Low Iron-Binding Capacity as a Risk Factor for Myocardial Infarction

Magnus K. Magnusson, MD; Nikulas Sigfusson, MD, PhD; Helgi Sigvaldason, PhD; Gudmundur M. Johannesson, MD; Sigmundur Magnusson, MD; Gudmundur Thorgeirsson, MD, PhD

Background In a recent Finnish study, ferritin was suggested to be an independent risk factor for acute myocardial infarction. This study suggested that high levels of iron stores might thus be atherogenic and possibly explain partly the sex difference in the incidence of ischemic heart disease.

Methods and Results A randomly selected group (n=2036), men and women aged 25 to 74 years, were examined between June and September 1983. All classic risk factors for coronary artery disease were measured as well as basic hematologic parameters and the parameters of iron metabolism, ie, iron, total iron-binding capacity (TIBC), and ferritin. During the follow-up for 8.5 years, 81 subjects experienced acute myocardial infarction (63 men and 18 women). The differences in the iron parameters between men and women were almost exclusively seen in ferritin values (198 μg/L in men and 91 μg/L in women), whereas small differences were seen in TIBC. The Cox proportional hazards model was used to estimate the contribution of independent variables to the risk of myocardial infarction. TIBC was found to be a strong independent negative risk factor in men (RR=0.95; 95% CI, 0.92 to 0.98), whereas ferritin (RR=0.999; 95% CI, 0.997 to 1.001) or other iron parameters had not significant predictive power. Each increase in TIBC of 1 μmol/L was associated with a 5.1% decrease in the risk of myocardial infarction. The classic major risk factors, ie, blood pressure, smoking, total cholesterol, and high-density lipoprotein, had significant independent correlation with myocardial infarction. When Cox multivariate analysis was carried out on both sexes combined, TIBC was still an independent negative risk factor, and the logarithmic transform of ferritin had a weak negative correlation but was not statistically significant. Sex was in this group still a very strong risk factor after taking into account all classic risk factors as well as the parameters of iron metabolism.

Conclusions This study suggests that transferrin, measured as TIBC, is an independent negative risk factor for myocardial infarction. Other parameters of iron metabolism, including ferritin, were not found to contribute to the risk. (Circulation. 1994;89:102-108.)

Key Words • antioxidants • myocardial infarction population • transferrin

Substantial evidence has been put forth during the last few years that generation of free radicals might be involved in the pathogenesis of atherosclerosis. The postulated mechanism operates through oxidative modification of low-density lipoproteins (LDL), rendering them an increased pathogenicity. In concordance with this theory, the levels of antioxidant vitamins, especially vitamin E, have been shown to be inversely related to the incidence of ischemic heart disease. In a recent study by Salonen et al, the level of serum ferritin was suggested to be an independent risk factor for coronary heart disease. Free iron catalyzes the generation of free radicals. As serum ferritin partially reflects the iron stores in the body, high ferritin was postulated to reflect a state of increased free radical generation. The oxidation of LDL is thought to occur mostly in the subendothelial layer of the arteries prone to atherosclerosis. Atherosclerotic lesions have recently been shown to be rich in both iron and copper, and the crater from these lesions was found to induce lipid peroxidation that was inhibited by the iron chelator desferrioxamine.

The association of high iron stores and coronary heart disease was first suggested by Sullivan to explain the sex difference in heart disease risk. The mean serum ferritin among men is approximately twofold that of women, and the difference is greatest during the premenopausal stage. While the sex difference with respect to transferrin is not as great as for ferritin, this glycoprotein also has a potential role in atherogenesis. Along with coeuroplasmin, transferrin is one of the major serum antioxidants and thus might be protective against the generation of atherosclerosis.

In this prospective study we have measured the established major risk factors of coronary heart disease, including total serum cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, blood pressure, and smoking as well as ferritin, total iron-binding capacity (TIBC, representing transferrin), and iron in 2036 men and women and followed them for 8.5 years with regard to myocardial infarction.

Methods

Subjects
The study population consisted of the participants in the first risk factor survey of the Monica Iceland project, which was conducted from June to September 1983. Stratified randomization was used to select the subjects from a continuously updated population register, the National Roster. Stratification included...
TABLE 1. Clinical and Laboratory Coronary Risk Factors and Other Parameters Measured

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n=990)</th>
<th>Women (n=1046)</th>
<th>Whole Group (n=2036)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Value (SD)</td>
<td>Range</td>
<td>Mean Value (SD)</td>
</tr>
<tr>
<td>Age, y</td>
<td>51 (14)</td>
<td>25-74</td>
<td>50 (14)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>237 (40)</td>
<td>119-414</td>
<td>244 (49)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>50 (12)</td>
<td>18-132</td>
<td>59 (14)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>113 (65)</td>
<td>32-896</td>
<td>100 (62)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>83 (11)</td>
<td>56-120</td>
<td>80 (10)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>130 (18)</td>
<td>92-232</td>
<td>126 (21)</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>198 (171)</td>
<td>6-1800</td>
<td>91 (116)</td>
</tr>
<tr>
<td>Log (ferritin), µg/L</td>
<td>5.0 (0.81)</td>
<td>1.8-7.5</td>
<td>4.1 (0.92)</td>
</tr>
<tr>
<td>TIBC, µmol/L</td>
<td>55 (8)</td>
<td>30-93</td>
<td>57 (10)</td>
</tr>
<tr>
<td>Iron, µmol/L</td>
<td>18 (6)</td>
<td>2-45</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>152 (10)</td>
<td>78-181</td>
<td>136 (10)</td>
</tr>
<tr>
<td>Leukocyte count, 10^3/L</td>
<td>6.0 (1.8)</td>
<td>2.8-15.5</td>
<td>5.6 (1.7)</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; TIBC, total iron-binding capacity.

Sex and age (25 to 34, 35 to 44, 45 to 54, 55 to 64, and 65 to 74 years, respectively). In each stratum, 150 men and 150 women were randomly selected from an urban area (Reykjavik City) and an equal number from a rural area (Arnes County). The total number selected was 3000. Recruitment was by written invitation to participate in a study of cardiovascular disease risk factors. Those who did not respond were sent a repeat invitation and finally contacted by telephone by the Heart Preventive Clinic staff. The response rate in the male group was 70.2% and in the female group, 74.4%. No exclusion was made with regard to previous health, including cardiovascular diseases.

Risk Factor Measurements

Blood samples were drawn between 8:15 and 10:30 AM after 10 hours or more fasting. Samples were collected into Vacutainer tubes (Becton Dickinson, Rutherford, NJ) without venous stasis except in difficult cases. Subjects sat for 5 minutes before venipuncture. Basic hematologic parameters (B-hemoglobin, B-red cell count, and B-mean cell volume) were analyzed within 6 hours on a Coulter S-Plus IV. Serum samples for iron, TIBC, and ferritin were kept at −20°C until assayed in batches within several weeks of collection. Iron (day-to-day coefficient of variation [CV], 4%, ie, same specimens in the same laboratory) and TIBC (CV, 5%) were measured on a centrifugal analyzer (Multistat III, Instrumentation Laboratory Inc) using ferrozine as chromogen. Ferritin was assayed with a radioimmunoassay kit that uses antibodies against splenic ferritin (Amersham International plc, Amersham, UK). Total cholesterol was determined by a chemical colorimetric method, and triglycerides were determined fluorometrically (Technicon AutoAnalyzer method N24-a). HDL was determined by the heparin-manganese method. Blood pressure was measured with a mercury sphygmomanometer (Erka) after a 5-minute rest. Body weight was measured on a lever balance and was recorded to the nearest 0.1 kg. Body mass index was calculated as weight (kg) divided by the square of the height (m²). All participants completed a health questionnaire on smoking habits and use of antihypertensive agents. No data on socioeconomic status were obtained, nor data on alcohol intake.

End-Point Determinations

During 1983 to 1991, all episodes of acute myocardial infarction in people aged 25 to 74 years anywhere in Iceland were registered by the Icelandic study group of the World Health Organization’s MONICA project. As the oldest individuals in our material were older than 74 years during follow-up, hospital files were searched for all episodes of acute myocardial infarction occurring in this age group in the whole country as well as reports on sudden death occurring outside the hospital. The data were registered and analyzed in accordance with the method of the MONICA project. The diagnostic criteria have been published previously and included symptoms, ECGs, enzyme activities, and necropsy findings leading to the diagnostic categories of definite or possible myocardial infarction (categories I and II). External control of the quality of event registration was performed by the World Health Organization reference center in Dundee.

Statistical Methods

Product moment correlation coefficients (r) were computed to find correlation between variables. Their significance was tested by assuming the statistic \( t = r \sqrt{(n-2)/(1-r^2)} \) to have a distribution with \( n-2 \) degrees of freedom.

Cox proportional hazards model (simultaneous regression) was used to calculate β-coefficients for prediction of risk of myocardial infarction. The program package EGRET was used.

Results

In Table 1, the coronary risk factors as well as the basic iron parameters for men and women and the sexes combined are shown. As published earlier, iron and TIBC decreased slightly with increasing age in men, whereas ferritin increased steadily with age, on average, 2.2 µg/L per year. In women, TIBC did not change with increasing age, whereas ferritin decreased slightly with increasing age in premenopausal women, while increasing sharply in postmenopausal women, on average, 3.9 µg/L per year. The differences between the sexes were most evident in the ferritin values (men higher than women, 198 versus 91 µg/L), whereas TIBC was similar, with female values slightly higher (57 versus 55 µmol/L). Total cholesterol was slightly higher in the men than in the women (244 versus 237 mg/dL), representing the higher HDL cholesterol in women (59 versus 50 mg/L).
Tables 2 and 3 show the correlation coefficients between different variables in the study for men and women. As shown, strong correlation does not exist between variables except between systolic and diastolic blood pressures ($r=.635, men; r=.622, women$), systolic blood pressure and age ($r=.413, men; r=.545, women$).

HDL and log (triglycerides) in men ($r=-.408$), and age and log (ferritin) ($r=.498$) and cholesterol ($r=.513$) in women. As ferritin and triglycerides have a positively skewed distribution, a logarithmic transform (natural logarithm) of ferritin and triglycerides also was used. As expected, TIBC and ferritin were inversely related, but the inverse correlation was not very strong, at $-.153$ for men and $-.188$ for women, but increased to $-.25$ and $-.334$, respectively, for log (ferritin).

During the 8.5-year follow-up, 81 individuals suffered myocardial infarction (63 men and 18 women). In the
group of men, the mean values for TIBC in the cases and control subjects were 51.8 μmol/L (SD, 7.0) and 54.7 μmol/L (SD, 8.6), respectively (P<.001). The mean values for ferritin in the cases and control subjects were 219 μg/L (SD, 192) and 196 μg/L (SD, 170), respectively (not significant). In the group of women, the mean values for TIBC in cases and control subjects were 55.2 μmol/L (SD, 6.7) and 56.5 μmol/L (SD, 10.5), respectively (not significant). The mean values for ferritin in the cases and control subjects were 105 μg/L (SD, 119) versus 90 μg/L (SD, 117), respectively (not significant).

In an age-adjusted univariate analysis, the relative risk for men of suffering myocardial infarction was 0.958 (95% CI, 0.929 to 0.989; P=.009) for TIBC and 1.000 (95% CI, 0.999 to 1.001; P=.85) for ferritin. Because of interdependence of variables, Cox multivariate regression analysis (simultaneous modeling) was carried out with myocardial infarction (n=63) as the dependent variable. The results are listed in Table 4. Classic risk factors that reached significance in the multivariate regression analysis were age, diastolic blood pressure, HDL cholesterol, total cholesterol, and smoking. As shown, TIBC had a negative correlation with myocardial infarction, with an RR of 0.949 (95% CI, 0.917 to 0.981), i.e., for each increment in TIBC of 1 μmol/L, the risk of suffering myocardial infarction decreased by 5.1%. Neither ferritin nor log (ferritin) reached significance but had weak negative correlation to the risk of myocardial infarction in the multivariate analysis: RR=0.999; 95% CI, 0.997 to 1.001 for ferritin and RR=0.781; 95% CI, 0.540 to 1.129 for log (ferritin). Iron, transferrin saturation (iron divided by TIBC), hemoglobin, leukocyte count, triglycerides, and body mass index had no independent predictive power for myocardial infarction in the Cox multivariate analysis. No significant interactions between the variables were found.

Table 5 shows the results of Cox multivariate regression analysis for the whole group (men and women), with myocardial infarction (n=81) as the dependent variable as before. Importantly, after taking all iron parameters into account as well as classic risk factors, female sex was still a very important protective factor (RR=0.26). The risk of suffering myocardial infarction by women was only 26% of the risk by men. The relative risk for female sex increased by 7% when parameters of iron metabolism were not taken into account in the regression analysis (data not shown). As before, TIBC was a statistically significant negative risk factor (RR=0.962). Ferritin was not a significant predictor, but its logarithmic transform was close to being a significant negative risk factor (RR=0.752, P=.07). In a univariate analysis, log (ferritin) had a positive correlation with myocardial infarction (RR=1.559, P<.001), but after taking sex and age into account, it lost all predictive power (RR=0.966, P=.78).

Of the women participating in this study, only 18 suffered myocardial infarction, limiting the power of multivariate regression analysis for the women as a separate group. However, by the application of the Cox model, age (RR=1.176; 95% CI, 1.099 to 1.259), triglycerides (RR=1.007; 95% CI, 1.001 to 1.013), and smoking (RR=4.09; 95% CI, 1.28 to 13.01) (current smokers vs. never-smokers) were found to be statistically significant risk factors for sustaining myocardial infarction among women. TIBC had a very weak negative correlation, with an RR of 0.995 (95% CI, 0.941 to 1.057). Log (ferritin) also had a negative correlation, with an RR of 0.575 (95% CI, 0.321 to 1.032).

**Discussion**

In this prospective study of 2036 randomly selected Icelandic men and women aged 25 to 74 years who were followed for 8.5 years, we have detected a strong
negative relation between TIBC and the risk of myocardial infarction, not only in univariate analysis but also when several classic risk factors of coronary heart disease as well as other iron parameters have been accounted for. This study does not confirm the recently published results by Salonen et al\(^6\) on the positive relation between ferritin and the risk of myocardial infarction. On the contrary, after taking into account age, classic cardiovascular risk factors, and other iron parameters, ferritin had a weak inverse relation with myocardial infarction. In the study by Salonen et al, no other iron parameters than ferritin were measured.

Over 90% of the serum iron-binding capacity is accounted for by the iron transport protein transferrin. Transferrin is a 678-amino acid residue glycoprotein produced mostly in the liver. It can bind two ferric (Fe\(^{3+}\)) ions and is taken up into cells by receptor-mediated endocytosis. The ferric ions are incorporated into cellular ferritin, and the apotransferrin is released back for further iron transport in serum. The production of transferrin is increased by low hepatocyte ferritin concentration, representing iron stores at a low level, whereas high cellular ferritin decreases its production.\(^19\) Other factors known to depress transferrin production are inflammation, infection, malignancy, liver disease, and malnutrition, whereas pregnancy and oral contraceptives tend to increase its production.\(^20\) Apart from its function as an iron-transporting protein, transferrin has long been known to be an antioxidant,\(^11\) and its antioxidant property is believed to be related to its capacity to bind iron.\(^21\) Whether this function has any relevance in vivo is unknown.

The generation of oxygen free radicals, especially the highly reactive hydroxyl radical (OH), is dependent on the presence of transition metal ions.\(^7\) This is supported by the fact that substances that bind transition metals are highly active antioxidants. The most important transition metals in vivo are believed to be iron and copper.\(^7\) To support the possible role of iron in the generation of free radicals in vivo, it has been shown that coronary reperfusion damage, a process thought to be partially mediated by reactive oxygen species, has been shown to be increased by iron load, and this damage was partially reversed by iron-chelating agents in experimental animal models.\(^22,23\)

During the last few years, oxidative modification of LDL has come into focus as an important step in rendering pathogenicity to LDL in atherosclerotic lesions.\(^1\) There is considerable experimental evidence indicating that this modification increases the atherogenic effects of the LDL molecule. Several epidemiological studies have shown dietary antioxidants (especially vitamin E) to be inversely correlated with the incidence of coronary artery disease.\(^2-5\) Although most attention has been paid to dietary antioxidants, the most potent inhibitors of LDL oxidation in vitro are agents complexing copper and/or iron.\(^24\) Among these are the serum proteins transferrin and coeruloplasmin and the iron-chelating drug desferrioxamine. Recently, iron and copper have been shown to be present in free form in atherosclerotic lesions. Furthermore, the content of these lesions was shown to stimulate the peroxidation of rat liver microsomes, and this oxidation was inhibited by desferrioxamine.\(^9\) Recently, serum copper has been shown to be positively related to the risk of acute myocardial infarction.\(^25\) Finally, the recently published study on the relation between serum ferritin and the risk of myocardial infarction\(^6\) and the present results further suggest that iron might be important as a prooxidant in atherogenesis.

Although the study presented here and the study by Salonen and coworkers\(^6\) both indicate that iron metab-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Risk</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>1.101</td>
<td>1.073-1.130</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Female sex</td>
<td>0.260</td>
<td>0.130-0.501</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>High-density lipoprotein, mg/dL</td>
<td>0.970</td>
<td>0.950-0.990</td>
<td>.004</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>1.008</td>
<td>1.005-1.011</td>
<td>.006</td>
</tr>
<tr>
<td>TIBC, (\mu)mol/L</td>
<td>0.962</td>
<td>0.935-0.990</td>
<td>.007</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>1.030</td>
<td>1.008-1.052</td>
<td>.007</td>
</tr>
<tr>
<td>Log (ferritin), (\mu)g/L(^*)</td>
<td>0.752</td>
<td>0.553-1.023</td>
<td>.070</td>
</tr>
<tr>
<td>Ferritin, (\mu)g/L(\dagger)</td>
<td>0.999</td>
<td>0.998-1.001</td>
<td>.23</td>
</tr>
</tbody>
</table>

Smoking

Never

1.000

Former

1.020

Pipe/cigars

2.090

1-14 cigs/day

2.250

15-24 cigs/day

3.990

>24 cigs/day

2.820

TIBC indicates total iron-binding capacity; cigs, cigarettes.

\(^*\)When ferritin is not included.

\(\dagger\)When log (ferritin) is not included.
olism contributes independently to the generation of coronary heart disease, they differ with respect to which parameter of iron metabolism predicts the risk. In the present study, all serum iron parameters were taken into account, ie, iron, TIBC, and ferritin, whereas in the Finnish study, only ferritin measurements were reported. It is well established that ferritin is a much more reliable indicator of total iron stores than TIBC. The present results, showing low TIBC rather than ferritin to be the risk factor, do not support the concept that high iron stores per se are an important determinant of coronary heart disease. If iron increases, the risk of coronary artery disease by oxidizing LDL, as has been postulated, a critical step in its pathogenetic pathway would be the accumulation of free iron in the subendothelial space. The serum iron-binding capacity might be a more reliable predictor of this accumulation of free iron in the vessel wall than the total iron stores. Because the Finnish study did not take into account TIBC, it is possible that part of the strong correlation reported in their study is due to the protective role of transferrin, as these parameters are weakly inversely correlated (Tables 2 and 3).

Preliminary results have been published regarding the role of iron parameters in coronary artery disease in the United States. Stamper and coworkers did not detect any correlation between ferritin and the risk of myocardial infarction, which is in concordance with our results. In another study, Cooper and Liao found no relation between TIBC and the incidence of myocardial infarction or coronary heart disease, whereas iron was inversely associated with myocardial infarction in women and iron and transferrin saturation inversely associated with coronary heart disease in both sexes. Neither of the studies measured all three parameters of iron metabolism, ie, ferritin, TIBC, and iron. Further comparison between the studies must await more detailed reports.

As stated earlier, the concentration of transferrin decreases in several disease states unrelated to iron metabolism, especially in chronic inflammatory processes, malignancy, and malnutrition. It could be argued that the epidemiological association reported here only represented transferrin as a negative immunological marker. It is well established that activation of macrophages is one of the key steps in atherogenesis, and it has been suggested that the correlation between the parameters of iron metabolism and coronary artery disease merely represented the cellular immune activation. However, ferritin would then be expected to have a positive correlation with myocardial infarction, not a negative one, as found in the present study. Furthermore, there was no correlation between TIBC and the white blood cell count ($r=.005$).

In the early eighties, Sullivan suggested that the sex difference in the incidence of ischemic heart disease might be explained by the differences in iron stores. Premenopausal women have much lower iron stores than men of the same age because of regular blood loss through menstruation (Table 1). However, since ferritin did not correlate with myocardial infarction, these differences do not account for the marked sex differences in risk found in the present study. The relative risk for female sex only increased by 7% when TIBC was left out of the analysis (data not shown). As shown in Table 5, sex was still a very strong risk factor for myocardial infarction after all the iron parameters had been accounted for. However, this needs further study, since the group of women suffering myocardial infarction in this study only comprised 18 individuals.

The study presented here indicates that the serum antioxidant and iron-binding protein transferrin is an independent negative risk factor for coronary heart disease. The findings support the concept that iron being an important transition metal might contribute to atherogenesis, along with the classic risk factors, although arguing against the recent hypothesis that iron stores per se increase the risk. The results also are in agreement with the concept that the concentration of oxidized LDL is a crucial pathogenic factor in atherosclerosis and that the level of antioxidants has an important protective role. In this study, male sex remained a major independent risk factor of myocardial infarction when all the iron parameters had been accounted for.

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