

Iron Status Is Associated with Carotid Atherosclerotic Plaques in Middle-Aged Adults^{1,2}

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Abstract

Although the iron-heart disease hypothesis is prevalent, the epidemiological findings are incongruent. The relationship of serum ferritin with early cardiovascular disease (CVD), particularly atherosclerosis, has not been evaluated extensively, particularly with accounting for inflammation. We examined this association in a case-control study of 124 age- and sex-matched pairs embedded in the population-based random sample (MONICA survey) in Southwest France, taking into account inflammation status. Cases had ≥ 2 carotid atherosclerotic plaques and controls had none. Inflammation was assessed using several markers, including serum α -1 acid glycoprotein (AGP) and high sensitivity C-reactive protein. There was an interaction of inflammation with group (case/control) for serum ferritin. In adults without elevated AGP, serum ferritin was significantly greater in atherosclerotic cases than in adults in the control group. In models adjusted for CVD risk factors, the odds of atherosclerosis increased with the increase in serum ferritin in individuals without elevated AGP; for every 10- μ g/L increase in serum ferritin, the risk for atherosclerosis increased by 3% (odds ratio [95% CI]: 1.03 [1.01–1.06]). In conclusion, carotid atherosclerosis was positively associated with serum ferritin in individuals free from subclinical inflammation based on AGP. Further prospective and/or experimental studies are needed to corroborate the observed association of iron status with atherosclerosis. *J. Nutr.* 140: 812–816, 2010.

Introduction

A putative role of iron in atherosclerosis has been proposed to account in part for the sex differences in cardiovascular disease (CVD)⁷ and for the higher incidence of heart disease in older persons related to age-associated increase in iron stores (1,2). The biologically grounded “iron hypothesis” of heart disease (3–5) has not been confirmed, however, with the mixed findings from epidemiological studies (6,7). Certain cross-sectional and case-control studies that have used serum ferritin to assess iron stores have not found an association with CVD (8–10), although a positive association has been reported by others (11–13). Similarly, prospective studies also provide equivocal findings regarding serum ferritin and subsequent CVD; some studies

reported no association (14,15) whereas others found a positive association (16–19). Furthermore, this association was generally nonsignificant or even negative when transferrin saturation, serum iron, or total iron binding capacity were used to assess iron status (8,19,20).

The discrepancies in findings relating iron stores to CVD may be due to the use of varying outcomes; few studies have focused on subclinical CVD evaluated by atherosclerotic plaques (12,13,16) or intima media thickness (8–11,13) whereas others examined later stages of CVD (14–20). In addition, differences in age, sex, and health status of participants across studies as well as the small number of cases examined in certain studies may have contributed to differential findings. Another important confounder across studies could be underlying inflammation. The majority of the studies did not account for inflammation in their design (8–10,12–18,20). Few studies (11,19,21) that considered inflammation in their analysis used C-reactive protein (CRP) as the only marker of inflammation.

Most studies have used traditional markers such as serum ferritin, transferrin saturation, and serum iron to define iron status and iron stores, and these markers are recognized to be independently affected by inflammation (22,23). In fact, individuals' iron stores based on ferritin in normal ranges may be difficult to interpret in the presence of inflammation without

¹ Supported by grants from the National Institute for Health and Medical Research (INSERM), France, National Health Surveillance System, France, la Fédération Française de Cardiologie, la Fondation pour la Recherche Médicale, la Fondation de France, and the Direction de la Recherche Clinique, Centre-Hospitalier Universitaire, Toulouse (grant no. 0604702).

² Author disclosures: N. Ahluwalia, A. Genoux, J. Ferrieres, B. Perret, M. Carayol, L. Drouet, and J. B. Ruidavets, no conflicts of interest.

⁷ Abbreviations used: AGP, α -1-acid glycoprotein; Apo, apolipoprotein; CRP, C-reactive protein; CVD, cardiovascular disease; OR, odds ratio; sICAM-1, serum intercellular adhesion molecule-1.

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further investigation involving bone marrow or liver biopsy and iron staining (23,24). On the other hand, although raised serum iron and transferrin saturation are suggestive of iron overload, they demonstrate high day-to-day, within-person variability and are also affected by inflammation (25,26).

Thus, we conducted a case-control study to examine the association of iron status with subclinical atherosclerosis, assessed using carotid atherosclerotic plaques, in apparently healthy, middle-aged persons taking into account their inflammation status.

Methods

This study had a 1:1 pair-matched, case-control design; cases and controls were selected from the 3rd French Monitoring of Trends and Determinants in Cardiovascular Disease survey on cardiovascular risk factors (27,28). A population-based sample of 972 middle-aged Caucasian men and women (35–64 y) was randomly selected in the Toulouse region (Southwest France). Participants provided informed consent following protocols approved by the Ethics Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Lille, France). Participants were free from overt disease such as cancer, renal, and liver disease.

Atherosclerotic cases were defined based on the presence of at least 2 carotid atherosclerotic plaques as described below. Controls were defined as the absence of carotid atherosclerotic plaques and were matched to cases according to sex and age. A total of 124 age- and sex-matched pairs (124 cases and 124 controls; 73.2% men and 26.8% women) were drawn from the initial sample ($n = 972$).

Participants provided information on demographic variables, socioeconomic status, and medical history, including CVD risk factors, medication use, smoking and alcohol consumption, and physical activity using questionnaires with the help of trained medical staff (28). Height, weight, and waist and hip circumference were recorded by trained staff using standardized methods. Arterial blood pressure was measured by the medical staff according to standardized protocols (28).

Identification of atherosclerotic plaques. High-resolution B-mode ultrasonography was conducted using an ATL UM9 system (Advanced Technology Laboratories Ultramark 9 High Definition Imaging) with a 7.4-MHz transducer. The potential presence of atherosclerotic plaques was investigated in the right and left common carotid arteries and internal carotid arteries (including carotid bulbs). A plaque was defined as a distinct zone identified with either a focal area of hyperechogenicity relative to adjacent segments or a focal protrusion into the lumen of the vessel, composed of only calcified deposits or a combination of calcified and noncalcified material (28).

Laboratory procedures. Fasting blood samples were obtained and centrifuged within 4 h to obtain serum. Serum samples were aliquoted and stored at -80°C until analyses for thorough evaluation of CVD risk factors, inflammation, and iron status markers. Internal controls and calibrators when available were used to ensure quality control; analytical variation for samples was $<5\%$.

Traditional risk factors for CVD. Serum total cholesterol and triglycerides were measured using enzymatic assays (Roche Diagnostics). HDL cholesterol was determined after sodium phosphotungstate-magnesium chloride precipitation of apolipoprotein (Apo)-B-containing lipoproteins (Roche Diagnostics). LDL cholesterol was determined using the Friedewald formula (29). Apo-A1 and Apo-B were measured by immunoturbidimetric assays in an automated analyzer (Roche Diagnostics). Glucose concentrations were measured using enzymatic reagents (Dade Behring Marburg).

Markers of inflammation. Serum α -1 acid glycoprotein (AGP) was measured using an immunonephelometric method with a Beckman Immage analyzer (Beckman Coulter). High-sensitivity CRP was mea-

sured by the immunonephelometric method (Dade Behring Marburg). Serum intercellular adhesion molecule-1 (sICAM-1) concentrations were measured by an immunoenzymatic method (Immunotech).

Markers of iron status. Serum iron was determined by a colorimetric method using Olympus reagents and analyzer (Olympus Diagnostics). Serum ferritin and transferrin were assayed by immunoturbidimetric methods using Olympus reagents and analyzer.

Statistical analysis. We compared the distributions of case and control characteristics using a paired t test for continuous variables. When basic statistical assumptions were not satisfied for variables even after logarithmic transformation, the Wilcoxon's rank-sum test was used instead. For log-transformed data, geometric means and 95% CI were computed; these values are provided after converting them back to the original scale for ease of interpretation.

Unadjusted comparisons between cases and controls were conducted on iron status markers using a generalized linear model. A systematic interaction case-control group \times AGP (dummy variable using the cutoff of 1 g/L) was tested for each iron status marker. Similarly, interaction of case-control group with CRP (dummy variable using the cutoff of 1 mg/L) was also tested for iron status tests. When significant interactions ($P < 0.10$) were noted for certain iron status markers, analyses were stratified on inflammation status (based on AGP and CRP in separate analyses). Age- and sex-matched case-control pairs without inflammation were compared using general linear models on these iron status markers (crude model) and upon adjustment for age, alcohol intake, smoking, BMI, systolic blood pressure, serum glucose, Apo-A1, and Apo-B (multivariate-adjusted model). The latter model was rerun by adding either CRP or sICAM-1 when inflammation status was defined using AGP, and vice versa, by adding AGP or sICAM-1 when inflammation status was defined using CRP. The association of serum ferritin with atherosclerosis was examined in logistic regression models; odds of atherosclerosis were computed for every 10- $\mu\text{g/L}$ increase in serum ferritin in crude and multivariate-adjusted models as described above. In addition, the risk of atherosclerosis was compared for individuals in the highest tertile compared with those in the lowest tertile for serum ferritin; the odds of atherosclerosis and corresponding 95% CI were computed using crude and adjusted models, as described above. $P \leq 0.05$ was used as the level of significance. Statistical analyses were performed using the SAS release 9.1 (SAS Institute).

Results

Cases had a more adverse cardiovascular risk profile than controls (Table 1). Cases smoked more and had significantly greater BMI, waist circumference, systolic blood pressure, and serum ApoB, CRP, and sICAM-1. Systolic blood pressure, CRP, and sICAM-1 differed between cases and controls without inflammation (AGP ≤ 1 g/L).

Cases and controls did not differ in any of the iron status indicators examined when inflammation status was not taken into account (Table 2). These results were unchanged when other traditional factors known to affect CVD risk (age, BMI, alcohol intake, smoking, systolic blood pressure, ApoA1, ApoB, glucose) were considered in the statistical models (data not shown). For ferritin, a significant interaction between group (case/control) and inflammation status (yes/no) was noted irrespective of whether inflammation was defined on the basis of AGP or CRP. Thus, analyses were repeated among case-control pairs without inflammation (elevated AGP and CRP concentrations) (Table 3).

Among individuals without elevated AGP concentrations, cases had significantly higher ferritin concentrations (Table 3). These findings were unchanged in multivariate-adjusted models that included traditional CVD risk factors (Table 3) as well as CRP or sICAM-1 (data not shown).

TABLE 1 Characteristics and serum biochemistry of cases and controls¹

	All cases and controls			Cases and controls with AGP ≤1 g/L		
	Cases	Controls	P ²	Cases	Controls	P ²
<i>n</i>	124	124		93	93	
Age, <i>y</i>	56.3 (56.1–56.5)	55.7 (55.5–55.9)	0.001	56.2 (55.9–56.4)	55.7 (55.4–55.9)	0.006
Alcohol, ³ <i>g/d</i>	15.4 (1.8–39.1)	15.4 (2.6–36.9)	0.27	15.4 (2.1–40.6)	15.4 (0–30.8)	0.25
Smoking, ³ <i>pack/y</i>	9.4 (0–33.6)	0 (0–17.8)	0.04	7.3 (0–32.5)	0 (0–23.0)	0.23
BMI, <i>kg/m²</i>	27.4 (26.7–28.1)	26.2 (25.5–26.8)	0.02	26.9 (26.2–27.6)	26.1 (25.4–26.7)	0.09
Waist circumference, <i>cm</i>	94.3 (92.4–96.2)	91.1 (89.2–93.0)	0.02	93.3 (91.2–95.3)	90.5 (88.6–92.4)	0.06
Systolic blood pressure, <i>mm Hg</i>	144.1 (141.3–146.9)	131.1 (128.3–133.8)	0.001	143.9 (140.5–147.4)	131.5 (128.3–134.7)	0.001
Glucose, ⁴ <i>mmol/L</i>	5.87 (5.70–5.99)	5.75 (5.58–5.93)	0.43	5.81 (5.64–5.90)	5.70 (5.53–5.87)	0.49
Triglycerides, ⁴ <i>mmol/L</i>	1.22 (1.13–1.31)	1.14 (1.05–1.23)	0.24	1.17 (1.07–1.28)	1.12 (1.03–1.22)	0.45
ApoA1, <i>g/L</i>	1.60 (1.56–1.65)	1.62 (1.58–1.66)	0.63	1.64 (1.59–1.69)	1.62 (1.57–1.67)	0.61
ApoB, <i>g/L</i>	1.32 (1.28–1.37)	1.23 (1.19–1.27)	0.003	1.30 (1.25–1.35)	1.24 (1.19–1.29)	0.10
Total cholesterol, <i>mmol/L</i>	6.18 (6.01–6.35)	5.96 (5.79–6.13)	0.08	6.15 (5.94–6.36)	6.02 (5.83–6.22)	0.39
HDL cholesterol, <i>mmol/L</i>	1.37 (1.30–1.44)	1.37 (1.30–1.45)	0.94	1.44 (1.36–1.52)	1.38 (1.31–1.46)	0.33
CRP, ³ <i>mg/L</i>	1.3 (0.6–2.7)	0.8 (0.4–1.8)	0.004	1.0 (0.5–2.2)	0.7 (0.4–1.4)	0.02
AGP, ⁴ <i>g/L</i>	0.78 (0.75–0.81)	0.76 (0.73–0.79)	0.39	0.72 (0.69–0.74)	0.73 (0.70–0.75)	0.76
sICAM-1, ⁴ <i>g/L</i>	275.9 (259.8–290.0)	244.7 (232.8–259.8)	0.005	270.4 (252.1–290.0)	239.8 (225.9–254.7)	0.02

¹ Values are means (95% CI) unless noted otherwise.

² *P* obtained with paired *t* test or Wilcoxon's signed rank-sum test.

³ Median and 25th and 75th percentiles as measures of dispersion.

⁴ Analyzed using log-transformed data.

Similar results regarding cases having higher iron stores, based on serum ferritin were noted, when analyses were conducted on case-control pairs without elevated CRP concentrations (Table 3). However, in multivariate-adjusted models whereby potential confounders including traditional CVD risk factors were considered, cases and controls (without elevated CRP concentrations) no longer differed on ferritin. These findings were unaltered upon further adjustment for AGP or sICAM-1 (data not shown).

In crude and multivariate-adjusted analyses with cases and controls without inflammation based on AGP (≤1 g/L), there was a significant positive association between serum ferritin concentrations and atherosclerotic plaques (Table 4). Specifically, each 10-μg/L increase in serum ferritin was associated with a 3% increased probability of having ≥2 atherosclerotic plaques (*P*-trend = 0.05). In addition, there was a borderline significant trend for increased odds of atherosclerosis for individuals in the highest tertile of serum ferritin compared with those in the lowest tertile; the odds ratio (OR) and 95% CI were 2.27 and 0.98–5.30, respectively (*P* = 0.06). When inflammation was defined on the basis of CRP (≤1 mg/L),

individuals in the highest tertile of serum ferritin still tended to have a greater risk of atherosclerosis compared with those in the lowest tertile (*P* < 0.10).

Discussion

Serum ferritin is considered the best single indicator of total body iron stores (30), particularly in the absence of inflammation. In this case-control study of middle-aged men and women, serum ferritin was associated with carotid atherosclerotic plaques in individuals free from inflammation based on elevated AGP. In previous studies, mixed results have been reported on the association of ferritin with CVD (8–13,16,17,19). A significant positive association between serum ferritin and subclinical atherosclerosis has been reported in some studies (11–13,16,17), whereas others did not find any association (8–10,19). These discrepancies may be partly related to differential control of inflammation and choice of inflammatory markers used in these studies.

Multiple lines of evidence support an important role of iron in atherosclerosis. The proatherogenic properties of iron are due

TABLE 2 Markers of iron status in atherosclerotic cases and controls¹

	Cases	Controls	<i>P</i>	Interaction of group (case/control) × inflammation (yes/no)	
				Group × AGP, <i>P</i>	Group × CRP, <i>P</i>
<i>n</i>	124	124			
Serum ferritin, ² <i>μg/L</i>	179.5 (157.6–202.4)	165.7 (145.5–186.8)	0.39	0.001	0.02
Serum transferrin receptor, ² <i>mg/L</i>	4.48 (4.35–4.62)	4.44 (4.31–4.62)	0.74	0.50	0.36
Serum iron, <i>μmol/L</i>	18.9 (17.6–20.1)	19.0 (17.8–20.3)	0.85	0.28	0.39
Transferrin saturation, %	30.3 (28.2–32.2)	30.3 (28.3–32.2)	0.97	0.14	0.48
Hemoglobin, <i>g/L</i>	143.2 (141.7–144.6)	142.5 (141.1–143.9)	0.52	0.54	0.79

¹ Values are means (95% CI).

² Analyzed using log-transformed data.

TABLE 3 Serum ferritin ($\mu\text{g/L}$) in atherosclerotic cases and controls free of inflammation based on serum AGP and serum CRP^{1,2}

	Cases	Controls	<i>P</i>
93 case: control pairs with AGP ≤ 1 g/L			
Crude	200.3 (174.2–230.6)	151.4 (132.9–172.4)	0.006
Adjusted ³	204.4 (174.2–239.9)	146.9 (126.5–172.4)	0.009
36 case: control pairs with CRP ≤ 1 mg/L			
Crude	202.4 (159.2–257.2)	142.6 (119.1–172.4)	0.05
Adjusted ³	206.4 (154.5–278.7)	156.0 (126.5–190.6)	0.14

¹ Values are means (95% CI).² Analyzed using log-transformed data.³ Adjusted for age, BMI, alcohol intake, smoking, systolic blood pressure, Apo A1, Apo B, and glucose.

to its ability to generate highly active free radicals, oxidize lipoproteins, and activate platelets (1,3–5). Ferritin, the iron storage protein, has been described in human atherosclerotic lesions and in diseased coronary arteries from patients with coronary artery disease (31,32). Localization of ferritin in the arteries may contribute to the oxidation of lipids within the vessel wall and formation of oxidized LDL, leading to the development of foam cells and progression of atherosclerosis (5). Animal studies have confirmed these observations (33) and shown that chronic iron administration accelerates thrombus formation and increases vascular oxidative stress (34). In a recent randomized, controlled study with peripheral arterial disease patients, reduction in body iron via repeated phlebotomy, however, did not affect all-cause mortality (35), even though the risk of a composite outcome, including all-cause death and nonfatal coronary heart disease, was reduced among younger patients (35).

In the current study, the association of serum ferritin with atherosclerosis depended on inflammation status. When inflammation was defined based on elevated AGP concentrations, the association of serum ferritin and atherosclerosis was significant in both crude and multivariate-adjusted models. However, when inflammation was defined using CRP, the positive association was not significant ($P < 0.10$). Both CRP and AGP are positive acute phase reactant proteins; however, their half-lives are quite different. The time course of increase in ferritin following the onset of inflammation and the duration that its levels stay elevated resemble more closely the changes in AGP than those in CRP (36). Therefore, the use of AGP may be more valid to correct for the presence of subclinical inflammation when examining associated changes in iron stores. In addition, in our study, more individuals had elevated CRP (>1 mg/L) than

elevated AGP (>1 g/L), thus leaving fewer case-control pairs without inflammation based on CRP concentrations. Therefore, it is likely that the association of ferritin with atherosclerosis may have been underestimated in the current study when CRP was used to define inflammation.

We controlled for subclinical inflammation in the statistical models in several ways. First, we used several markers of subclinical inflammation, including CRP and AGP. Because there was an interaction between iron status and inflammation (based on CRP or AGP), we restricted analysis to cases and controls without inflammation. In addition, we also adjusted for sICAM-1, which is involved in the development and progression of atherosclerosis (37); the results of the association of atherosclerosis with serum ferritin were unchanged (data not shown). Finally, we took the conservative approach to define inflammation by considering CRP and AGP simultaneously. Thirty-five cases and control pairs did not have inflammation based on this strict criteria; the association of iron stores with atherosclerosis was attenuated and only a statistical trend was observed ($P < 0.10$). Because serum ferritin can be affected by inflammation, we could not evaluate the relationship of iron status with atherosclerosis when inflammation was present (38). However, to our knowledge there is no biological reason that would diminish the association of iron status with CVD in the presence of inflammation. This remains to be tested through future clinical studies involving invasive yet confirmatory bone marrow biopsy to quantify iron stores, as feasible.

The residual confounding due to other factors such as dietary regimen, treatment for diabetes, hypertension, and dyslipidemia, or physical activity should be considered. When these factors were added to the multivariate-adjusted models, however, the results were unchanged. Few participants (4%) took nutrient supplements in the current study; thus, supplement use was probably not an important confounder. The observational nature of the study, however, precludes drawing any cause and effect conclusions. Nevertheless, because the study focused on subclinical CVD among apparently healthy persons, it is unlikely that individuals changed their diets or lifestyles that could affect their exposure or outcome variables. Due to the smaller number of women in the study, we could not examine associations for gender separately; data on both genders were pooled for analyses, because no gender interaction was noted ($P > 0.20$).

In summary, carotid atherosclerosis was positively associated with serum ferritin in apparently healthy middle-aged men and women who were free from subclinical inflammation, independently of traditional CVD risk factors. Further prospective and/or experimental studies that take into account subclinical inflammation, assessed using several markers notably AGP, are

TABLE 4 Odds of atherosclerosis (at least 2 carotid plaques) in individuals free from inflammation for every 10- $\mu\text{g/L}$ increase in serum ferritin¹

	Model	OR ²	95% CI ²	<i>P</i>
AGP ≤ 1 g/L	Crude	1.03	1.01–1.05	0.02
	Adjusted ³	1.03	1.01–1.06	0.03
CRP ≤ 1 mg/L	Crude	1.03	1.00–1.07	0.09
	Adjusted ³	1.05	0.99–1.12	0.10

¹ Analyzed using log-transformed data.² OR and 95% CI obtained with conditional logistic regression. Cases were defined on the basis of presence of at least 2 atherosclerotic carotid plaques whereas controls had no carotid plaques.³ Adjusted for age, BMI, alcohol intake, smoking, systolic blood pressure, Apo B, Apo A1, and glucose.

needed to establish the direction of the observed association and to better understand the underlying mechanisms.

Acknowledgments

N.A., J.F., and J.B.R. designed research and obtained funding; A.G., B.P., and L.D. conducted research and supervised data collection; J.B.R. analyzed data with input from N.A.; N.A. wrote the paper with input from J.B.R.; M.C. assisted with bibliographic research and data presentation; and N.A. and J.B.R. had primary responsibility for final content. All authors read and approved the final manuscript.

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