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Misconceptions in the debate on the iron hypothesis

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1. Introduction

In their review of the iron hypothesis [1–3], Sempos and Looker [4] conclude that "the data do *not* support the hypothesis that body iron stores are a risk factor for CHD." However, in a recent review on the same subject, de Valk and Marx [5] draw the opposite conclusion, i.e. that "strong epidemiological evidence is available that iron is an important factor in the process of atherosclerosis." At the least, this extreme incompatibility of opinion about the meaning of the data suggests that treating the debate on the iron hypothesis as "an example of the use of nutritional epidemiology" [4] is premature. In my view, the disagreement resolves in favor of the iron hypothesis if misconceptions in the discussion by Sempos and Looker [4] are remedied.

2. Getting the hypothesis right

A key problem is that the authors argue against the wrong hypothesis. Sempos and Looker [4] do not present an accurate statement of the hypothesis. According to them, "in 1981 Dr. Jerome Sullivan proposed that body iron stores are directly or positively related to CHD risk, i.e. the higher your body iron stores the greater your CHD risk." This statement of the hypothesis colors their presentation. The strongest statement of the hypothesis is that iron depletion protects against ischemic heart disease. This form of the hypothesis does not attempt to define precisely the relationship between stored iron and disease. The findings that led me to propose the iron hypothesis do not permit an accurate prediction of the form of the relationship between stored iron level and disease. To hypothesize that iron depletion is a protective factor, leaves unspecified the precise epidemiological behavior of ferritin as a risk factor. I discussed these issues in detail in a 1992 paper [6] that was overlooked by Sempos and Looker [4]:

"The epidemiological findings and the accumulating data on the protective effects of deferoxamine in myocardial injury are consistent with the iron depletion hypothesis. The hypothesis implies that ferritin is a risk factor for IHD but does not precisely define the epidemiological behavior of ferritin as a risk factor. The hypothesis focuses on protective effects of iron depletion, a state in which there is essentially no ferritin present. The hypothesis does not, and with available data, cannot adequately address the quantitative aspects of the relationship between ferritin and incidence of IHD. The strength of serum ferritin as a risk factor for IHD may vary considerably over its clinically observed range.

"The unresolved question involves the amount of iron required for promotion of IHD. Perhaps the key protective factor is the maintenance of iron depletion. Nearly maximal promotion of IHD may require relatively small amounts of storage iron. After iron sufficiency is achieved, further increments may be associated with progressively less added risk per additional mg of storage iron. If this is the case, serum ferritin would behave as a strong risk factor, but only over the extreme low end of its observed range. This implies that iron repletion or iron sufficiency exerts a permissive effect in the development of IHD. So long as iron depletion, or near iron depletion, is maintained the other risk factors may not promote IHD. . . .

"Above the extreme low end of its observed range, serum ferritin may also be a significant, though weaker, risk factor. Clearly there are many more iron-replete men and postmenopausal women without clinical evidence of IHD than with. If iron depletion is protective and if the other risk factors promote disease only in iron-replete subjects, serum ferritin may appear to be a risk factor only insofar as it is a marker for the number of years a given subject has been iron replete.

"These considerations should be kept in mind in future epidemiological work on iron and heart disease. Care should be taken to design studies that do not overlook effects of iron depletion. A study of only iron replete subjects would not be expected to reveal protective effects of iron depletion. . . ."

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Sempos and Looker [4] present a "straw man" version of the hypothesis. Upon cursory analysis, the hypothesis that iron depletion protects against CHD looks similar to the authors' version: "the higher your body iron stores the greater the risk of CHD" [4]. However, this alternative rendering of the idea has major implications for study design. Studies, such as many of those cited [4], that include few or no iron depleted subjects cannot test the protective effects of iron depletion as noted previously [6].

The studies reviewed by Sempos and Looker [4] on the epidemiological relationship between serum ferritin and risk are compatible with the core hypothesis. The findings collectively suggest that, in some circumstances but not in others, there is a relationship between serum ferritin and risk even *among iron replete subjects.* Counting the number of papers that report either an effect or no effect, and then treating each paper as a vote for or against the hypothesis is not an adequate approach to the determination of fact. Some studies have failed to find a relationship, though other papers have demonstrated an effect even with a population composed almost entirely of iron replete subjects. Taken together, these studies are compatible with a complex relationship between serum ferritin and risk. The variability in the findings may reflect the presence of unknown modifying factors that influence the dependence of risk on stored iron. However, the studies on serum ferritin and risk do not directly address the core hypothesis which concerns iron depletion and risk.

3. Confirmation of the hypothesis versus identification of possible mechanisms

The authors ignore the distinction between studies designed to answer the question "Does iron depletion protect (by whatever mechanism)?" and those studies that look for a role of iron in one proposed mechanism. As an example of this point consider studies on iron and atherosclerosis. Some studies see a role for iron, others do not. There has not been a definitive clinical study addressing the question "Does iron depletion protect against atherosclerosis?" However, even a conclusive finding that iron depletion had no role whatsoever in protecting against *atherosclerosis* would not invalidate the hypothesis that iron depletion protects against *ischemic heart disease.* Atherosclerosis and ischemic heart disease are not synonymous terms. Iron depleted persons may have lower rates of myocardial infarction despite significant atherosclerosis because of a direct protective effect of iron depletion against ischemic myocardial injury. Evidence against one mechanism does not invalidate the central hypothesis. Sempos and Looker should consider this crucial distinction.

In their discussion of how iron might promote atherosclerosis, the authors focus excessively on one potential mechanism: catalysis of LDL oxidation by iron. There may well be other mechanisms by which iron promotes atherosclerosis that do not directly involve LDL, including, for example, a role for iron in promoting vascular smooth muscle cell proliferation [6].

4. Appropriate measures of stored iron

Comments by Sempos and Looker [4] on the value of various measurements of iron status to assess the stored iron level are misleading. They note correctly that "less direct and sensitive measures of body iron stores [than serum ferritin] are serum iron, total iron binding capacity (TIBC), and transferrin saturation (TS)" [4]. However they do not point out that these measures other than serum ferritin are essentially without merit in designing observational studies to evaluate the iron hypothesis. Serum iron, TIBC and TS as single measurements or taken together cannot reliably differentiate iron depletion from either iron deficiency or anemia of chronic disorders. In otherwise healthy subjects with iron depletion, as distinct from iron deficiency, these measures of iron status are typically within normal limits. Low values for serum iron and TS and high values for TIBC are generally not seen in iron depletion but only in frank iron deficiency. There is a gray zone between iron depletion and what everyone would agree is iron deficiency in which some lowering of serum iron and TS with an increase in TIBC may be observed in otherwise healthy subjects. However, in anemia of chronic disorders, serum iron is often low in the presence of *elevated* stored iron.

Using one or more of these measurements as the sole criterion for detecting iron depletion will miss many iron depleted subjects with normal values for serum iron, TIBC and TS while at the same time misclassify as iron depleted those with elevated iron stores associated with anemia of chronic disorders. These issues were presented in the 1992 paper cited above [6]:

"An additional caveat for future epidemiological investigations concerns the study of spontaneously iron depleted subjects. . . . In affluent societies there are few causes of iron depletion in completely healthy adult subjects. Menstrual blood loss and regular frequent blood donation are by far the most common causes in Western countries. Middle-aged men with low ferritin are unusual. In this group low ferritin is often an important sign of poor health. Low ferritins can be associated with chronic occult blood loss, chronic malabsorption or the chronic use of certain drugs. A study of IHD incidence in spontaneously iron depleted subjects might be invalid because of high prevalences of these disorders, especially if all cause mortality is one of the end points of interest."

Uncomplicated iron deficiency can lower serum iron and TS and raise TIBC. But some of these changes also occur in the wide range of conditions associated with anemia of chronic disorders. As noted above [6], studies of spontaneously iron deficient subjects have a built in bias toward finding an association of low serum iron, low TS and/or

high TIBC with higher mortality. This is because, in the industrialized countries, spontaneously iron deficient persons are likely to be sick, unless they are menstruating women or regular blood donors. For example, chronic bleeding from the tumor in a case of colon cancer often causes severe iron deficiency over time. A case of colon cancer associated with iron deficiency at the time of diagnosis in no way supports the conclusion that the iron deficiency caused the cancer. Rather, the cancer caused the iron deficiency through chronic blood loss, and the cancer also caused an increased risk of mortality. To then attribute the increased mortality to iron deficiency is an obvious error.

One of the most notable examples of this misconception is the study of Corti et al [7], cited prominently by Sempos and Looker [4]. Corti et al [7] used only serum iron determination to investigate the role of stored iron in mortality in elderly patients. Unsurprisingly, they found that low serum iron is associated with increased mortality. The result is unremarkable in light of what has been known about clinical iron metabolism for decades. Corti et al [7] then drew a conclusion that the association of low serum iron with mortality in the elderly is evidence against the iron hypothesis, a non-sequitur. Corti et al [7] should have been aware of the facts regarding use of serum iron to measure stored iron, and also the built-in bias in such a study design toward finding a spurious causal association between low stored iron and excess mortality.

(The authors suggest that my citation of a study on iron and cancer [2] endorses the use of the lesser measurements as valid indicators of stored iron burden. This is a misreading of my comments. In the paper in question [2], I made a brief allusion to work on iron and other disorders, such as cancer and infectious diseases. My citation of a study that used one of the lesser measures of iron status was intended to give the reader an introduction to the large literature on iron and cancer.)

5. Cardiovascular disease and inherited iron overload

Sempos and Looker [4] note the lack of proof that full blown hemochromatosis is associated with ischemic heart disease and conclude that this is evidence against the iron hypothesis. In fact, no properly designed study has examined this issue. However, recently two studies were jointly published showing that an increase in cardiovascular events is significantly associated with heterozygosity for the most common known genetic polymorphism for hemochromatosis [8,9]. Association between carrier status and cardiovascular events rebuts the criticism of the iron hypothesis on this point. The implications of the new findings with regard to this criticism have been reviewed in detail in an accompanying editorial [3].

6. Cardiovascular disease and dietary iron

Iron depletion can occur despite high iron intake. As with a bank account, the balance cannot be determined only from the deposits, withdrawals must also be considered. Any form of chronic blood loss, e.g. aspirin use, menstruation, or blood donation, may cumulatively cause iron withdrawals in excess of iron deposits. Extremely high intakes of iron can cause an increase in stored iron in people and in experimental animals. However, among most people in the iron replete countries, the association of iron intake and the amount of stored iron in the body is tenuous. Very little of the dietary iron consumed is absorbed. Iron intake is woefully inadequate as a sole indicator of the presence of iron depletion or iron deficiency. On the other hand, the heme iron component of total iron intake may correlate better with both stored iron burden and mortality from myocardial infarction [10], an effect compatible with the iron hypothesis. Dietary iron intake is only relevant to the iron hypothesis to the extent that it affects iron storage levels. The discussion of dietary iron and heart disease [4] does not address these important distinctions.

7. Supportive studies not cited

Sempos and Looker [4] do not cite many recent studies (including references 11–42) that provide experimental or observational support for various aspects of the iron hypothesis. In particular, the direct effect of iron as a promoter of ischemic myocardial injury is not presented by these authors. In my view, the totality of evidence now available favors the iron hypothesis.

8. Support or invalidate?

A key deficiency of the Sempos and Looker [4] brief is that they do not address the question of whether or not the findings prove the iron hypothesis wrong. In fact, even the incomplete data reviewed by these authors remain entirely consistent with the iron hypothesis. “The data do *not* support” [4] is not the same conclusion as “the data invalidate.” The data, in fact, do not invalidate the hypothesis. Nothing in the available data rules out a protective effect of iron depletion strong enough to explain the low rate of myocardial infarction in menstruating women [1–3].

9. Conclusions

The iron hypothesis has not been proved wrong in nearly 20 years of observation, experimentation and sometimes rancorous debate. The literature cited by Sempos and Looker [4] is consistent with the idea and many of the cited papers as well as a multitude of uncited studies provide

strong support for it [1–3,5,11–42]. At issue in this debate is a robust, unrefuted hypothesis with broad explanatory power that offers the possibility of low risk, low cost preventive therapy against the leading cause of death in the industrialized countries. Sempos and Looker [4] seem ready to reject the hypothesis on the basis of flawed studies, while at the same time opposing a prospective, randomized trial to properly test the hypothesis. Their urging that no large scale randomized trial be done [4] may delay a definitive test of primary prevention of ischemic heart disease by iron depletion. A trial of secondary prevention in patients with established peripheral vascular disease has been initiated [43]. Preliminary findings appear compatible with a significant decrease in cardiovascular events in phlebotomized patients [43].

Every area of biomedical science requires careful thinking and attention to detail, however, we have a particularly heavy burden to take pains in the investigation of our leading cause of death. Superficial analysis may lead to delay in implementing a new preventive therapy. Each day of delay could potentially take thousands of lives.

Even in the absence of properly completed trial, physicians have an obligation to use medical judgment [3]. On the basis of the existing data, many physicians may conclude that it is prudent to help patients lower their stored iron burden. The risk/benefit ratio appears to be favorable. On the risk side of the equation, iron depletion is a benign condition and iron removal by phlebotomy under medical supervision is safe. The potential benefit of lowering the risk from our leading cause of death is large in relation to the minimal risk of stored iron removal.

References

[1] Sullivan JL, Iron and the sex difference in heart disease risk, Lancet, 1 (1981) 1293–4.

[2] Sullivan JL, Iron versus cholesterol–perspectives on the iron and heart disease debate, J Clin Epidemiol, 49 (1996) 1345–52.

[3] Sullivan JL, Iron and the genetics of cardiovascular disease (Editorial). Circulation 1999;100:1260–1263.

[4] Sempos CT, Looker AC, Iron status and the risk of coronary heart disease. An example of the use of nutritional epidemiology in chronic disease research. J Nutr Biochem, 12 (2001) (submitted).

[5] de Valk B, Marx JJM, Iron, atherosclerosis, and ischemic heart disease Arch Intern Med. 1999;159:1542–8.

[6] Sullivan JL, Stored iron as a risk factor for ischemic heart disease. In: Lauffer RB (Ed.). Iron and Human Disease. Boca Raton: CRC Press 1992.

[7] Corti M-C, Guralnik JM, Salive ME, Ferrucci L, Pahor M, Wallace RB, Hennekens CH, Serum iron level, coronary artery disease, and all-cause mortality in older men and women, Am J Cardiol, 79 (1997) 120–127.

[8] Roest M, van der Schouw YT, de Valk B, Marx JJM, Tempelman MJ, de Groot PG, Sixma JJ, Banga JD, Heterozygosity for a hereditary hemochromatosis gene is associated with cardiovascular mortality in women, Circulation, 100 (1999) 1268–1273.

[9] Tuomainen T-P, Kontula K, Nyyssönen K, Lakka TA, Heliö T, Salonen JT, Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation: a prospective cohort study in men in eastern Finland, Circulation, 100 (1999) 1274–1279.

[10] Ascherio A, Willett WC, Rimm EB, Giovannucci EL, Stampfer MJ, Dietary iron intake and risk of coronary disease among men, Circulation, 89 (1994) 969–974.

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

[12] Araujo JA, Romano EL, Brito BE, Parthe V, Romano M, Bracho M, Montano RF, Cardier J, Iron overload augments the development of atherosclerotic lesions in rabbits, Arterioscler Thromb Vasc Biol, 15 (1995) 1172–80.

[13] Bolli R, Patel BS, Jeroudi MO, Li XY, Triana JF, Lai EK, McCay PB. Iron-mediated radical reactions upon reperfusion contribute to myocardial "stunning, Am J Physiol, 259 (6 Pt 2) (1990) H1901–11.

[14] Chopra K, Singh M, Kaul N, Andrabi KI, Ganguly NK, Decrease of myocardial infarct size with desferrioxamine: possible role of oxygen free radicals in its ameliorative effect, Mol Cell Biochem, 113 (1992) 71–6.

[15] DeBoer DA, Clark RE, Iron chelation in myocardial preservation after ischemia- reperfusion injury: the importance of pretreatment and toxicity, Ann Thorac Surg, 53 (1992) 412–8.

[16] Dulchavsky SA, Davidson SB, Cullen WJ, Devasagayam TP, Diebel LN, Dutta S, Effects of deferoxamine on H2O2-induced oxidative stress in isolated rat heart, Basic Res Cardiol, 91 (1996) 418–24.

[17] Ely D, Dunphy G, Dollwet H, Richter H, Sellke F, Azodi M, Maintenance of left ventricular function (90%) after twenty-four-hour heart preservation with deferoxamine, Free Radic Biol Med, 12 (1992) 479–85.

[18] Evans PJ, Smith C, Mitchinson MJ, Halliwell B, Metal ion release from mechanically-disrupted human arterial wall. Implications for the development of atherosclerosis, Free Radic Res, 23 (1995) 465–9.

[19] Ferreira R, Burgos M, Milei J, Llesuy S, Molteni L, Hourquebie H, Boveris A, Effect of supplementing cardioplegic solution with deferoxamine on reperfused human myocardium, J Thorac Cardiovasc Surg, 100 (1990) 708–14.

[20] Jian Y, Lesnefsky EJ, Incremental iron overload during reperfusion progressively augments oxidative injury, Chin Med J (Engl), 109 (1996) 450–8.

[21] Katoh S, Toyama J, Kodama I, Akita T, Abe T, Deferoxamine, an iron chelator, reduces myocardial injury and free radical generation in isolated neonatal rabbit hearts subjected to global ischaemia-reperfusion, J Mol Cell Cardiol, 24 (1992) 1267–75.

[22] Kobayashi S, Tadokoro H, Wakida Y, Kar S, Nordlander R, Haendchen RV, Corday E, Coronary venous retroinfusion of deferoxamine reduces infarct size in pigs, J Am Coll Cardiol, 18 (1991) 621–7.

[23] Lee FY, Lee TS, Pan CC, Huang AL, Chau LY, Colocalization of iron and ceroid in human atherosclerotic lesions, Atherosclerosis, 138 (1998) 281–8.

[24] Lee TS, Shiao MS, Pan CC, Chau LY. Iron-deficient diet reduces atherosclerotic lesions in apoE-deficient mice, Circulation, 99 (1999) 1222–9.

[25] Lesnefsky EJ, Repine JE, Horwitz LD, Deferoxamine pretreatment reduces canine infarct size and oxidative injury, J Pharmacol Exp Ther, 253 (1990) 1103–9.

[26] Liu LL, Zhao BL. A study on the protective effect of metallic chelators on ischemia-reperfusion injury of isolated rat heart, Chung Hua Hsin Hsueh Kuan Ping Tsa Chih, 1993;Oct;21:304–6, 317.

[27] Maruyama M, Pieper GM, Kalyanaraman B, Hallaway PE, Hedlund BE, Gross GJ, Effects of hydroxyethyl starch conjugated deferoxamine on myocardial functional recovery following coronary occlusion and reperfusion in dogs, J Cardiovasc Pharmacol, 17 (1991) 166–75.

[28] Marzabadi MR, Llvaas E, Spermine prevents iron accumulation and depresses lipofuscin accumulation in cultured myocardial cells, Free Radic Biol Med, 21 (1996) 375–81.

[29] Matthews AJ, Vercellotti GM, Menchaca HJ, Bloch PH, Michalek VN, Marker PH, Murar J, Buchwald H, Iron and atherosclerosis: inhibition by the iron chelator deferiprone (L1), J Surg Res, 73 (1997) 35–40.
[30] Menasche P, Grousset C, Mouas C, Piwnica A. A promising approach for improving the recovery of heart transplants. Prevention of free radical injury through iron chelation by deferoxamine, J Thorac Cardiovasc Surg, 100 (1990) 13–21.
[31] Morita K, Ihnken K, Buckberg GD, Sherman MP, Young HH, Studies of hypoxemic/reoxygenation injury: without aortic clamping. IV. Role of the iron-catalyzed pathway: deferoxamine. J Thorac Cardiovasc Surg 1995;110:1190–9.
[32] Paolicchi A, Minotti G, Tonarelli P, Tongiani R, De Cesare D, Mezzetti A, Dominici S, Comporti M, Pompella A. Gamma-glutamyl transpeptidase-dependent iron reduction and LDL oxidation–a potential mechanism in atherosclerosis, J Investig Med, 47 (1999) 151–60.
[33] Pool GF, van Jaarsveld H, Dietary iron elevates LDL-cholesterol and decreases plasma antioxidant levels: influence of antioxidants, Res Commun Mol Pathol Pharmacol, 100 (1998) 139–50.
[34] Pratico D, Pasin M, Barry OP, Ghiselli A, Sabatino G, Iuliano L, FitzGerald GA, Violi F. Iron-dependent human platelet activation and hydroxyl radical formation: involvement of protein kinase C, Circulation, 99 (1999) 3118–24.
[35] Sullivan JL, Till GO, Ward PA, Newton RB, Nutritional iron restriction diminishes acute complement-dependent lung injury, Nutr Res, 9 (1989) 625–34.
[36] Thong PSP, Selley M, Watt F, Elemental changes in atherosclerotic lesions using nuclear microscopy, Cell Molec Biol, 42 (1996) 103–110.
[37] Tiede R, Sareen S, Singal PK, Transferrin delays oxygen radical induced cardiac- contractile failure, Can J Physiol Pharmacol, 68 (1990) 480–5.
[38] van Jaarsveld H, Pool GF, Barnard HC, Dietary iron concentration alters LDL oxidatively. The effect of antioxidants, Res Commun Mol Pathol Pharmacol, 99 (1998) 69–80.
[39] Van Lenten BJ, Prieve J, Navab M, Hama S, Lusis AJ, Fogelman AM, Lipid- induced changes in intracellular iron homeostasis in vitro and in vivo, J Clin Invest, 95 (1995) 2104–10.
[40] Williams RE, Zweier JL, Flaherty JT, Treatment with deferoxamine during ischemia improves functional and metabolic recovery and reduces reperfusion-induced oxygen radical generation in rabbit hearts Circulation 1991;83:1006–14.
[41] Yuan XM, Anders WL, Olsson AG, Brunk UT, Iron in human atheroma and LDL oxidation by macrophages following erythrophagocytosis, Atherosclerosis, 124 (1996) 61–73.
[42] Horwitz LD, Rosenthal EA. Iron-mediated cardiovascular injury, Vascular Medicine, 4 (1999) 93–99.
[43] Zacharski LR, Chow B, Lavori PW, Howes PS, Bell MR, DiTommaso MA, Carnegie NM, Bech F, Amidi M, Muluk S, The Iron (Fe) and Atherosclerosis Study (FeAST): A pilot study of reduction of body iron stores in atherosclerotic peripheral vascular disease, Am Heart J, 139 (2000) 337–45.