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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 $<30\,\mu g\,l^{-1}$. 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 peroxidation due to its pro-oxidant properties in generating free radicals[1]. 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 development[2–5]. Most cross-sectional and case–control studies using serum ferritin as an indicator of iron stores[6] have not found an association with CVD[5,7–16], although some did[17,18]. However, it has to be taken into account that in these types of studies the disease itself (post-myocardial damage and associated chronic inflammation), 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 studies[19–32] have been reported, and only a Finnish study[19] found a statistically significant association between serum ferritin and CVD; after a 3-year follow-up, serum ferritin concentrations $>200\,\mu g\,l^{-1}$ were associated with a 2.2-fold increase in the incidence of acute myocardial infarction compared with serum ferritin concentrations $<200\,\mu g\,l^{-1}$. An Italian prospective study examining intermediate endpoints observed an association between serum ferritin and an ultrasound measure of atherosclerosis[18].

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

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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 used[33].

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 elsewhere[34,35]. In brief, 12 741 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 120–124[36] 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 12 h. Haemoglobin was measured immediately (cyanmethaemoglobin 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 10 min, 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 absorption[12]: depleted and low status, $<30\,\mu g\,l^{-1}$ (taken as the referent group); non-replete and borderline normal status, $31–70\,\mu g\,l^{-1}$; replete and adequate status, $71–160\,\mu g\,l^{-1}$; and replete and elevated status, $>160\,\mu g\,l^{-1}$. 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.

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Table 1 Risk factors for cardiovascular disease and markers of iron status in the study population

	Men	Women
n	3223	6694
Age (years)	51.8 (4.7)	47.0 (6.6)
Smoking (%)		
Non-smokers	34.0	55.0
Former smokers	51.7	29.2
Current smokers	14.3	15.8
Body mass index ($kg\,m^{-2}$)	25.6 (3.2)	23.4 (4.0)
Blood pressure (mmHg)		
Systolic	129.1 (13.9)	119.7 (13.6)
Diastolic	83.4 (8.4)	76.9 (8.5)
Total cholesterol ($mmol\,l^{-1}$)	6.2 (1.0)	5.9 (1.0)
Serum triglycerides ($mmol\,l^{-1}$)	1.41 (1.1)	0.90 (0.5)
Haemoglobin ($g\,l^{-1}$)	149.7 (10.3)	135.0 (10.5)
Serum ferritin ($\mu g\,l^{-1}$)	195.1 (154.6)	54.9 (54.8)
	144.3*	36.3*

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 $<30\,\mu g\,l^{-1}$; and serum ferritin level in the range $30–70\,\mu g\,l^{-1}$ 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, Sullivan[37] 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 radicals[2,3]. In 1992 a Finnish study confirmed this hypothesis, showing a positive relationship between serum ferritin and risk of acute myocardial infarction in men[19], after which interest in this theory grew. However, most other studies do not support the theory[2–5] and a recent meta-analysis[38] of prospective studies comparing subjects with serum ferritin concentration $>200\,\mu g\,l^{-1}$ versus those having serum ferritin $<200\,\mu g\,l^{-1}$ 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

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

	Women			Men		
	IHD	No IHD	*P*-value	IHD	No IHD	*P*-value
n	39	6655		148	3075	
Age (years)	51.0 (6.9)	46.9 (6.5)	0.0001	54.0 (4.8)	51.7 (4.7)	<0.0001
Smoking (%)						
Non-smokers	55.3	55.0	0.59	28.1	34.3	0.0002
Former smokers	23.7	29.2		45.9	52.0	
Current smokers	21.1	15.8		26.0	13.7	
Body mass index ($kg\,m^{-2}$)	25.0 (5.0)	23.4 (4.0)	0.02	26.5 (3.4)	25.50 (3.2)	0.0007
Blood pressure (mmHg)						
Systolic	128.2 (16.9)	119.6 (13.5)	0.0004	135.3 (15.9)	128.8 (13.8)	<0.0001
Diastolic	82.1 (10.3)	76.9 (8.5)	0.0007	86.2 (8.8)	83.3 (8.4)	0.0003
Total cholesterol ($mmol\,l^{-1}$)	6.5 (1.1)	5.9 (1.0)	<0.0001	6.6 (1.0)	6.2 (1.0)	<0.0001
Serum triglycerides ($mmol\,l^{-1}$)	1.1 (0.5)	0.9 (0.5)	0.03	1.8 (1.5)	1.4 (1.0)	<0.0001
Haemoglobin ($g\,l^{-1}$)	137.1 (10.3)	135.0 (10.5)	0.24	151.0 (11.5)	149.7 (10.2)	0.17
Serum ferritin ($\mu g\,l^{-1}$)	47.8*	36.3*	0.08	165*	143*	0.05

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

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Table 3 Relative risk for ischemic heart disease (IHD) according to serum ferritin concentration

	Men			Women		
	No. IHD cases/total no. of subjects	RR (95% CI)		No. IHD cases/total no. of subjects	RR (95% CI)	
		Crude	Adjusted*		Crude	Adjusted†
Serum ferritin						
$< 30\,\mu g\,l^{-1}$	11/318	1.00	1.00	19/3977	1.00	1.00
$30-70\,\mu g\,l^{-1}$	19/609	0.77 (0.29–2.05)	0.66 (0.21–1.96)	12/1832	0.88 (0.41–1.88)	0.70 (0.28–1.76)
$70-160\,\mu g\,l^{-1}$	55/1125	0.96 (0.41–2.24)	0.85 (0.33–2.18)	4/727	1.14 (0.49–2.64)	0.95 (0.35–2.56)
$> 160\,\mu g\,l^{-1}$	63/1171	1.45 (0.64–3.33)	1.31 (0.52–3.27)	4/158	2.42 (0.80–7.38)	2.18 (0.64–7.43)

RR – relative risk; CI – confidence interval.
* Adjusted for age, smoking, body mass index, total cholesterol, serum triglycerides and group of supplementation.
† Adjusted for age, smoking, body mass index, total cholesterol, serum triglycerides, menopausal status and group of supplementation.

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[39]. However, of three published studies on blood donation[39–41], two did not find any difference between the groups and one found a significant inverse relationship with CHD[41]. 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[19,42–44]. 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 of the development of IHD.

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