Association Between Body Iron Stores and the Risk of Acute Myocardial Infarction in Men

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**Background**—Epidemiological evidence concerning the role of iron, a lipid peroxidation catalyst, in coronary heart disease (CHD) is inconsistent. We investigated the association of the concentration ratio of serum transferrin receptor to serum ferritin (TfR/ferritin), a state-of-the-art measurement of body iron stores, with the risk of acute myocardial infarction (AMI) in a prospective nested case-control study in men from eastern Finland.

**Methods and Results**—Transferrin receptor assays were carried out for 99 men who had an AMI during an average 6.4 years of follow-up and 98 control men. Both the cases and the controls were nested from the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) cohort of 1931 men who had no clinical CHD at the baseline study. The controls were matched for age, examination year, and residence. AMIs were registered prospectively. Soluble transferrin receptors were measured by immunoenzymometric assay and ferritin concentration by radioimmunoassay from frozen baseline serum samples. The mean TfR/ferritin ratio was 15.1 (SE, 2.0) among cases and 21.3 (SE, 2.2) among controls ($P=.035$ for difference). In logistic regression models adjusting for other strongest risk factors for AMI and indicators of inflammation and alcohol intake, men in the lowest and second lowest thirds of the TfR/ferritin ratio had a 2.9-fold (95% CI, 1.3 to 6.6, $P=.011$) and 2.0-fold (0.9 to 4.2, $P=.081$) risk of AMI compared with men in the highest third ($P=.010$ for trend).

**Conclusions**—These data show an association between increased body iron stores and excess risk of AMI, confirming previous epidemiological findings. (*Circulation. 1998;97:1461-1466.*)

**Key Words:** coronary disease ■ diabetes mellitus ■ ferritin ■ myocardial infarction ■ population

Iron is a transition metal that can catalyze toxic redox reactions, and it has been suggested to be involved in many harmful biological processes and diseases in the human body.1,2 Excessive iron has been proposed to be a potent risk factor for CHD, especially for AMI.3–5 Supporting evidence comes from in vitro lipid peroxidation and lipoprotein modification studies.6–8 from cholesterol-fed iron-overloaded animal models,9,10 and from analyses of the composition of human atherosclerotic lesions.11,12 The evidence from prospective human population studies is inconsistent. In these, increased estimated body iron stores have been associated with increased risk of CHD death or AMI in some4,5,13–15 but not in all studies.16–22 However, the discrepancy may be largely a result of the vast biological and measurement variabilities in methods used in assessing the body iron stores and, to some extent, study outcomes.23–25 Thus, the question of whether or not body iron is an independent risk factor for CHD and AMI is still unanswered.

Because recent methodological development has allowed valid and reliable assessment of body iron stores, we carried out a case-control study, in a prospectively examined cohort, to test the hypothesis that increased body iron stores are associated with an excess risk of first AMI in men. We used the ratio of serum TfR concentration to serum ferritin concentration, notably the best currently available noninvasive measure of body iron stores,26,27 which has not been used previously in population studies.

**Methods**

**Subjects**

The present study is a prospective nested case-control study among participants of the KIHD, a prospective population study to investigate previously unestablished risk factors for AMI, carotid atherosclerosis, and other related outcomes.28 The study protocol was approved by the Research Ethics Committee of the University of Kuopio. The baseline examinations were conducted during 1984 to 1989. The study sample comprised 3235 men in eastern Finland 42, 48, 54, or 60 years old at baseline. Of these, 198 were excluded because of death, serious disease, or migration, and 2682 (82.9%) participated. All participants gave a written informed consent. Men with prevalent CHD at baseline (n=677) were excluded. Prevalent CHD was defined as either a history of AMI or angina pectoris, positive angina pectoris on effort, or use of nitroglycerin tablets at least weekly. Of the remaining 2005 men, data on serum ferritin and...
blood hemoglobin concentrations were available for 1931 men. The cases of the present study are all 99 subjects who had a registered AMI by the end of 1992. Ninety-nine control subjects, matched according to age, examination year, and place of residence, were drawn from the same cohort. Of the control subjects, one was excluded because of missing data.

**Collection and Coding of AMI Data**
The province of Kuopio participated in the multinational MONICA project, in which detailed diagnostic information on all heart attacks during 1982 to 1992 was collected prospectively. The diagnostic classification was made by the FINMONICA investigator group. The present study is based on all heart attacks classified as definite or possible AMI or prolonged chest pain within the KIHD cohort between March 1984 and December 1992. The follow-up time for the 197 subjects to December 31, 1992, or death was 1.0 to 8.8 years; mean, 6.4 years. Of the 99 cases, 45 had a definite AMI, 36 a possible AMI, 14 a prolonged chest pain, and 4 insufficient diagnostic data.

**Examination Protocol**
The KIHD examination protocol and measurements have been described in detail earlier. Subjects came to give fasting venous blood samples and hair specimens in the morning and were instructed to abstain from ingesting alcohol for 3 days and from smoking and eating for 12 hours. Blood was drawn after a 30-minute supine rest. Hair samples were taken and blood pressure was measured at the same visit.

**Chemical Measurements**
Serum TfR concentrations were measured from frozen serum samples by use of the IDEA Transferrin Receptor Immunoenzymometric Assay (ORION Diagnostica). The test is based on a noncompetitive sandwich-type assay technique using mouse monoclonal antibodies in immobilized and enzyme-labeled forms against the human TfR. The interassay coefficient of variation was 4.0% to 6.1%, based on four samples from duplicate results in 10 subsequent assays, with mean concentrations from 1.5 to 5.5 mg/L. Serum samples for present TfR analyses were kept frozen for 7.4 to 13 years before the assays. Serum TfR concentration decreased over storage time ($P=.0007$ for linear trend). For this reason, the examination year was adjusted for in all statistical analyses.

Serum ferritin concentrations were measured from frozen serum samples with a radioimmunoassay (Amersham International) based on a double-antibody technique. The interassay coefficients of variation for serum ferritin levels of 52, 172, and 490 μg/L were 6.4%, 6.0%, and 10.9%, respectively, for 20 samples. No association ($P=.6982$) was detected between serum ferritin concentration and storage time. The TfR/ferritin ratio was computed as serum TfR concentration ($\mu$g/L) divided by serum ferritin concentration ($\mu$g/L). The correlation between TfR and ferritin concentrations was determined enzymatically. HDL$_2$ and HDL$_3$ subfractions were separated as previously described. Plasma fibrinogen concentrations were measured from fresh plasma samples (Coagulometer KC4, Heinrich Amelung GmbH) on the basis of clotting of diluted plasma with excess thrombin. Blood leukocytes were counted by the Coulter DN cell counter. Serum γ-glutamyl transferase activities were measured according to the Scandinavian recommendation. Serum C-reactive protein and apolipoproteins B and A-1 concentrations were measured with immunoturbidimetric methods (ORION Diagnostics for C-reactive protein and KONE Oy for apolipoproteins B and A-1). The detection limit of the method for C-reactive protein is 10 mg/L, which was used as a cutoff value to construct a binary variable for statistical analyses. Apolipoprotein(a) concentration was measured by radioimmunoassay (Merodia). Serum copper concentrations were measured with atomic absorption spectrometry with flame atomization. Hair mercury contents were measured by flow injection analysis with cold vapor atomic spectrometry and amalgamation.

**Physical Fitness**
Physical fitness was estimated by maximal oxygen uptake of the subject. The respiratory gas exchange was measured directly during a symptom-limited exercise test. The highest average 8-second oxygen uptake during a linear workload increase of 20 W/min was defined as the maximal oxygen uptake.

**Questionnaires and Interviews**
The number of cigarettes, cigars, and pipefuls of tobacco smoked daily and the duration of regular smoking in years and history of diseases were recorded on a self-administered questionnaire, checked by an interviewer. The consumption of alcohol in the previous 12 months was assessed with a quantity-frequency method, the Nordic Alcohol Consumption Inventory. The patterns of drinking, eg, binge drinking, were assessed. The dietary intake of nutrients, including iron, was estimated by use of Nutrica software and a 4-day food recording. Total leisure time energy expenditure was estimated with a modified 12-month questionnaire.

**Statistical Methods**
Differences in risk factors between cases and controls were tested for statistical significance with Student’s $t$ test allowing for unequal variances. Risk-factor–adjusted odds ratios for AMI were estimated by multivariate logistic regression models. Missing values in covariates were replaced by means, separately for cases and controls. To assess consistency of findings, TfR/ferritin was tested both as a continuous variable and by constructing indicator variables from the two lowest thirds of its distribution. Tertiles were defined a priori as cutoff points to ensure a sufficient number of subjects in all categories. All tests of significance were two-sided. All statistical analyses were performed with SPSS software in an IBM RS/6000 workstation.

**Results**
The distribution of the main risk factor characteristics among the case and control subjects are shown in Table 1. The mean serum TfR/ferritin ratio was 28.6% (95% CI, 2.2% to 54.8%) lower among the cases than the controls ($P=.035$ for differ-
en in a t test). Other risk factors with statistically significant differences between the cases and controls were cigarette pack-years ($P = .002$), serum HDL cholesterol ($P = .007$), maximal oxygen uptake ($P = .001$), plasma fibrinogen concentration ($P = .020$), and serum copper concentration ($P = .044$). Four cases and three controls had serum C-reactive protein of $\geq 10$ mg/L.

TfR/ferritin had a statistically significant crude correlation with serum LDL cholesterol ($r = -.225, P = .002$) and with the average weekly alcohol consumption ($r = -.169, P = .017$). In a step-up regression model, none of the indicators of inflammation, including blood leukocyte count, serum copper, and serum C-reactive protein, had any association at all with the ratio. Diabetics (mean, 6.2; $n = 13$) had a lower ratio than nondiabetics (mean, 19.1; $n = 184$; $P < .001$ for difference).

In a multivariate logistic regression model adjusting for other strongest risk factors for AMI and indicators of inflammation and alcohol intake (shown in Table 2), a low TfR/ferritin ratio was significantly associated with an increased risk of AMI ($P = .0404$).

To illustrate the magnitude of the relationship, TfR/ferritin was divided into thirds of its distribution (<7.4, 7.4 to 18.6, >18.6) (Figure). On average, the AMI risk was 71.2% (95% CI, 13.9% to 157.4%) higher in each lower third compared with the next ($P = .0097$ for trend). Men in the lowest and second lowest thirds of the TfR/ferritin ratio had a 2.91-fold (95% CI, 1.28 to 6.58; $P = .0105$) and 1.97-fold (95% CI, 0.87 to 4.22; $P = .0807$) risk-factor–adjusted risk of AMI, respectively, compared with men in the highest third (Table 2). The respective adjusted odds ratios of definite AMI ($n = 45$) were 3.17 ($P = .0426$) and 2.47 ($P = .0897$), $P = .0405$ for trend.

In addition to serum LDL and HDL cholesterol concentrations, serum total, VLDL, HDL₂, and HDL₃ cholesterol, apolipoproteins B and A-I, total triglycerides, and apolipoprotein(a) concentrations were also measured. None of these lipid measurements either provided additional information in the logistic model shown in Table 2 or influenced the association between TfR/ferritin and AMI risk. The same applied to blood leukocyte count, mean systolic blood pressure, years of hypertension, use of antihypertensive or anti-
dyslipidemic medications (yes versus no), measures of obesity (body mass index in kg/m\(^2\) and waist-to-hip circumference ratio), presence of ischemia in exercise test, claudication, history of any cardiovascular disease, family history of CHD, history of any chronic inflammatory disease, calories expended in conditioning leisure time physical activity, and alcohol abuse (binge drinking and frequency of hangovers).

To test whether any extreme values had an influence on the observed associations, we repeated the analyses after the exclusion of such values. One subject had an extreme value in the TfR/ferritin ratio (167). When this case was excluded, the adjusted odds ratios for the two lowest thirds of TfR/ferritin ratio were 2.94 (\(P=.0098\)) and 2.01 (\(P=.0737\)), \(P=.0090\) for trend. In serum ferritin, there were 10 markedly elevated values (\(\geq 500 \mu g/L\)), 6 among the cases and 4 among the controls. After the exclusion of these, the adjusted odds ratios of AMI for the two lowest thirds of TfR/ferritin were 2.74 (\(P=.0214\)) and 1.97 (\(P=.0802\)), \(P=.0176\) for trend.

In addition, we repeated the logistic models after excluding regular users of prostaglandin-inhibiting analgesics (eg, aspirin), \(n=13\), and regular users of antioxidative vitamin supplements (vitamins C and E), \(n=12\). The adjusted odds ratios for AMI in the two lowest thirds of TfR/ferritin were 3.39 (\(P=.0088\)) and 2.50 (\(P=.0348\)), \(P=.0081\) for trend. Also, we repeated the logistic models after the exclusion of eight subjects who were regular alcohol users, defined as weekly alcohol consumption of \(\geq 500 \text{ g of absolute ethanol or serum } \gamma\text{-glutamyltransf erase of } \geq 95 \text{ IU/L.}\) For the two lowest thirds of TfR/ferritin, the adjusted odds ratios were 3.24 (\(P=.0063\)) and 1.95 (\(P=.0915\)), \(P=.0058\) for trend.

We also repeated the logistic models after either the exclusion of or adjustment for diabetes. Among the 184 nondiabetic subjects, the adjusted odds ratios in the two lowest TfR/ferritin thirds were 2.83 (\(P=.0175\)) and 1.78 (\(P=.1443\)), \(P=.0162\) for trend. After adjustment for diabetes in 197 subjects, the respective odds ratios were 2.55 (\(P=.0290\)) and 1.89 (\(P=.1019\)), \(P=.0260\) for trend.

To study the synergism of iron stores and LDL cholesterol, the association of TfR/ferritin ratio with the risk of AMI was analyzed separately among men with serum LDL cholesterol below and above the median (4.2 mmol/L). The adjusted odds ratios in the two lowest thirds of TfR/ferritin were 4.20 (\(P=.0226\)) and 3.09 (\(P=.0548\)), \(P=.0219\) for trend in men with high cholesterol and 2.43 (\(P=.1480\)) and 1.44 (\(P=.5093\)), \(P=.1508\) for trend in men with low cholesterol.

When the estimated dietary intake of iron was added into the logistic model shown in Table 2, iron intake had a statistically significant residual association with the risk of AMI, whether the TfR/ferritin ratio was in the model or not. For each \(1 \text{ mg of iron daily, there was an increment in the risk of AMI of } 8.4\% (95\% CI, 1.7\% to 15.5\%; } P=.0136\) in a model including TfR/ferritin as a continuous variable. The risk-factor–adjusted odds ratios for the two highest thirds (>19.4 mg/d and 16.0 to 19.4 mg/d) of iron intake were 2.41 (95\% CI, 1.03 to 5.63; \(P=.0416\)) and 2.40 (95\% CI, 1.07 to 5.39; \(P=.0335\)), with the lowest third (<16.0 mg/d) as the reference (Figure). An additional adjustment for any dietary variable (eg, intakes of energy, saturated fat, and cholesterol) did not appreciably affect the association between iron intake and AMI risk.

**Discussion**

High body iron was first hypothesized to be involved in CHD >15 years ago.\(^4\) Several studies have been conducted since then to assess the association of iron and CHD or AMI.\(^5\)\(^,\)\(^13\)\(^-\)\(^22\) Results of some studies have been in favor of iron being a risk factor; others have not. Unfortunately, just a few studies have used the same exposure and outcome measures, and so most of the studies are not comparable.\(^23\)\(^-\)\(^25\) In our previous study, we investigated whether excess body iron, estimated as serum ferritin concentration, is associated with an increased risk of AMI (from the prospective MONICA AMI registry) in middle-aged eastern Finnish men.\(^4\) The results showed that men with serum ferritin concentration \(\geq 200 \mu g/L\) had a 2.2-fold (95\% CI, 1.2 to 4.0; \(P<.01\)) risk–factor–adjusted risk of AMI compared with those with serum ferritin <200 \(\mu g/L\).

In most other studies, the more traditional clinical measurements, serum iron or serum transferrin saturation, have been used. Both of these measurements are very responsive to inflammation and various disease processes and have a large biological and analytical variability.\(^27\)\(^,\)\(^36\)\(^,\)\(^37\) Since our original study, the association of body iron and the risk of fatal CHD or AMI has been studied in at least nine other prospective epidemiological studies, of which only two have used serum ferritin as the measure of body iron and published a full report.\(^14\)\(^,\)\(^19\)

It is conceivable that the impact of increased body iron stores on the risk of AMI is greater in the Finnish male population than in North Americans. The use of antioxidative vitamin supplements and aspirin, which is also antioxidative,\(^38\) is rare in Finland (9\% in our KIHD baseline data, \(n=2682\)), whereas almost half of Americans have reported the use of either or both.\(^39\)\(^,\)\(^40\) In addition, the mean LDL cholesterol level among eastern Finnish men is higher (4.04 mmol/L in KIHD) than that reported in the North American populations.\(^41\) If iron contributes to CHD and AMI through a pro-oxidative effect, a large proportion of antioxidative users in the study population would antagonize the risk-increasing effect of high iron stores and thus attenuate the observed association. In our present data, the association between TfR/ferritin ratio and AMI risk was stronger among men who did not use either antioxidative vitamins or aspirin. It would be informative to present a separate analysis in nonusers of aspirin and antioxidants in all epidemiological studies concerning the role of body iron stores in CHD. Also, the synergism between iron stores and LDL cholesterol levels should be studied.

We recently conducted another study in the KIHD cohort, in which we analyzed the association of voluntary blood donation (loss of iron >200 mg per donation) and AMI risk. In a multivariate model adjusted for the main coronary risk factors, blood donors had a relative AMI risk of 0.14 (95\% CI, 0.02 to 0.97; \(P=.047\)) compared with nondonors.\(^4^2\) These results provide support for the iron-CHD hypothesis from an additional perspective. Whereas other short-term minor changes in blood constituents take place after blood donation,
the reduction in serum ferritin concentration, indicating loss of iron, is the most marked consequence.\textsuperscript{42,43}

Serum ferritin concentration has been by far the best noninvasively measurable indicator of body iron stores.\textsuperscript{44} The use of serum ferritin in assessing iron stores, however, is complicated, because ferritin is also an acute-phase protein that may become elevated in inflammation, in severe liver diseases, and in cancer.\textsuperscript{37,44} The way to rule out potential confounding by these conditions has been either the exclusion of subjects with these conditions or the statistical adjustment for indexes of inflammation (C-reactive protein, blood leukocyte count) and liver damage (serum \(\gamma\)-glutamyl transferase, serum alanine transaminase). This problem also can be overcome to some extent by measurement of intracellular ferritin, for example, in erythrocytes.\textsuperscript{45}

Recently, an improved measure of body iron has been introduced, the serum soluble TfR concentration.\textsuperscript{46} Iron delivery to erythroblasts is mediated by the interaction of plasma transferrin with cell surface TfRs,\textsuperscript{26} and soluble TfR present in human serum reflects the availability of iron in the body.\textsuperscript{26,37,46,67} Because low iron stores result in the induction of TfR synthesis,\textsuperscript{47} the number of TfRs on the cell surface also reflects iron requirement.\textsuperscript{47} Several authors have suggested that the ratio of serum concentrations of the soluble TfR and ferritin should be a preferred measure of body iron stores.\textsuperscript{26,27,37} According to Cook, it “quantitatively reflects body iron over the entire spectrum of iron balance encountered in humans.”\textsuperscript{27} Because of its noninvasiveness, the serum TfR/ferritin ratio is also very usable in population studies.

According to our present data, this ratio is virtually independent of inflammation. It is, however, somewhat affected by alcohol abuse. For this reason, we carried out exhaustive statistical analyses to examine whether the association between TfR/ferritin ratio and AMI risk could be caused by the correlation of the ratio with alcohol consumption. If anything, controlling for alcohol tended to strengthen the observed association between TfR/ferritin ratio and AMI risk. This was also the finding in analyses excluding subjects with any inflammatory diseases, whereas either the exclusion of diabetics or an adjustment for diabetes weakened the association slightly. This is in accordance with the hypothesis that increased body iron stores might predispose to non–insulin-dependent diabetes.\textsuperscript{48} If this were the case, then the statistical control for diabetes would represent overadjustment. Also, in the present study, diabetics had 67.5% lower TfR/ferritin ratio than nondiabetics, supporting the role of body iron stores in a predisposition to diabetes.

In conclusion, our present findings suggest that men with high body iron stores (low TfR/ferritin ratio) are at a twofold to threefold increased risk of the first AMI, confirming our original observation that was based on serum ferritin measurement alone. In our view, the presently available evidence speaks in favor of a role of increased body iron stores in the development of the first myocardial infarction in men. Randomized preventive trials concerning the effect of iron depletion on coronary events are necessary to ultimately verify or refute the iron-CHD hypothesis.

Acknowledgments

This study was supported by the city of Kuopio, the Academy of Finland, and the Ministry of Education of Finland. Dr Tuomainen was funded by the Graduate School of Public Health, University of Kuopio, and Dr Salonen was Academy Professor of the Academy of Finland. We are indebted to Rainer Rauramaa, MD, PhD, for the participation of the Kuopio Research Institute of Exercise Medicine in data collection; to Riitta Salonen, MD, PhD, Esko Taskinen, MD, and Juha M. Venäjäinen, MD, for exercise tests; to Timo Lakka, MD, PhD, for data coding; to Mariatta Kantola, MSc, and Kari Seppänen, MSc, for chemical analyses; to Kalevi Pyörälä, MD, PhD, and Jaakko Tuomilehto, MD, PhD, for the FINMONICA registry data; and to Kimmo Ronkainen, MSc, for data analyses.

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Circulation. 1998;97:1461-1466
doi: 10.1161/01.CIR.97.15.1461

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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