Serum ferritin, cardiovascular risk factors and ischaemic heart
diseases: a prospective analysis in the SU.VI.MAX
(SUpplementation en Vitamines et Minéraux AntioXydants) cohort

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Abstract

Background: Iron has been suggested to play a role in the development of
cardiovascular disease (CVD) through its pro-oxidant properties. However,
epidemiological studies on iron status and the risk of CVD have yielded conflicting
results. We therefore carried out a prospective study to evaluate the relationship
between iron status and CVD in a middle-aged French population.

Methods: In total, 9917 subjects (3223 men aged 45–60 years and 6694 women aged
35–60 years) included in the SU.VI.MAX (SUpplementation en Vitamines et Minéraux AntioXydants) cohort were followed prospectively for 7.5 years. All cases of
ischaemic heart disease (IHD) were identified and validated. CVD risk factors,
haemoglobin and serum ferritin concentrations were measured at baseline.

Findings: Of men 4.3%, and of women 37.8%, presented at baseline a serum ferritin
concentration
30
mgl
2
. During the follow-up, 187 subjects (148 men, 39 women)
developed IHD. Serum ferritin was positively associated with total cholesterol, serum
triglycerides, systolic and diastolic blood pressure, body mass index and
haemoglobin. No linear association was found between serum ferritin and IHD risk
in men or in women.

Conclusion: Our data do not support a major role of iron status in the development of
IHD in a healthy general population.

Keywords

Iron
Serum ferritin
Ischaemic heart disease
Prospective study

Oxidative stress and free-radical damage to tissues may be
involved in the development of cardiovascular disease (CVD). Iron has been suggested to be one of the factors
implicated in ischaemic heart damage and lipid peroxi-
dation due to its pro-oxidant properties in generating free
radicals1. Although there is a strong hypothesis for the
mechanism explaining a possible relationship between
iron and CVD, the accumulated epidemiological evidence
is inconsistent and most studies do not support the role of
iron in CVD development2–5. Most cross-sectional and
case–control studies using serum ferritin as an indicator of
iron stores6 have not found an association with CVD5–16;
although some did17,18. However, it has to be taken into
account that in these types of studies the disease itself
(post-myocardial damage and associated chronic inflam-
mation), its treatment (such as aspirin) and behavioural
changes (healthier diet and more physical activity) could
influence serum ferritin concentrations and thereby
obscure the true relationship. Prospective cohort studies
measuring serum ferritin in blood samples collected
before the occurrence of CVD avoid these methodological
biases. Fourteen prospective studies19–32 have been
reported, and only a Finnish study19 found a statistically
significant association between serum ferritin and CVD;
after a 3-year follow-up, serum ferritin concentrations
> 200 μg l−1 were associated with a 2.2-fold increase in the
incidence of acute myocardial infarction compared with
serum ferritin concentrations < 200 μg l−1. An Italian
prospective study examining intermediate endpoints
observed an association between serum ferritin and an
ultrasound measure of atherosclerosis18.

Even though we have an accumulation of studies, the
issue of the relationship between iron status and cardiovascular diseases still remains controversial. This
may be due to the fact that studies differed in their design
(cross-sectional, case–control and prospective studies), the
chosen endpoints (occurrence of CVD or intermediate endpoints), the population (men, women, healthy or sick people) and the assessment of iron status (serum ferritin, serum transferrin, etc.). Therefore, we carried out a prospective study to evaluate the relationship between iron status, CVD risk factors and the incidence of CVD in a middle-aged population, living in France, where iron supplementation and iron-fortified foods are rarely used33.

**Materials and methods**

**Study population**

Subjects were part of the SU.VI.MAX (SUpplementation en Vitamines et Minéraux AntiOxydants) study, a double-blind, placebo-controlled, primary prevention trial evaluating the effect of antioxidant supplementation on chronic diseases. Details concerning study rationale, design, methods and participant characteristics have been reported elsewhere34,35. In brief, 12741 French adults (7713 females aged 35–60 years and 5028 males aged 45–60 years) were recruited by a multimedia campaign to be randomly allocated to receive either a combination of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, 100 μg selenium (as selenium-enriched yeast) and 20 mg zinc (as gluconate)) or a matching placebo, in a single daily capsule. Participants did not have known diseases likely to threaten 5-year survival.

The current analysis includes 9917 subjects (3223 men and 6694 women) for whom serum ferritin measurements at baseline were available and who did not have known major inflammatory diseases. The protocol was approved by a medical ethics committee and the national committee for the protection of privacy and civil liberties.

**Ascertainment of ischaemic heart disease**

Participants were asked to complete a monthly questionnaire, summarising treatment compliance and health events, via Minitel (a phone-based French terminal), the Internet or mail. If there was no contact with the participant for a long period, or if the participant failed to appear at the yearly visit, an investigation was launched to determine the reasons. Once a CVD event was suspected, all relevant records, including results of diagnostic tests and procedures, were collected from the physicians and hospitals involved or directly from the participant.

All data were reviewed and validated by an expert committee and International Classification of Diseases codes 120–12436 were used to define ischaemic heart disease (IHD). Causes of death were confirmed by information from relatives or physicians. At the end of the follow-up, vital status of all subjects and causes of death were checked at the national death registry.

**Measurement of CVD risk factors and markers of iron status**

Venous blood samples (drawn into mineral-free vacuum tubes; Becton Dickinson, Pont de Chaix, France) were obtained at enrolment from participants who had been fasting for 12h. Haemoglobin was measured immediately (cynmethaemoglobin method) and blood was kept at +4°C in the dark until centrifugation and preparation of aliquots. Aliquots of serum were frozen in polypropylene tubes and shipped to the coordination centre in Paris for storage.

Serum ferritin concentration was used as a marker of body iron stores and measured using automatic nephelometry (BNII nephelometer; Dade Behring, Paris La Défence, France). The laboratory quality assurance included analysis of serum from standard pools with each run and international standards.

Total cholesterol and serum triglycerides were measured using an enzymatic method (Technicon Dax 24; Bayer Diagnostic, Puteaux, France).

Blood pressure was measured at the first clinical examination (1995–1996), using a standardised procedure with a standard mercury sphygmomanometer. Blood pressure was measured once at each arm in subjects who had been lying down for 10min, and the mean of these two measurements was used for analyses. Body mass index (BMI) was also measured at 1 year and was calculated from measured weight and height.

**Statistical analyses**

Follow-up time for each subject was calculated from the date of randomisation until the date of IHD diagnosis, date of death or 1 September 2002.

The difference in CVD risk factors, haemoglobin and serum ferritin between cases and non-cases was evaluated using a t-test. The association between serum ferritin and CVD risk factors was evaluated by calculating the Spearman correlation coefficient. Because of skewed distributions, serum levels of ferritin were log-transformed for analysis and geometric means are presented. Cox proportional-hazards models were used to calculate the maximum likelihood estimates of the relative risk and its 95% confidence interval to evaluate the relationship between serum ferritin and IHD. For these analyses, serum ferritin concentrations were separated according to the following definition based on data regarding ferritin and iron absorption32; depleted and low status, < 30 μg l−1 (taken as the referent group); non-replete and borderline normal status, 31–70 μg l−1; replete and adequate status, 71–160 μg l−1; and replete and elevated status, > 160 μg l−1. In all analyses, the relative risks were adjusted for possible confounding factors (age, smoking, BMI, total cholesterol, serum triglycerides, supplementation group and menopausal status).

Statistical analyses were performed using SAS software version 8.2 (SAS institute, Inc., Cary, NC, USA) and were performed separately for men and women.
Table 1 Risk factors for cardiovascular disease and markers of iron status in the study population

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3223</td>
<td>6694</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.8 (4.7)</td>
<td>47.0 (6.6)</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smokers</td>
<td>34.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Former smokers</td>
<td>51.7</td>
<td>29.2</td>
</tr>
<tr>
<td>Current smokers</td>
<td>14.3</td>
<td>15.8</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.6 (3.2)</td>
<td>23.4 (4.0)</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>129.1 (13.9)</td>
<td>119.7 (13.6)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>83.4 (8.4)</td>
<td>76.9 (8.5)</td>
</tr>
<tr>
<td>Total cholesterol (mmol l⁻¹)</td>
<td>6.2 (1.0)</td>
<td>5.9 (1.0)</td>
</tr>
<tr>
<td>Serum triglycerides (mmol l⁻¹)</td>
<td>1.41 (1.1)</td>
<td>0.90 (0.5)</td>
</tr>
<tr>
<td>Haemoglobin (g l⁻¹)</td>
<td>149.7 (10.3)</td>
<td>135.0 (10.5)</td>
</tr>
<tr>
<td>Serum ferritin (µg l⁻¹)</td>
<td>195.1 (154.6)</td>
<td>54.9 (54.8)</td>
</tr>
</tbody>
</table>

Values are means (standard deviation) unless indicated otherwise.
* Geometric mean.

Table 2 Risk factors for cardiovascular disease and markers of iron status according to sex and ischaemic heart disease (IHD) status

<table>
<thead>
<tr>
<th></th>
<th>IHD</th>
<th>No IHD</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td>0.0001</td>
</tr>
<tr>
<td>Non-smokers</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Former smokers</td>
<td></td>
<td></td>
<td>0.02</td>
</tr>
<tr>
<td>Current smokers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
<td>0.0007</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol l⁻¹)</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serum triglycerides (mmol l⁻¹)</td>
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<td></td>
<td>0.03</td>
</tr>
<tr>
<td>Haemoglobin (g l⁻¹)</td>
<td></td>
<td></td>
<td>1.4 (1.0)</td>
</tr>
<tr>
<td>Serum ferritin (µg l⁻¹)</td>
<td></td>
<td></td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are means (standard deviation) unless indicated otherwise.
* Geometric mean.

Results

Baseline characteristics of the population are presented by sex in Table 1. Men were older but the percentage of current smokers was not different between sexes. As expected, mean BMI, total cholesterol and serum triglycerides, systolic and diastolic blood pressure, haemoglobin and serum ferritin concentrations were higher in men than in women.

Of men 4.3% and of women 37.8%, presented at baseline a serum ferritin concentration <30 µg l⁻¹, and serum ferritin level in the range 30–70 µg l⁻¹ was found in 11.2% and 26.3%, respectively. Serum ferritin was positively correlated with total cholesterol (r = 0.15; P < 0.001), serum triglycerides (r = 0.32; P < 0.001), systolic blood pressure (r = 0.26; P < 0.001), diastolic blood pressure (r = 0.24; P < 0.001), BMI (r = 0.27; P < 0.001) and haemoglobin (r = 0.41; P < 0.001).

During the median follow-up time of 7.54 years, 187 subjects (148 men, 39 women) developed IHD. In both sexes, subjects who subsequently developed IHD were older, more often current smokers, and had higher BMI, systolic and diastolic blood pressure, and higher concentrations of total cholesterol and serum triglycerides (Table 2). Furthermore, they had a higher mean serum ferritin concentration, although this difference was not statistically significant at the 5% level in women. No statistically significant differences were observed for haemoglobin.

No relationship was found between serum ferritin and IHD risk in men and in women before and after adjustment (Table 3).

Discussion

In this prospective study performed in a French population, the risk of IHD was not related to serum ferritin. In 1981, Sullivan37 proposed for the first time that body iron stores are positively related to coronary heart disease (CHD) risk. The theory was that production of free radicals that subsequently modify low-density lipoprotein cholesterol was important in the development of atherosclerosis and that iron stimulates the catalysis of oxidation reactions that produce free radicals2–5. In 1992 a Finnish study confirmed this hypothesis, showing a positive relationship between serum ferritin and risk of acute myocardial infarction in men19, after which interest in this theory grew. However, most other studies do not support the theory2–5 and a recent meta-analysis38 of prospective studies comparing subjects with serum ferritin concentration >200 µg l⁻¹ versus those having serum ferritin <200 µg l⁻¹ reported a combined risk ratio for CHD of 1.03 (95% confidence interval: 0.83–1.29). In addition to the use of serum ferritin as a marker of iron...
status, the comparison of blood donors with non-donors appears to provide useful information on the iron-depletion hypothesis because of the marked contrast in body iron stores of regular donors compared with non-donors. However, of three published studies on blood donation, two did not find any difference between the groups and one found a significant inverse relationship with CHD. Our results are thus consistent with most of these studies that have failed to support the hypothesis that body iron stores are associated with risk of CHD.

On the other hand, in our study serum ferritin was related to established coronary risk factors, as has been shown in other studies. It is possible that ferritin may play a role through other risk factors such as cholesterol, triglycerides, obesity or blood pressure, but no association between serum ferritin and IHD was seen even before adjustment for CVD risk factors.

In conclusion, our results taken together with the accumulated evidence from previous prospective studies do not support a major role of iron in the development of IHD.

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Conflict of interest: None declared.

References


