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# Iron status and the risk of coronary heart disease: an example of the use of nutritional epidemiology in chronic disease research

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## 1. Introduction

Nutritional epidemiology is the science of how nutrition affects health. Based on the principals of nutritional science, and epidemiology, it is an essential component in the research to understand how diet affects risk of disease and in the development of public health policies and prevention strategies. Because nutritional epidemiologists have the unique opportunity to directly examine the association between nutrition and the risk of disease the results from their studies attract a great deal of attention in the popular press and they can have a powerful impact on the development of public policy. Unfortunately there is a great deal of misunderstanding about the nature of nutritional epidemiology -its strengths and its weaknesses. And while controversy occurs in all forms of science it has tended to increase the level of misunderstanding.

A currently controversial topic of research in nutritional epidemiology is the relationship of body iron stores to the risk of developing coronary heart disease (CHD). In 1981 Dr. Jerome Sullivan [1] proposed a new theory to explain the differences in CHD incidence and mortality between men and women. He noticed that as men and women age the gaps between them in heart disease incidence and in body iron stores both decrease [2,3]. Lower stores of iron in women are due mostly to menstrual blood loss and with menopause the differences in iron stores decrease. As a result, he theorized 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. The hypothesis was largely ignored until 1992 when it was reported that body iron stores were directly related to the risk of having a heart attack in Finnish men [4]. Since then, however, there has been an intense interest in this topic.

At present there is no generally agreed upon model for how body iron stores might directly affect the risk of CHD but it is thought that iron might indirectly promote the atherosclerosis leading to CHD by catalyzing the oxidation of low density lipoprotein (LDL) cholesterol [5–10]. The primary purpose of this article is to review the epidemiological evidence for and against the hypothesis that body iron stores may be directly associated with an increased risk of CHD or indirectly associated through the oxidation of LDL cholesterol. At the same time we hope to give you a brief introduction to the science of nutritional epidemiology and a sense of how it can complement traditional nutritional science.

## 2. Overview of nutritional epidemiology

Nutritional epidemiology is a specialized field within epidemiology. Epidemiology has been defined as the study of the patterns of disease occurrence in human populations and of the factors that influence those patterns [11]. Nutritional epidemiology is therefore the study of the nutritional determinants of disease [12]. Most of the subject matter is concerned with the effects of diet on chronic diseases that are multifactorial and that take years if not decades to develop.

Methods in nutritional epidemiology are designed to take those features into account. They focus on measuring exposure to nutritional factors, frequency and distribution of disease, and exposure to other factors that can confound the hypothesized association. Nutritional exposures may be the dietary intake of foods, nutrients, non-nutrients, additives, contaminants, chemicals formed during food processing or preparation, and other natural compounds. They also include biochemical measures of nutritional status, biomarkers of intake, biological intermediates influenced by diet, anthropometry, genetic markers and clinical measures [13].

The sequence of reasoning in epidemiology is, first, to determine statistically significant associations between nutritional exposures and the risk of disease and, second, to

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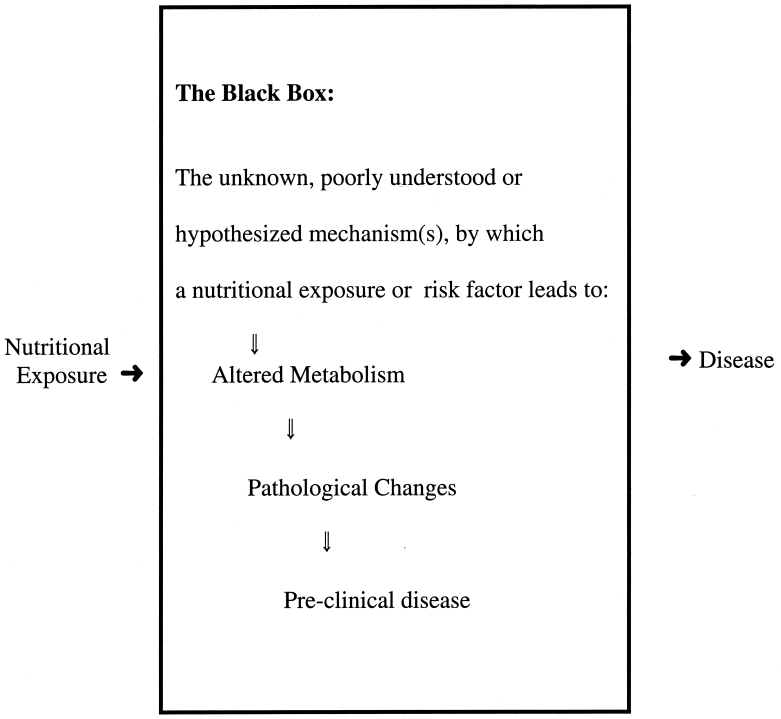


Fig. 1. The "Black Box" model of disease development and prevention in Nutritional Epidemiology.

determine biological inferences from the pattern of associations that emerges [11]. The paradigm currently used to investigate possible associations is known as the "Black Box" approach [14–16]. The "Black Box" (Figure 1) is a metaphor for the unknown, poorly understood or hypothesized mechanism by which an exposure or purported risk factor may lead to: 1) altered metabolism; 2) pathological changes; 3) pre-clinical disease; and eventually 4) to clinically apparent disease and possibly death. An association is first established by assessing if the incidence or mortality rate of the disease in question varies by the level of exposure. If it does and if it can be shown that the exposure precedes the development of the disease and that by changing the level of exposure you can significantly reduce the rate of disease then it may be possible to establish not only a causal relationship but a mechanism for disease prevention. The idea is that you may not need to understand precisely the mechanism by which an exposure leads to increased rates of disease in order to devise useful public health prevention strategies and clinical therapies. Three notable examples of the successful use of this approach are for: 1) cigarette smoking and lung cancer and other diseases; 2) high blood pressure and cardiovascular diseases; and 3) diet and heart disease [17].

## 3. Incidence, prevalence, risk and relative risk

Like all fields of study Epidemiology has its own set of terminology [18]. Four essential terms are: incidence, prevalence, risk, and relative risk.

The two most important measures of disease frequency in epidemiology are incidence and prevalence. The incidence rate (I) is the number of *new* cases of disease (d) that develop or are diagnosed in a specified time period ($\Delta t$) divided by the population at risk ($N_{risk}$) of developing the disease or condition, i.e. those who do not already have the disease of interest.

$$\text{Incidence (I)} = \frac{\text{Number of new cases of disease (d)}}{\text{Population at Risk } (N_{risk})} \text{ in time } (\Delta t)$$

Incidence includes both fatal and non-fatal cases of a disease or condition, a.k.a. events. Because incidence is defined as the rate of new or future events in the population at risk, it is *the* fundamental measure of disease frequency for the study of disease etiology [19,20].

The prevalence (P) of a condition or disease is defined as the total number of known or existing cases at a point in time divided by the size of the population at the same point in time.

$$\text{Prevalence (P)} = \frac{\text{Total number of existing cases of disease (n)}}{\text{Total population } (N_{Total})}$$

at a point in time

This definition of prevalence is also called the *point* prevalence.

Notice that both incidence and point prevalence are proportions. That is the numerator is a subset of the denominator. As a result, both are estimates of probability or risk. Risk is a synonym for probability. With incidence we estimate the probability of *developing* a disease within a certain time period while with prevalence we estimate the probability of *having* a disease at a particular point in time.

Using incidence data, relative risk is estimated as the ratio of the incidence rate in the exposed group, e.g. the CHD incidence rate in those *with* high serum ferritin levels, divided by the incidence rate in the unexposed group, e.g. the CHD incidence rate in those *without* high serum ferritin levels. A similar measure of relative risk exists for prevalence as well.

## 4. Types of studies

There are two general types of studies in epidemiology: observational and experimental [12]. In observational studies, the nutritional exposure is measured but not manipulated, the frequency and patterns of disease are observed, and the statistical association between a suspected nutritional exposure and disease risk is estimated. In experimental studies, one set of individuals is randomly assigned to a treatment or intervention group and the other to a control group.

For operational and ethical reasons, most epidemiologic research is observational [13]. The most common types of observational studies are the cohort, case-control and cross-sectional studies [12]. Cohort studies are referred to also as prospective, incidence, follow-up, and longitudinal studies [21]. The question being asked in cohort studies is - Do persons with the risk factor develop or die from the disease more frequently or sooner than those who do not have the risk factor? Cohort studies are initiated with a cross-sectional survey where the presence or absence of the disease of interest , dietary intake and other measures of exposure are ascertained. After prevalent cases of, for example, CHD are excluded the population is then followed over time to see who develops CHD and when it develops. The persons remaining in the study are those who are at risk of developing the disease. That is, they are the participants for whom it can be reasonably established that the nutritional exposure preceded the development of the disease. After a reasonable period of follow-up, analyses designed to look at the association between, say, serum ferritin and the risk of developing CHD, i.e. the incidence of CHD, would be conducted. Because risk factor exposure is measured prior to the onset of clinically apparent disease and since incidence is directly measured, cohort studies are considered to be the strongest observational study design in epidemiology [22,23].

A type of cohort study that is a hybrid of the cohort and case-control study designs is the nested case-control study [12,23]. The term implies that a case-control study (see below) is inserted into a cohort study. In such a study the researcher compares all the cases that have developed over a defined period of time with a random sample of controls selected from the cohort. For example, because of cost concerns a researcher may collect 7-day food records or serum samples for all the participants in the study at the first or baseline exam but stores the data for future use instead of analyzing it immediately [24]. A portion of those stored data may then be analyzed in the future to compare cases that have developed since the first exam with a random sample of controls. Such a study is prospective in design (exposure precedes diagnosis) and it is cost effective because the analysis of samples takes place only in a subset of the entire cohort. This design allows the researcher the flexibility to investigate hypotheses that would have been unknown at the start of the study and therefore extends the utility of cohort studies.

The case-control study is also know as a retrospective study [19,20]. There are many variations of the case-control study design but, classically, in this type of study persons with the disease of interest are located, a suitable group of controls is found and the exposure of interest is measured [23]. The objective is to measure exposure status prior to the onset of clinical symptoms. Many times this is possible but often exposure is ascertained through questionnaire or laboratory analysis concurrently with disease diagnosis. In all instances it must be assumed that the disease did not affect the assessment or measurement of exposure status [22].

Cross-sectional or prevalence studies share many similarities with case-control studies. In cross-sectional studies a sample of persons, often a random or representative sample, is selected and then physical and laboratory measurements along with assessments to enable diagnosis of the disease of interest are performed. The most widely known example of this type of study is the periodic National Health and Nutrition Examination Surveys or NHANES [25]. In contrast to a case-control study, persons are invited to participate regardless of whether or not they have a particular disease in order to interview and examine a representative sample of the reference population. In both case-control and cross-sectional studies, however, cases with the disease of interest are compared to non-cases (controls) and the question being asked is: “Are cases more likely to have higher (lower) levels of the nutritional exposure than non-cases?”

Case-control and cross-sectional studies are not generally considered to be as rigorous as cohort studies [20,22]. Two disadvantages with both types of studies are that incidence is not directly measured and both disease status and exposure are generally measured at the same time. For example, the disease itself may alter measurements related to the exposure. Additionally, people who already know that they have the disease or who know they are sick before they are examined may change their behavior so as to alter their exposure level [22]. As a result, it is difficult to establish if the exposure led to the disease or vice versa.

Experimental studies are also conducted in epidemiology [12,26]. Two examples of experiments in epidemiology and nutrition are randomized clinical trials, such as the DASH clinical trial on effects of dietary patterns on blood pressure [27] and the classic feeding studies of Keys et al. [28] and

Hegsted et al. [29] looking at the effects of fatty acid and dietary cholesterol intake on serum total cholesterol levels. But they are much less common than the observational studies.

## 5. Interpretation of epidemiologic data and assessment of causality

Interpretation of data from observational epidemiology studies can be difficult. Because these studies are not experiments there is always the possibility that the results are affected by measurement error or confounding. A major consideration in the design of nutritional epidemiology studies is the need to reduce those forms of bias. Confounding also may be reduced in data analysis. The development of the multivariate discriminant model (and later the logistic regression model) was one of the most important advances in modern epidemiology [30]. To help reduce the potential for confounding, epidemiologists use multivariate models to simulate an experiment [31]. By using multivariate regression models that adjust for possible confounding factors, it is possible to evaluate the association between the exposure of interest and the outcome variable while holding all other variables in the model constant [32]. One feature of diet, however, cannot be minimized by statistical adjustment: components in the diet are highly correlated. This is also referred to as collinearity or multicollinearity.

As a result of multicollinearity, it is usually not possible to determine the effects of a particular nutrient independent of the other nutrients with which it is highly correlated [33,34]. For example, McGee et al. [35] showed that because the components of energy intake were highly correlated, the separate effect of fat from protein cannot be demonstrated in observational studies: "If the question is simply whether the dietary variables as a group predict CHD and we are not interested in the exact relationship of a particular diet variable to CHD, solutions clearly exist. If, on the other hand, we are interested in interpreting how a particular diet variable relates to outcome controlling for other diet variables, the collinearity of the data appears to be a structural rather than a mathematical problem with no apparent solution [35]." This is the fundamental limitation in observational nutritional epidemiology. We believe that the same may also be true in the situation where the metabolism of nutrients is highly interrelated.

There is a great deal of debate on what constitutes evidence of causality [36,37]. In rare instances epidemiological data alone may be sufficient to demonstrate causality. However, since most of the data from those studies are observational rather than experimental, it is generally only through an iterative exchange of hypotheses and results among the laboratory, clinical, and the epidemiologic sciences that progress can ultimately be made in understanding whether the nutritional exposure is a causal factor and, given the multifactorial nature of chronic disease, what role nutrition or, more precisely, diet can play in disease prevention.

## 6. Iron and heart disease: the epidemiological data

### *6.1. Serum measures of body iron stores*

In order to evaluate the research data on this subject it is important to understand how body iron stores are measured in epidemiological studies. Serum ferritin is currently the best measure of body iron stores that is feasible to use in epidemiological studies [38]. It is a fairly sensitive indicator of changes in body iron stores as you move along the stages of iron status from deficient to replete to iron overload in healthy individuals, e.g. not suffering from an infection, inflammation or cancer. As stores increase so do serum ferritin levels. The opposite trends occur as body iron stores decrease [39]. A serum ferritin level of $<$12–15 $\mu$g/L has been used as an indicator of iron deficiency in both men and women [38,40]. Separate upper limits have been suggested for adult men (400 $\mu$g/L), menstruating women (200 $\mu$g/L) and postmenopausal women (300 $\mu$g/L) [40].

Less direct and sensitive measures of body iron stores are serum iron, total iron binding capacity (TIBC) and transferrin saturation (TS) which is calculated as the ratio of serum iron to total iron binding capacity [38]. Like serum ferritin, TS and serum iron levels are positively related to body iron stores while TIBC levels tend to decrease as stores increase. At very high levels of body iron stores as in homozygous hemochromatosis (TS $>$ 60%), or at depleted levels (TS $<$ 16%), i.e. iron deficiency, TS is considered to be good measure of body iron stores. Within the normal range of TS, i.e. 20%–60%, TS is a relatively weak indicator of stores [41]; within that normal range, the correlation between TS and ferritin is about 0.2 [42].

Serum iron status measures are also affected by inflammation, cancer, liver damage, and infection [43–45]. Serum ferritin levels tends to increase in response to inflammation while TS, TIBC and serum iron levels decrease. For example, in response to a heart attack ferritin levels are initially raised while TS, TIBC and serum iron levels decrease [46, 47]. In the study by van der Schouw et al. [48] serum ferritin levels returned to control levels 6 weeks after a heart attack while TS, and serum iron levels continued to be depressed.

### *6.2. Hypotheses and possible mechanisms*

There are three possible hypotheses related to the iron-heart disease debate. The first hypothesis might be termed the "Low or No Threshold" hypothesis. In this hypothesis, stored iron at any level promotes myocardial ischemia or ischemic heart disease and that iron depletion will protect against that ischemia [2]. The second hypothesis could be termed the "High Threshold" hypothesis. The rationale for this hypothesis stems from the results in Finnish men where

those with serum ferritin levels at or above 200 $\mu$g/L had a greater than 2 fold increased risk of heart attack compared to men with levels below 200 $\mu$g/L [4]. As mentioned above a serum ferritin of 200 $\mu$g/L is in the high normal range for men [40]. The final hypothesis - the "Linear or Graded Risk" hypothesis- is that risk of CHD increases linearly with increased body iron stores and also is a result of the initial findings from Finland [4].

The possible effects of body iron stores on CHD risk may be viewed as direct or indirect. It has been suggested that body iron stores might directly lead to CHD through a mechanism which might involve oxidative damage to the myocardium [1–3]. Contrariwise, it has been proposed that body iron stores might indirectly lead to CHD. The indirect model by which body iron stores might effect CHD risk is a modification of the classical model for the pathogenesis of CHD. In the classical model a diet high in saturated fatty acids and cholesterol leads to increases in serum total and LDL cholesterol, atherosclerosis, and often to clinical coronary heart disease resulting in heart attack and possibly premature death [49,50]. In the modified model the oxidation of LDL cholesterol is an important step for its absorption by macrophages and the subsequent development of foam cells leading to fatty streak formation and atherosclerosis [6,7,9]. The role of iron in the model is as a catalyst for the free radical oxidation of LDL cholesterol [8,9]. Finally, it has been proposed that any relationship between serum ferritin and CHD risk may be as a result of inflammation. Some consider atherosclerosis to be an inflammatory disease [51]. In general, however, inflammation has generally been considered a confounder or nuisance variable because of its effect in raising serum ferritin levels. But with the coming together of the injury and lipid hypotheses of atherosclerosis [7] it may be more important to focus on whether iron may promote the inflammation leading to atherosclerosis rather than viewing inflammation as a side issue.

Regardless of the possible mechanism, using the "Black Box" approach with both observational and experimental epidemiological data it is possible to design studies and analyses that can test both the direct and indirect mechanisms for the "Low"or "High" threshold hypotheses and the "Linear or Graded Risk" hypothesis.

### *6.3. The direct impact of body iron stores on CHD risk*

#### *6.3.1. Serum ferritin and CHD risk - cohort studies*

The relevant question being asked in cohort studies designed to assess the relationship between body iron stores and CHD is - are persons who have high body iron stores more likely to develop CHD in the future than are persons who do not? Using various measures of body iron stores, a number of researchers have attempted to address this question (Table 1).

The first such study was by Salonen et al. [4] and it was based on the Finnish Kuopio Ischemic Heart Disease Risk Factor Study (KIHD). They reported finding a statistically significant positive linear association between serum ferritin level and the risk of heart attack ($z = 2.64$, $p < 0.01$) in men after adjusting for possible confounding. Thus, as serum ferritin levels increased so did the risk of heart attack. The more surprising finding was, however, that men with a serum ferritin 200 $\mu$g/L, which is considered to be in the high but normal range, had a greater than two fold higher risk of heart attack compared to those with lower serum ferritin values. The difference was statistically significant (relative risk = 2.2, 95% CI 1.2–4.0, $p < 0.01$). Additionally, they reported finding that compared to men with serum ferritin levels $<$ 200 g/L, men with a ferritin of 200–399 g/L had a nearly identical risk of heart attack as did men with ferritin levels 400 g/L [4]. Moreover, the association between serum ferritin and heart attack was not attenuated when the analysis was repeated after removing heart attacks which occurred within the first six months following blood collection. Serum ferritin levels go up after a heart attack but return to baseline levels within six weeks afterwards, as stated earlier [48].

In a letter to the editor, they presented data to indicate that the relationship was still significant after an average of five years of follow-up and 83 heart attacks - relative risk = 2.0, 95% CI = 1.2–3.1, $p = 0.004$ [52]. They also found, in a subsample of their cohort, that the ratio of transferrin to ferritin was positively related to CHD risk [53]. This is not surprising given the strong finding in the larger cohort. Moreover, because the three studies by their group were based on the same set of individuals from the KIHD cohort we have considered them as one study in support of the hypothesis (Table 1).

The results from eight other cohort studies [54–60,62] on the association between serum ferritin and CHD have been reported (Table 1). Only one of them found a consistent association between serum ferritin and CHD [58]. In that study Kiechl et al. [58] reported that the 5-year progression of carotid stenosis was significantly related to serum ferritin levels. The study consisted of 826 men and women 40 to 79 years of age who were randomly selected from the population of Bruneck, Italy. Carotid atherosclerosis was assessed by repeated carotid ultrasound evaluation. The authors further reported that changes in iron stores were associated with changes in the progression of carotid atherosclerosis in that lowering of stores was associated with a decreased risk of progression while increases in stores were associated with an increased risk. It would important to see if those interesting results can be replicated.

The studies which found no association between serum ferritin and CHD each addressed slightly different aspects of the iron hypothesis. In the study by Magnusson et al. [54] 2,036 Icelandic men and women ages 25–74 years were followed for an average of 8.5 years. In their multivariate models, Magnusson et al. included serum ferritin, in the normal units or log transformed as continuous variables to test if a statistically significant positive linear association

Table 1
Serum ferritin and the risk of heart disease: cohort studies

| Authors (reference) | Age (y) | Sex | Sample size | Mean years of followup | Incident cases | | Iron/disease association |
|---|---|---|---|---|---|---|---|
| | | | | | Type | Number | |
| *Kupio Ischemic Heart Diease Risk Factor (KIHD) Study** | | | | | | | |
| Salonen (4) | 42,48,54,60 | M | 1,931 | 3 | MI | 51 | + |
| Salonen (52) | 42,48,54,60 | M | 1,931 | 5 | MI | 83 | + |
| Toumainen (53)** | 42,48,54,60 | M | 197 | 6.4 | MI | 99 | + |
| Magnusson (54)† | 25–74 | M | 990 | 8.5 | MI | 63 | None |
| | | W† | 1,046 | 8.5 | MI | 18 | None |
| Stampfer (55)** | 40–84 | M | 476 | 8 | MI | 238 | None |
| Mänttäri (56)** | 40–55 | M | 268 | 5 | CHD | 134 | None |
| Frey (57) | 30–89 | M | 298 | 5.2 | MI | 32 | None |
| Kiechl (58) | 40–79 | M/W | 780 | 5 | CHD | 375 | + |
| Aronow (59) | 62+ | M/W | 577 | 3 | MI | 235 | None |
| Marniemi (60) | 65+ | M/W | 344 | 13 | CVD death | 142 | None |
| Klipstein-Grobusch** (62) | 55+ | M/W | 172 | 4 | MI | 60 | Mixed none total + smokers |

Abbreviations: Y = years, M = men, W = women, MI = Myocardial Infarction or Heart Attack, CHD = Coronary Heart Disease, CVD = Cardiovascular Disease (Heart Disease + Stroke).

* All three studies are from the same cohort—*Kuopio Ischemic Heart Disease Risk Factor Study (KIHD).* The difference between the two papers by Salonen et al. is that the first consisted of the results after of 3-yr follow-up and the second after a 5-yr follow-up of the same individuals. The third paper is a study on a subset of the cohort in the first two studies looking at the transferrin/ferritin ratio and risk of heart attack.

† Because of the small number of heart attacks among the women the authors concentrated on results for the men alone and for the combined sample of men and women.

** Nested case-control study, i.e a case-control or retrospective study which is ''nested'' within a cohort or cohort study [23]. Serum transferrin/ferritin ratio (Tuomainen [53]), or ferritin (Stampfer [55], Mänttäri [56], Klipstein-Grobusch [62]) were determined on frozen sera collected at the beginning of the study. Cases accrued during the follow-up period and controls were selected from the pool of individuals who were at risk of having CHD at the time a case was diagnosed. Because of the efficient sample design only small sample sizes are required [24].

exists between serum ferritin and risk of heart attack - the "Linear or Graded Risk" hypothesis. Neither serum ferritin (RR = 0.999, 95% CI 0.997–1.001) or log ferritin (RR = 0.781, 95% CI 0.540–1.129) were significantly related to the risk of heart attack.

Stampfer et al. [55] directly addressed the issue of a threshold at 200 $\mu$g/L. Using a variant of the cohort study design - a nested case-control design [23,24], 238 men participating in the U.S. Physicians Health Study had a heart attack during the period after the 1982 baseline. Stored serum for those men and for 238 controls matched for age and smoking status were analyzed for serum ferritin concentrations. And after adjustment for other CHD risk factors, men with serum ferritin levels 200 $\mu$g/L did not have a higher risk of heart attack (RR = 1.1, 95% CI 0.7–1.6).

Similar results were found also in another nested case-control study by Mänttäri et al. [56]. The participants in this study were a subset of men from the Finnish Helsinki Heart Study - a randomized clinical trial of the lipid lowering drug gemfibrozil. They looked to see if the threshold for ferritin occurs at much lower levels. Mänttäri et al assessed the risk of developing CHD in two groups of men with serum ferritin levels of 43–84 $\mu$g/L or 85 $\mu$g/L compared with men with serum ferritin levels of 42 $\mu$g/L and found that it was not different.

Three smaller studies also were conducted to examine the relationship between serum ferritin and CHD risk by: a) comparing mean ferritin levels between cases and non-cases [57,59,60]; b) testing for a positive linear association between serum ferritin and CHD [59,60]; or c) evaluating the risk of heart attack for those having a serum ferritin level above 200 $\mu$g/L [57]. No association was found between serum ferritin and CHD risk in any of these studies. Two of the studies, however, appear to be case series reports [57, 59], which is a weaker type of cohort study. In a case series study a group of patients from a physician's practice is followed over time. Although the studies were prospective in design the reasons why serum ferritin was measured in a group of patients may be related in some unknown way with the development of CHD and the results must therefore be interpreted cautiously [61]. In addition, Frey and Krieder did not appear to adjust results for age or for other CHD risk factors [57].

One study reported mixed results [62]. Klipstein-Grobusch et al. found no association between serum ferritin and risk of MI in the total sample but they did report finding one in the subgroup of smokers. Designed as a nested case-control study with 202 cases and 202 controls the sample sizes were eventually reduced to 60 cases and 112 controls. Given the large loss of sample the possibility of biased results can not be discounted [63].

Table 2
Serum ferritin and the risk of heart disease. Cross-sectional, and case-control studies

| Authors (reference) | Age (y) | Sex | Sample | Heart disease cases | | Type of association |
|---|---|---|---|---|---|---|
| | | | | Type | Number | |
| Cross-sectional studies | | | | | | |
| Aronow (64) | 62–100 | M | 171 | CHD | 74 | None |
| | 62–100 | W | 406 | CHD | 172 | None |
| Solymoss (65) | ? | M | 225 | CAD | 195 | None |
| | ? | W | 74 | CAD | 48 | None |
| Rauramaa (66)* | 50–60 | M | 206 | CVD | 82 | None |
| Kiechl (67)† | 40–59 | M/W | 431 | CAA | ? | + |
| | 60–79 | M/W | 416 | CAA | ? | None |
| Case-control study | | | | | | |
| Duthie (68) | 39 mean | M/W | 225 | Stable angina | 25 | None |
| Rengström (69) | 40 mean | M | 194 | MI | 94 | None |
| Moore (70)** | 45–64 | M/W | 730 | CAA | 365 | None |
| Van der Schouw (71) | <75 | M/W | 162 | MI | 84 | None |
| Eicher (72) | ? | M | 457 | CAD | Vessel score | None |
| | ? | W | 114 | CAD | Vessel score | None |
| Endbergs (73) | 55 mean | M | 208 | CAD | Vessel score | None |
| | | W | 67 | CAD | Vessel score | None |

Abbreviations: Y = years, M = men, W = women, MI = myocardial infarction or Heart Attack, CHD = Coronary Heart Disease, CAA = Carotid Artery Atherosclerosis; CAD = Coronary Artery Disease, CVD = Cardiovascular disease, ? = data not reported.

* Authors also reported finding no association between serum transferrin and CVD.

† Results not shown for men and women separately but the authors state that serum ferritin was an independent predictor for CAA for both men and women 40–59 years of age.

** From the multicenter Atherosclerosis Risk in Communities (ARIC) Study. Cases matched on study center, race, sex, 10-year age group, and 6-month baseline examination period.

### *6.3.2. Serum ferritin and CHD risk - case-control or cross-sectional studies*

There have been a number of studies [64–73] that have used a case-control or cross-sectional study design to examine the relationship between ferritin and CHD risk (Table 2). Only one [67] of the studies reported finding a significant positive association between serum ferritin and CHD and then in only the youngest of two age groups examined, i.e. ages 40–59 years.

As stated before, serum ferritin levels are increased in response to inflammation, infection, cancer and heart attack [43–48]. Since inflammation occurs in CHD, one might expect that a false-positive relationship between ferritin and CHD risk would be found more often than not in these types of studies. Given this, it is somewhat surprising that only one of studies in this category found an association between ferritin and CHD. On the other hand, including persons with CHD in these studies who have change their diets to lower their serum cholesterol levels would tend to produce false negative results, since a reduction in the intake of meat and animal products may lead to reduce levels of body iron stores and, as a result, serum ferritin levels. However, seven out of the ten studies addressed this possibility by assessing the association between CHD and serum total cholesterol, LDL cholesterol, and serum ferritin concurrently [65–70, 73]. In those studies, LDL cholesterol was positively associated with having CHD which suggest that it is unlikely that changes in behavior have produced false negative results.

### *6.3.3. Serum TS and the risk of CHD, stroke and all causes mortality*

The association between TS [74–78], serum iron [56,58, 62,75,77,79,80] or TIBC [54,75,77,78] and CHD risk has been investigated in seven different cohorts (Table 3). Only the study by Morrison et al. [79] reported finding a significant positive association. In contrast, Coti et al. [80] reported finding a significant *inverse* association: the higher a persons serum iron level the lower their risk of death from CHD or cardiovascular disease (CVD).

The above papers that found no association between iron status and CHD have been criticized for using TS as a measure of body iron stores [52,81,82]. It is interesting to note that the same criticism has not been applied to studies often cited to support the possibility that high body iron stores are related to cancer or CHD [82,83], even though they used serum iron or TS as well. Clearly, if TS is an indicator of body iron stores in one setting it must also be in the other.

In a few cohort studies TS has been used to look at the association between iron status and risk of stroke and all causes mortality. A u-shaped association between TS and stroke was reported for white women [84] while no association was found for white men or blacks. No association has been found between TS and all causes mortality [42,74,80,85].

Table 3
Serum transferrin saturation and the risk of heart disease. Cohort studies

| Authors (reference) | Sample Age (y) | Sex | Size | Mean years of followup | Incident cases | | Type of association |
|---|---|---|---|---|---|---|---|
| | | | | | Type | Number | |
| *Transferrin saturation* | | | | | | | |
| *NHANES I Epidemiologic Followup Study** | | | | | | | |
| Sempos (74) | 45–74 | M | 1,345 | 14.6 | MI | 166 | None |
| | | | | | CHD | 384 | None |
| | | W | 1,750 | 14.6 | MI | 139 | None |
| | | | | | CHD | 328 | None |
| Liao (75) | 40–74 | M | 1,827 | 13 | MI | 289 | None |
| | | | | | CHD | 633 | — |
| | | W | 2,410 | 13 | MI | 200 | None |
| | | | | | CHD | 518 | — |
| Baer (76) | 30+ | M | 15,167 | 14.1 | MI | 969 | None |
| | | W | 31,765 | 14.1 | MI | 871 | None |
| Reunanen (77) | 45–64 | M | 6,086 | 14 | CHD death | 739 | — |
| | | W | 6,102 | 14 | CHD death | 245 | U† |
| Van Asperen (78) | 64–87 | M | 129 | 17 | CHD death | 27 | None |
| | | W | 131 | 17 | CHD death | 23 | None |
| *Serum Iron* | | | | | | | |
| Morrison (79)** | 35–79 | M | ? | ? | MI death | 141 | + |
| | | W | ? | ? | MI death | 83 | + |
| Corti (80) | 71+ | M | 1,385 | 4.3 | CHD death | ? | — |
| | | W | 2,551 | 4.8 | CHD death | ? | — |
| Mänttäri (56) | 40–55 | M | 268 | 5 | CHD | 134 | None |
| Kiechl (58) | 40–79 | M/W | 780 | 5 | CHD | 375 | None |
| Klipstein-Grobusch** (62) | 55+ | M/W | 172 | 4 | MI | 60 | None |
| *TIBC* | | | | | | | |
| Magnusson (54)‡ | 25–74 | M | 990 | 8.5 | MI | 63 | — |
| | | W‡ | 1,046 | 8.5 | MI | 18 | None |

Abbreviations: Y = years, TS = Transferrin saturation (%), SI = Serum Iron ($\mu$mol/L), TIBC = Total Iron Binding Capacity ($\mu$mol/L), M = men, W = women, MI = Myocardial Infarction or Heart Attack, ChD - Coronary Heart Disease, ? = data not reported.

* Both studies based on the NHANES I Epidemiologic Follow-up Study (NHEFS) with follow-up through 1987. The NHEFS is a follow-up of the first National Health and Nutrition Examination Survey (NHANES I) which was conducted in 1971–75.

† Women with TS or serum iron in the second and third quintiles were at lower risk of CHD death than women first or lowest quintile and they also appeared to be at lower risk than women in the highest quintile.

** The sample consisted of 9,920 men and women. The numbers of men and women were not reported; nor was the average length of followup reported.

‡ Because of small numbers of heart attacks among the women the authors concentrated on results for men alone and for the combined sample of men and women.

### *6.3.4. Dietary iron and risk of CHD*

Salonen et al. [4] also reported that dietary iron intake was positively associated with the risk of having a heart attack. Other researchers have not been able to corroborate this finding [62,75,77,86–88] while to date only one other study [87] has found a positive association between dietary iron intake and CHD risk. Additionally, two papers have reported finding an association between heme iron intake and risk of heart attack but not with total iron intake [86,88] while a third found no association with heme iron [77].

### *6.3.5. Blood donation and the risk of CHD*

Blood donation has been hypothesized as a way of decreasing body iron stores to reduce the risk of heart disease [89,90]. Frequent blood donation will reduce body iron stores and, as a result, serum ferritin levels [91]. An indirect way to test the iron-heart disease hypothesis is to look at the risk of CHD in voluntary blood donors and non-donors in existing epidemiologic studies. There have been two such studies [92–94] as well as a study looking at the effects of blood donation on cancer incidence [95]. The results were mixed. Meyers et al. [92] reported that blood donation was associated with a reduced risk of CHD in non-smoking men but not in male smokers or in women while Salonen et al. [93,94] reported a significant reduction in risk of heart attack in the KIHD cohort of Finnish men.

While interesting there are several concerns about these studies. The principal one is that volunteer blood donors are healthier than non-donors and that any association may be a result of some unmeasured selection bias [96,97]. The data from both studies do indicate that the volunteers were healthier. For example, in contrast to the usual practice in cohort studies, Salonen et al. [93,94] did *not* eliminate persons with pre-existing clinical CHD at baseline from their analyses as they had done in their previous paper from the same cohort [4]. In their study over a quarter of the 2,529 non-donors (26.3%) had pre-existing disease compared to 8.5% in the 153 voluntary donors. As a result, it is

impossible to determine if blood donation was influenced by the presence of heart disease; that is a potential bias that can not be reduced or eliminated by statistical analysis.

#### *6.3.6. Iron overload and CHD risk*

If excess body iron stores are related to an increased risk of CHD then you would expect to see increased rates of the disease in persons with iron overload, e.g. hemochromatosis and hemosiderosis. But such does not appear to be the case. For example, there seems to be no evidence of higher rates of atherosclerosis or CHD in persons with hemochromatosis [98]. Patients with hemochromatosis may develop cardiomyopathy which can result in arrhythmia, bradycardia, congestive heart failure and death [99,100]; but liver diseases are the most common causes of death [101,102].

Another piece of evidence comes from studies of dietary iron overload in Sub-Saharan Africa. There is a high prevalence of iron overload among Sub-Saharan blacks which appears to be related, in part, to the consumption of traditional beer which is made in steel drums coupled with a genetic defect that may be different from the HLA-linked trait seen in whites with hemochromatosis [103]. But to date there have been no reports that iron overload in Africa was associated with an increased risk of CHD [104,105].

### *6.4. The indirect impact of body iron stores on CHD risk*

#### *6.4.1. Serum ferritin and LDL cholesterol oxidation*

Is there any evidence that body iron stores are directly or positively associated with the in vivo oxidation of LDL cholesterol? In what might be a direct test of this issue, Salonen et al. [83] used a Latin Squares design to look at the effects of donating 500 ml of blood three times over a 14 week period in 14 men who were heavy smokers on measures of non-HDL (very low density lipoprotein [VLDL] plus LDL cholesterol) cholesterol oxidizability. The authors reported finding that serum ferritin levels were reduced by 44% while the maximal oxidation velocity was decreased by 20% and the lag time to start oxidation was lengthened by 33%.

While interesting, the meaning of those results, even if replicated, are uncertain [106]. Currently the LDL oxidation theory is an interesting and attractive but as yet unproved hypothesis [9]. But the primary issue lies with the measurement of LDL oxidation itself [5]. The susceptibility of LDL to oxidation is usually measured by using LDL and exposing it to oxidative stress. The question is whether such a marker corresponds to the extent of oxidation in vivo and whether it predicts risk of CHD [107]. The results of the few epidemiological studies which have look at the association between LDL oxidation susceptibility and markers of atherosclerosis or CHD risk are mixed [108–111]. There are also mixed results concerning the association between autoantibodies against oxidized LDL and atherosclerosis [108, 112,113]. As a result, the available markers for oxidized LDL cannot yet be regarded as valid predictors of CHD risk [107].

Putting aside the possible problems with the measurement of oxidized LDL, several observational studies have looked at the association between serum ferritin and the susceptibility of LDL to oxidation [110,114,115]. No association was found in any of the studies. In fact, Craig et al. reported that serum ferritin account for about 1.6% of the variability in measures of LDL susceptibility to oxidation while serum copper accounted for 21% of that variability [115]. Serum copper also is considered as a possible catalyst for the oxidation of LDL [116,117]. In another experiment, reported only in abstract form, Derstine et al. [118] looked at the susceptibility of LDL to oxidation in the plasma samples of 77 men and women ages 20–65 years of age who were participating in one of three feeding studies. No association was found between serum ferritin and the measures of the susceptibility of LDL to oxidation.

Given that several mechanisms are probably involved in the oxidation of LDL and that even the same cell type may use different mechanisms including copper [119], it is not altogether surprising that body iron stores as measured by serum ferritin may not be related to measures of LDL susceptibility to oxidation or of autoantibodies to oxidized LDL.

Alternatively, a relationship observed between serum ferritin and CHD risk could be reflecting serum ferritin's role as an indicator of inflammation rather than an indicator of body iron stores [120]. Several markers of inflammation have been found to be associated with increased CHD risk [121]. Fibrinogen, C-reactive protein, albumin, WBC and erythrocyte sedimentation rate have all be found to associated with increased CHD risk [121,122]. With so many measures of inflammation associated with CHD risk the important question may not be: Is serum ferritin - the measure of body iron stores - related to CHD risk? but: Why isn't serum ferritin - the acute phase reactant - related to CHD risk?

In any event, it is important to remember Professor Dormany's admonition that "Cells and tissues are protected against oxidizing free radicals by a complexity of antioxidant mechanisms. *In disease these mechanisms may fail or the mechanisms may fail and cause disease*" [123]. The case of body iron stores and CHD risk appears to be a situation of the mechanisms protecting against free radical oxidation having failed as a result of the disease atherosclerosis and not the other way around. At present the epidemiological, clinical and laboratory data all appear to be pointing in the same direction. There appears to be no evidence that body iron stores play a direct role in the development of CHD and any role it *may* have in affecting CHD risk appears to be secondary to the primary underlying cause of atherosclerosis and CHD - a diet high in saturated fatty acids and cholesterol leading to high blood cholesterol [124–127].

## 7. Summary and conclusions

In 1981 Dr. Jerome Sullivan [1] 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. Then in 1992 Salonen et al. [4] reported finding a positive relationship between serum ferritin levels and risk of heart attack in Finnish men. While a plausible hypothesis was proposed by Dr. Sullivan to define a role for iron in the development of CHD, possibly by catalyzing the free radical oxidation of LDL cholesterol, the vast majority of the epidemiologic results published since the study by Salonen et al. [4] have failed to support the original hypothesis. Whether looking at the direct relationship between serum ferritin and CHD risk, serum ferritin and measures of atherosclerosis, serum TS and CHD risk, iron intake and CHD risk, iron overload and CHD risk or indirectly at the association between serum ferritin and measures of LDL oxidizability the results are the same: the data do *not* support the hypothesis that body iron stores are a risk factor for CHD. In addition, the affect of blood donation on serum levels of oxidized LDL remains an open question as does the measurement of oxidized LDL and the much larger question of whether oxidized LDL is a risk factor for CHD at all.

Sound clinical guidance and public health recommendations must be based on reasonably solid evidence that what is being recommended is both safe and effective. Given the results to date concerning the iron hypothesis, we agree with Corti, Gaziano and Hennekens who stated that: "Further research, including basic research and large-scale epidemiologic studies, is needed to fully assess the association between iron status and the risk of CVD and other adverse outcomes. At present the currently available data do not support radical changes in dietary recommendations or screening to detect high normal levels nor do they support the need for large-scale randomized trials of dietary restriction or phlebotomy as a means of lowering iron stores [102]."

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