is mm-LDL that induces endothelial cells to release inflammatory compounds and it is these inflammatory compounds which cause monocyte infiltration. When the monocyte differentiate into macrophages, they begin to do what macrophages do best - phagocytize cellular garbage/foreign invaders. In this case, the macrophages take up the oxidized LDL (ox-LDL) using a receptor known as the scavenger receptor (*Note: mm-LDL became ox-LDL under the oxidizing agents secreted by macrophages). The macrophage that has filled itself with fat is now called a foam cell. It is the slow build-up of these foam cells which leads to the plaque, initially called a fatty streak. The key feature to this mechanism is that the LDL needs to be oxidized for any of this atherogenesis to occur (8).

## Iron and the Atherosclerotic Plaque

Up to this point, the story is building upon two key ideas: iron has pro-oxidant properties and oxidation is a key event in atherogenesis. Sullivan hypothesized that iron might be able to promote plaque formation. However, in order for that to be feasible, human atherosclerotic plaques must have some catalytic (non-bound) iron and that iron must be capable of oxidizing LDL to its pathogenic form, mm-LDL. In fact, Smith et al. in 1992 showed that this was indeed the case(9). Using a bleomycin assay, they showed that human plaques contained detectable amounts of free iron (catalytic). Furthermore, they demonstrated that these iron-containing plaques were capable of oxidizing lipids that are similar to those found in LDL. Importantly, this oxidation was diminished in the presence of an iron-chelator, desferrioxamine, showing that iron was partly necessary for lipid oxidation. This in vitro work by Smith et al. has been continued by others with similar results (10) . We can now conclude that iron is capable of oxidizing LDL in vitro and might promote atherogenesis in vivo. The question is "Does it?"

## In Vivo Studies Supporting the Iron-Hypothesis

In 1992, Salonen et al. published one of the first reports to correlate coronary heart disease (CHD) with increased stores of iron(11). This prospective observational study examined a cohort (n=1,931) of Finnish men over the course of five years. This population was selected because of they have the highest mortality due to heart disease in the world. The experimenters gathered physical data about the cohort at the outset of the study. They recorded blood glucose levels, smoking history, LDL, HDL, age, triglycerides, family history and serum ferritin levels. Then, over the course of the experiment, the men were followed to see if they had an acute myocardial infarction (AMI). The results showed that those who had suffered an AMI had higher levels of serum ferritin than those who had not had an AMI. Importantly, the relative risk of ferritin was equalized between the two groups by correcting for differences in the other risk factors (ie. age, LDL, etc.). Moreover, the risk of high serum ferritin levels was

potentiated by having high LDL levels. In other words the risk with high LDL and high ferritin was more than if one simply added the risk of high ferritin only and the risk of high LDL only. Thus, there is a synergistic effect that is consistent with the model in which iron is only harmful if LDL is around to be oxidized.

Other observational studies have produced similar findings. Data was gathered on the Somali nomads, a tribe that has very low serum ferritin and atherosclerosis despite a high dietary intake of fat(12,13). The data showed that these groups had blood which had anti-oxidant properties when compared to age-matched Somali nomads who had been "westernized" with high-iron diets. It remains to be seen whether or not this "westernized" tribe has a higher incidence of CVD.

These past studies have correlated heart disease with body iron stores (serum ferritin). However, in order to validate the hypothesis, there should be evidence that this heart disease is caused by atherosclerotic changes (as opposed to iron accumulation within the heart muscle). In the most comprehensive studies, Kiechl et al. looked at a population of Italian men and women (n=826; 847) in the Bruneck Studies (14,15). Using ultrasonography, they measured the diameter of various parts of the carotid artery. Over the course of the five years, they determined whether patients had plaque formation, and thus a narrowing of the artery lumen. This was then correlated with the serum ferritin levels at the beginning of the study (15) (* Note: Again, the serum ferritin level was adjusted using the other risk factors for atherosclerosis). The results showed that serum ferritin was positively correlated with plaque formation (Fig. 3) and that this effect was potentiated by high serum LDL levels (data not shown).

It might seem that the data presented overwhelmingly supports the iron hypothesis. Unfortunately, these studies are not without weakness. For example, serum ferritin levels were measured only once in most cases and then correlated with five years of data. That leaves open the possibility of variation in ferritin values during the course of the five years. In addition, errors could result from unmeasured anti-oxidant intake(16).

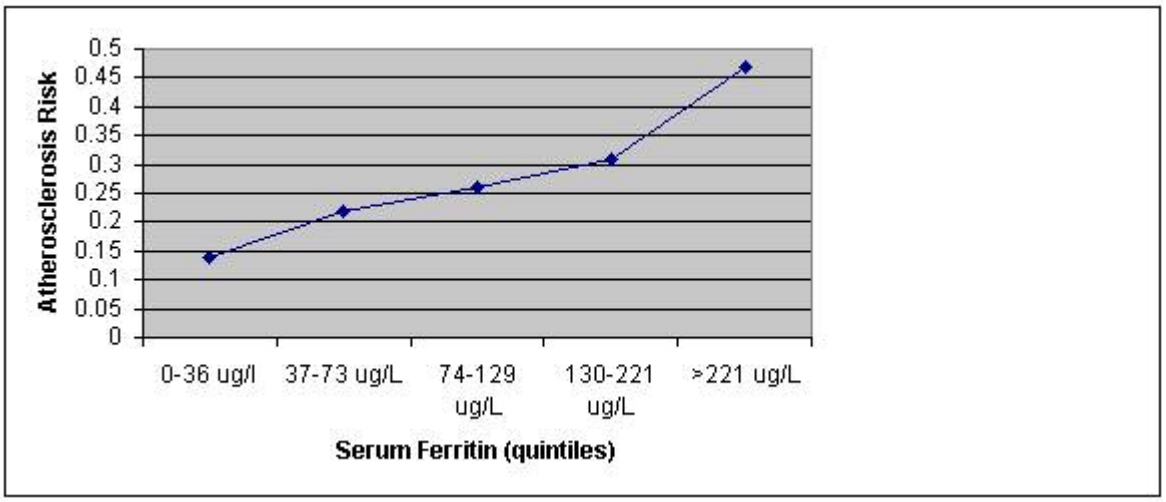



Fig. 3 Correlation between atherogenesis and ferritin levels (Adapted from Kiech)

In VivoStudies Which Oppose the Iron-Hypothesis

Indeed, the iron-hypothesis is far from being an accepted risk factor for CHD. This stems from the fact that several studies have found contradictory results in which high iron levels did not seem to predispose one to atherosclerosis or CHD. In 1994, many reports were published in response to Salonen's 1992 article. For instance, Liao et al. looked at serum iron levels and CHD in the NHANES (National Health and Nutrition Examination Survey) database (17). They found no correlation between high serum iron and heart disease, in fact, they found a slight negative correlation. Similarly Sempos et al. examined transferrin saturation in a part of the NHANES I data known as the NHEFS (National Health Epidemiological Follow-up Study). Again, only a slight negative correlation was found (18). Ascherio et al. demonstrated no correlation between iron intake and CHD (19) . As was mentioned earlier, measuring transferrin, serum iron and iron intake do not give as accurate information about body iron stores as does serum ferritin. Therefore, these studies, while possibly significant, do not really challenge the iron-hypothesis.

However, Rauramaa et al. measured carotid diameter using ultrasonography and correlated it to serum ferritin levels in a group of Finnish men (n=206). Remarkably, he found no association between iron stores and carotid atherosclerosis (r=0.01, not significant). Whether or not these negative results were influenced by the smaller sample size and other possible biases, it still casts some doubt on the iron hypothesis.

Future Possibilities

All of the studies thus far have provided correlative evidence suggesting that ferritin might be a risk factor for CVD. Also, there have been conflicting reports arguing that this might not be the case. Therefore, there must be further studies to settle the issue. Ideally, one would like to do experiments where a homogenous group of people is randomly divided into two arms, one with high serum ferritin and the other which has low serum ferritin. The two groups would be followed and examined for atherosclerotic changes. The caveat is to change someone's ferritin value. In fact, this can be done with repeated blood donation (phlebotomy). However, as of today, no one has done this experiment(16).

So, will giving blood, help you live longer and healthier? At this point, it is safe to say that no one really knows. What is known is that the evidence for the iron hypothesis is strong enough that phlebotomy should at least be investigated as a means of lowering one's risk for cardiovascular disease.

REFERENCES

1. Sullivan JL. Iron and the sex difference in heart disease risk. Lancet. 1981;1:1293-1294.

2. Hamilton M. Pers. Comm. 2/19/98 UCLA Department of Cardiology.

3. Meyers DG. The iron hypothesis: does iron cause atherosclerosis? Clin. Cardiol. 1996;19:925-929.

4. Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. Am J Clin Nutr. 1974;27:681-687.

5. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990's. Nature. 1993;362:801-809.

6. Libby P. Atherosclerosis. Harrison's Principles of Internal Medicine (14th ed.) McGraw-Hill, New York: 1998;1345-1352.

7. Berliner JA, Navab M, Fogelman AM, Frank JS, Demer LL, Edwards PA, Watson AD, Lusis AJ. Atherosclerosis: basic mechanisms - oxidation, inflammation, and genetics. Circulation. 1995;91:2488-2496.

8. Novab M, Berliner JA, Watson AD, Hama SY, Territo MC, Lusis AJ, Shih DM, Van Lenten BJ, Frank JS, Demer LL, Edwards PA, Fogelman, AM. The yin and yang of oxidation in the development of the fatty streak: a review based on the 1994 George Lyman Duff Memorial Lecture. Arterioscler Thromb Vasc Biol. 1996;16:831-842.

9. Smith C, Mitchinson MJ, Aruoma OI, Halliwell B. Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions. Biochem J. 1992;286:901-905.

10. Fuhrman B, Oiknine J, Aviram M. Iron induces pipid peroxidation in cultured macrophages, increases their ability to oxidatively modify LDL, and affects their secretory properties. Atheroscler. 1994;111:65-78.

11. Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation. 1992;86:803-811.

12. Murray MJ, Murray AB, Murray NJ. Do iron and copper supplementation of the diet impair antioxidant activity and speed atherogenesis? Arterioscler Thromb Vasc Biol. 1991a;11:1479a (abstract).

13. Murray MJ, Murray AB, Murray NJ. Nutritional iron and copper deficiency may protect against the atherogenesis of high-fat diets. Arterioscler Thromb Vasc Biol. 1991b;11:1479a (abstract).

14. Kiechl S, Aichner F, Gerstenbrand F, Egger G, Mair A, Rungger G, Spögler F, Jarosch E, Oberhollenzer F, Willeit J. Body iron stores and presence of carotid atherosclerosis: results from the Bruneck Study. Arterioscler Thromb Vasc Biol.

1994;14:1625-1630.

15. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and presence of carotid atherosclerosis: prospective results from the Bruneck Study. Circulation. 1997;96: 3300-3307.

16. Gillum RF. Body iron stores and atherosclerosis. Circulation. 1997;96:3261-3263.

17. Liao Y, Cooper RS, McGee DL. Iron status and coronary heart disease: negative findings from the NHANES I epidemiologic follow-up study. Am J Epidem. 1994;139: 704-712.

18. Sempos CT, Looker AC, Gillum RF, Makuc DM. Body iron stores and the risk of coronary heart disease. N Engl J Med. 1994;330:1119-1124.

19. Ascherio A, Willett WC, Rimm EB, Giovannucci EL, Stampfer MJ. Dietary iron intake and risk of coronary disease among men. Circulation. 1994;89:969-974.

i. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990's. Nature. 1993;362:801-809. ii. Salonen JT, Nyyssönen K, Korpela H, Tuomilehto J, Seppänen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation. 1992;86:803-811. iii. Kiechl S, Aichner F, Gerstenbrand F, Egger G, Mair A, Rungger G, Spögler F, Jarosch E, Oberhollenzer F, Willeit J. Body iron stores and presence of carotid atherosclerosis: results from the Bruneck Study. Arterioscler Thromb Vasc Biol. 1994;14:1625-1630. 4. Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and presence of carotid atherosclerosis: prospective results from the Bruneck Study. Circulation. 1997;96: 3300-3307. v. Steinberg D, Parthasapathy S, Carew TE, Khoo JC, Witztum, JL. Beyond cholesterol: modifications of low-density lipoproteins that increase its atherogenicity. N Engl J Med. 1989;320: 915-924. 6. Rauramaa R, Väisänen, Mercuri M, Rankinen T, Penttilä I, Bond MG. Association of risk factors and body iron status to carotid atherosclerosis in middle-aged eastern Finnish men. Eur Heart J. 1994;15: 1020-1027. 7. Liao Y, Cooper RS, McGeeDL. Iron status and coronary heart disease: negative findings from the NHANES I epidemiologic follow-up study. Am J Epidem. 1994;139: 704-712. 8. Sempos CT, Looker AC, Gillum RF, Makuc DM. Body iron stores and the risk of coronary heart disease. N Engl J Med. 1994;330:1119-1124.