

## Body Iron Stores and Presence of Carotid Atherosclerosis

### Results From the Bruneck Study

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**Abstract** We hypothesized that the formation of foam cells and fatty streaks requires a postsecretory oxidative modification of lipoproteins that targets them for rapid uptake by macrophages. Lipid peroxidation may in part depend on the concentration of tissue iron, one of the major oxidants in vivo. We analyzed the relation between sonographically assessed carotid atherosclerosis and body iron stores in a population sample of 847 men and women aged 40 to 79 years. In a logistic regression analysis adjusting for age, sex, and all major vascular risk markers, ferritin emerged as one of the strongest indicators of carotid artery disease in both sexes (40 to 59

years; odds ratio, 1.54 per 100  $\mu\text{g/L}$ ;  $P < .001$ ). The predictive significance of ferritin was found to be synergistic with that of hypercholesterolemia. Variations in body iron stores between sexes may partly explain evident sex differences in the expression of carotid atherosclerosis. In the elderly ( $\geq 60$  years) the predictive significance of ferritin was found to decrease parallel to that of apolipoprotein B. The current study suggests a possible role of body iron in early atherogenesis. (*Arterioscler Thromb.* 1994;14:1625-1630.)

**Key Words** • carotid atherosclerosis • ferritin • iron • population studies • oxidized lipoproteins

Recent advances in understanding vascular lipid metabolism have yielded new insights into the mechanisms that determine endothelial injury and plaque development. In particular, the postsecretory modification of lipoproteins has attracted increasing attention.<sup>1</sup> Oxidative changes in the surface structure of low-density lipoproteins enhance the affinity to macrophage scavenger receptors, giving rise to the formation of foam cells and fatty streaks.<sup>2,3</sup> Prominent iron stores may promote lipid peroxidation and accelerate atherogenesis and cardiovascular disease. This hypothesis recently received empirical support from a large population study (Kuopio Ischaemic Heart Disease Risk Factor Study) that demonstrated an enhanced risk of myocardial infarction in middle-aged men with elevated concentrations of serum ferritin.<sup>4</sup> These findings, however, do not necessarily imply a causal role of iron in atherogenesis. Enhanced reperfusion injury, direct cardiotoxicity of high myocardial iron deposits, vasospastic events, and increased blood viscosity may also be significant.<sup>5-7</sup> We designed the current study to investigate the relation between sonographically assessed carotid atherosclerosis and body iron stores as estimated by serum ferritin.<sup>8,9</sup> We also investigated physiologically normal iron status and addressed sex differences in the amount of body iron and the manifestation of atherosclerotic vascular disease.

## Methods

### Study Subjects

The study population was composed of an age- and sex-stratified random sample of 1000 men and women aged 40 to 79 years (The Bruneck Ischemic Heart Disease and Stroke Prevention Study<sup>10</sup>). The participation rate was 93.6%. To be eligible, subjects had to be free of symptoms or a history of transient ischemic attack and ischemic stroke. Further exclusion criteria were missing ultrasound and laboratory data or carotid endarterectomy. In the remaining population ( $n=909$ ) extensive screening was performed in an effort to identify subjects with no adequate indirect measure of the tissue iron concentration available, namely, those with neoplastic, inflammatory, or liver diseases. Data collection was composed of two consecutive steps, the first involving a self-reported medical history, clinical examination, and extensive laboratory screening. Individuals who were suspected of having a disease of interest were directed to a subsequent phase two evaluation. Based on detailed medical records available from previous external and hospital checkups and further optional examinations, a definite diagnosis was established applying standard diagnostic guidelines. In all, 62 subjects satisfied the screening criteria: autoimmune diseases were fairly rare ( $n=5$ ). Of the neoplastic diseases found in 11 subjects (leukemia,  $n=2$ ; breast cancer,  $n=2$ ; lung cancer,  $n=3$ ; oral cancer,  $n=1$ ; gastric cancer,  $n=2$ ; ovarian cancer,  $n=1$ ), 2 were previously unknown. An impairment of liver function was considered evident in the case of elevated liver enzymes ( $\gamma$ -glutamyl-transpeptidase and transaminases) and/or severe structural changes documented by ultrasonography. As the main underlying pathomechanism, we assessed in descending order severe alcohol consumption (usually  $>100$  g/d;  $n=26$ ), active hepatitis (B virus,  $n=5$ ; C virus,  $n=2$ ; other,  $n=1$ ), and hepatotoxic drugs ( $n=1$ ). Individuals with a carrier status of hepatic B surface antigen were considered eligible. In the case of normal liver enzymes, a weak association between average amounts of daily alcohol consumption and ferritin concentrations did not approach statistical significance. Acute infectious diseases were recognized at the baseline examination and blood sam-

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ples drawn up to 6 weeks later. Subjects with clinical or laboratory evidence of persistent infection were considered ineligible (pyelonephritis,  $n=1$ ; respiratory infection,  $n=2$ ; myocarditis,  $n=1$ ), as were 2 women with advanced renal failure. Localized urinary infections were found to exert no relevant influence on the level of serum ferritin.

### Clinical History and Examination

All participants underwent a structured in-person interview on their medical history including current and previous diseases, medication, and symptoms of cardiovascular, neoplastic, and infectious illness. All interviews were uniformly conducted by a single experienced physician and took one-half hour on average. Additional information on current or past exposure to vascular risk factors was collected with a standardized questionnaire. Initial data collection included full neurological and cardiological examinations.

Based on the responses given, the life-time years of regular smoking ("years smoked") were recorded for each smoker and ex-smoker. Mean diastolic and systolic blood pressures were calculated from two independent measurements, each of which was taken with the subject in a supine position after 10 minutes of rest. The body mass index (Quetelet index) was defined as weight (kilograms) divided by height (meters) squared. Heart insufficiency was graded according to the New York Heart Association criteria.

### Laboratory Methods

Venous blood samples were drawn after 12 hours of fasting and abstinence from smoking (7:30 to 9:30 AM).<sup>10</sup> Plasma and serum samples were separated and analyzed immediately or frozen at  $-70^{\circ}\text{C}$ . Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were analyzed by means of commercial enzymatic assays (CHOD-PAP and GOP-PAP methods, Merck). Apolipoproteins were measured with an immunonephelometric fixed-time method on a Behring nephelometric analyzer (Behringwerke AG). Low-density lipoprotein cholesterol was calculated with the Friedewald formula.<sup>11</sup> Plasma glucose, fibrinogen, transaminases, antithrombin III, and antinuclear antibodies were measured by standard methods. Serum ferritin was assessed with a fluorometric enzyme immunoassay ("sandwich assay") using a Stratus II Fluorometric Analyzer (Baxter Diagnostic Inc). The between-batch coefficients of variation were 5.0%, 5.1%, and 5.9% for ferritin levels of 66, 151, and 260  $\mu\text{g/L}$ , respectively ( $n=30$ ). Serum iron was measured with a centrifugal analyzer using ferrozine as chromogen. The laboratory protocol also included a standard urine analysis and screening for hepatitis B and C.

### Evaluation of Vascular Status

Sonographic assessment of the carotid arteries was performed with a duplex ultrasound system (ATL UM8, Advanced Technology Laboratories) using a 10-MHz imaging probe. The imaging protocol involved scanning of both left and right common (CCA) and internal (ICA) carotid arteries in multiple longitudinal and transverse planes. Measurements were taken by a single trained physician at the proximal CCA (15 to 30 mm proximal to the carotid bulb), distal CCA (<15 mm proximal to the carotid bulb), proximal ICA (carotid bulb and the initial 10 mm of the vessel), and distal ICA (>10 mm above the flow divider). The extent of carotid atherosclerosis was quantified by a sensitive and reproducible plaque score, which represented the sum of maximal near- and far-wall diameters of definite atherosclerotic plaques at each imaging site<sup>10</sup> (range, 0 to 33.2 mm).

### Statistical Analysis

Age-standardized relations between serum ferritin and cardiovascular risk markers were estimated with partial correlation coefficients. To evaluate the predictive significance of candidate cardiovascular risk factors, we developed multivari-

TABLE 1. Serum Ferritin in a Population of 847 Women and Men Aged 40 to 79 Years

Sex/Age, y	n	Mean±SEM, μg/L	Percentile		
			10th	50th	90th
Male					
40-49	106	164±10	53	151	309
50-59	103	196±18	51	151	352
60-69	110	201±14	62	165	372
70-79	97	202±16	45	162	412
Total	416	191±7	54	156	362
Female					
40-49	119	33±3	6	21	82
50-59	103	70±5	15	56	137
60-69	109	95±7	22	74	190
70-79	100	111±10	12	84	252
Total	431	76±4	11	52	176

able logistic regression models using either a step-forward selection procedure or simultaneous forced entry of a set of variables.<sup>12</sup> The outcome variable was dichotomized according to the carotid artery disease status (0 stands for a plaque score of 0 mm; 1 stands for plaque score >0 mm). In a random sample of 50 subjects with rescannings (by an independent sonographer), attribution to the dichotomized grouping variable was consistent in both assessments for all but 1 person ( $K=0.92$ ). A four-category polychotomous logistic regression analysis considered the severity of carotid atherosclerosis in the definition of the outcome variable (0...plaque score=0 mm, 1...0 mm<score $\leq$ 1.0 mm (first tertile), 2...1 mm<score $\leq$ 2.3 mm (second tertile), 3...score>2.3 mm). Analyses were performed separately in subjects aged 40 to 59 years and 60 to 79 years. The goodness of fit of each model was assessed by the test after Hosmer and Lemeshow.<sup>12</sup> Analysis was controlled for age by forcing it into the equation as an independent variable. On account of the clear age dependence of most risk markers, the definite models were complemented and confirmed by corresponding analyses in a sex- and age-matched environment.

Effect modification<sup>13</sup> of the relation between atherosclerosis and ferritin was estimated by fitting separate logistic regression models into subsamples according to the variable of interest. The difference in the regression coefficients of ferritin between both equations was tested for statistical significance using a pooled variance estimate.

### Results

Tables 1 and 2 summarize selected demographic and clinical information on the study population. In men, serum ferritin levels ranged from 10 to 1390  $\mu\text{g/L}$  compared with a range of 1.6 to 655  $\mu\text{g/L}$  in women. Median serum ferritin in men remained more or less constant (156  $\mu\text{g/L}$ ) over the entire age range (Table 1). In the female population iron accumulation occurred preferentially from age 50 to 59 years (median ferritin concentrations: 22  $\mu\text{g/L}$  [40 to 44 years], 21  $\mu\text{g/L}$  [45 to 49 years], 45  $\mu\text{g/L}$  [50 to 54 years], 70  $\mu\text{g/L}$  [55 to 59 years], and 74  $\mu\text{g/L}$  [60 to 69 years]), well in advance of the sharp postmenopausal rise in the frequency of carotid artery disease in the decade between ages 60 and 69. Iron overload as indicated by a serum ferritin level greater than 400  $\mu\text{g/L}$ <sup>8</sup> accounted for no less than



TABLE 2. Candidate Risk Factors for Carotid Atherosclerosis in a Population Aged 40 to 79 Years

Variable	40 to 59 Years (n=431)			60 to 79 Years (n=416)		
	Mean±SD		Odds Ratio (SD)*	Mean±SD		Odds Ratio (SD)*
	CA-	CA+		CA-	CA+	
Ferritin, $\mu\text{g/L}$	96±92	187±201	1.78§	136±114	160±143	1.11
Serum iron, $\mu\text{mol/L}$	17±6	18±6	1.15	17±5	17±6	1.04
Hemoglobin, g/L	147±22	152±11	1.03	147±11	149±13	1.07
Hematocrit, %	43±4	45±3	1.59†	43±5	44±4	1.17
Apolipoprotein B, g/L	1.1±0.3	1.3±0.4	1.56§	1.2±0.3	1.2±0.3	1.00
Triglycerides, mmol/L	1.3±0.8	1.8±1.4	1.55§	1.6±0.8	1.6±1.2	1.01
Total cholesterol, mmol/L	5.5±0.9	5.9±1.2	1.48‡	5.9±1.0	5.8±1.1	1.00
LDL cholesterol, mmol/L	3.4±0.9	3.7±1.1	1.34†	3.6±1.0	3.7±1.1	1.07
HDL cholesterol, mmol/L	1.4±0.3	1.4±0.4	0.93	1.5±0.4	1.4±0.4	0.93
Apolipoprotein A-I, g/L	1.6±0.3	1.6±0.3	1.06	1.7±0.3	1.6±0.3	0.98
Systolic BP, mm Hg	135±18	147±20	1.53‡	147±19	155±22	1.54§
Fibrinogen, g/L	2.4±0.5	2.5±0.6	1.07	2.7±0.5	2.9±0.6	1.02
Fasting glucose, mmol/L	5.3±0.5	5.7±1.3	1.32†	5.6±0.8	5.9±1.5	1.23
Years smoked	9±12	15±15	1.41†	12±17	20±22	1.39†
Antithrombin III, %	99±13	95±12	0.75†	98±14	96±14	0.99
ESR, mm/h	10±7	11±7	1.25	15±9	16±12	1.12
Body mass index, kg/m <sup>2</sup>	24±4	26±3	1.15	25±3	25±4	1.01

CA+ indicates subjects with carotid atherosclerosis; CA-, subjects without carotid atherosclerosis; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BP, blood pressure; and ESR, erythrocyte sedimentation rate.

\*Odds ratios for increase in 1 SD unit of given variables were derived from logistic regression analyses taking into account age, sex, and the variable of interest.

† $P < .05$ , ‡ $P < .01$ , § $P < .001$ .

7.4% in the male population (0.5% in women). The iron status of the current population sample and of previous population surveys in North America<sup>9,14</sup> and Europe<sup>4</sup> showed a particularly close agreement. Serum ferritin had a weak age-standardized correlation with blood glucose ( $r=.23$ ), triglycerides ( $r=.18$ ), and apolipoprotein B ( $r=.18$ ) in women and with triglycerides ( $r=.19$ ) in men. No significant relations were obtained for erythrocyte sedimentation rate, fibrinogen, blood leukocyte count, or various immune markers. In the population aged 40 to 59 years, the strongest predictors of carotid atherosclerosis when adjusting for age and sex were serum ferritin, apolipoprotein B, and triglycerides. In a step-forward logistic regression analysis allowing for all significant risk markers in Table 2, serum ferritin remained independently significant (odds ratio, 1.54 per 100  $\mu\text{g/L}$ ;  $P < .001$ ). The corresponding polychotomous logistic regression analysis (see above) yielded similar results, with the predictive significance of ferritin tending to be even higher in this equation (odds ratio, 1.63 per 100  $\mu\text{g/L}$ ;  $P < .0001$ ). This procedure took into account the severity of carotid artery disease and indicated a dose-response relation between serum ferritin and atherosclerosis. A logarithmic transformation of the skewed ferritin distribution resulted in the selection of essentially the same prognostic factors. Logistic regression analyses with the same set of independent variables were also fitted separately in men and women. In both equations, ferritin was found to be highly indicative of carotid atherosclerosis, the relative increment of risk

being slightly more pronounced in women. When blood hematocrit was analyzed jointly with serum ferritin and the risk markers of Table 3, its significant relation to carotid atherosclerosis (see Table 2) disappeared, whereas the odds ratio associated with ferritin remained more or less unchanged (odds ratio, 1.52 per 100  $\mu\text{g/L}$ ).

TABLE 3. Multiple Logistic Regression Analysis of Carotid Atherosclerosis on Major Cardiovascular Risk Factors in a Population Aged 40 to 59 Years

Variable	LRC	Odds Ratio (SD)	95% CI	P
Constant	-13.066			
Age, y	0.170	2.60	1.86-3.64	<.001
Ferritin, $\mu\text{g/L}$	0.004	1.71	1.25-2.35	<.001
Apolipoprotein B, g/L	1.270	1.47	1.11-1.94	<.01
Systolic BP, mm Hg	0.011	1.24	1.01-1.52	<.05
Glucose, mmol/L	0.361	1.31	1.00-1.71	.05
Antithrombin III, %	-0.028	0.70	0.51-0.96	<.05
GOF $P=.74$				

Odds ratios for increase in 1 SD unit of given variables, logistic regression coefficients (LRC), and 95% confidence intervals (95% CI) were derived from multivariate logistic regression analysis. BP indicates blood pressure; GOF  $P$ , probability value for Hosmer-Lemeshow goodness of fit test.



To test whether hyperlipidemia modifies the relation between body iron stores and carotid atherosclerosis, we established separate logistic regression functions in two subpopulations defined by their total cholesterol. The cutoff value was set at the overall median of 5.5 mmol/L. In both subgroups, the age and sex distributions were fairly consistent. The risk factor-adjusted odds ratios for serum ferritin were 1.22 per 100  $\mu\text{g/L}$  in the low-cholesterol and 1.86 per 100  $\mu\text{g/L}$  in the high-cholesterol group when ferritin was entered jointly with age and the independent risk variables in Table 3 ( $P=.05$  for difference). For comparison, the relative risk associated with ferritin was not appreciably different in smokers and nonsmokers, normotensive or hypertensive individuals, and subsamples categorized according to glucose tolerance.

In the population aged 60 to 79 years, the comparatively low variation of serum ferritin still accounted for some of the differences in the atherosclerosis risk. As expected, the discriminative power was far less pronounced in this age group, although generally high iron stores may be one reason for the equally high prevalence of atherosclerotic lesions.

### Discussion

The possibility that iron overload might play a role in atherosclerosis was first postulated by Sullivan<sup>15,16</sup> as a potential explanation for a wide range of empirical phenomena, including the sex difference in cardiovascular disease. The documentation of a postsecretory modification of lipoproteins *in vivo* provided a promising pathophysiological basis for the empirical hypothesis.<sup>1,17</sup> Native low-density lipoprotein experiences a series of postsecretory oxidative changes that target it for a rapid uptake by macrophages through the scavenger receptors.<sup>1-3</sup> Apart from foam cell formation, the chemical properties of oxidized lipoproteins include all atherogenic effects required for the development of plaques. Oxidized lipoproteins were found to be chemotactic to blood monocytes, facilitate the entry of lipoproteins by a cytotoxic endothelial injury, and give rise to smooth muscle cell proliferation.<sup>1,5,18</sup> Native low-density lipoprotein, in contrast, lacks all these atherogenic potentials.<sup>1,3,18</sup> Lipid peroxidation therefore may constitute an initiating and crucial step in the development of fatty streaks and plaques. This procedure in turn is absolutely dependent on at least low concentrations of iron or copper.<sup>1</sup> Catalytic iron converts poorly reactive into highly reactive radicals, including the  $\text{OH}\cdot$  ions (Haber-Weiss reaction<sup>19,20</sup>), and triggers an amplification of the oxidative burst in rough proportion to the actual iron stores. Once initiated, this process is autocatalytically expanded by a radical-induced release of new catalytic iron from tissue ferritin and hemin.<sup>21,22</sup> *In vitro*, sufficient amounts of iron strikingly shorten the time required for lipid peroxidation and foam cell formation to occur.<sup>20,22</sup> Iron deficiency *in vivo* has been reported to exert an inhibitory effect on the oxidative modification of lipoproteins, with the antioxidant defense lost during experimental dietary iron supplementation.<sup>23,24</sup>

Our study yielded a particularly strong relation between sonographically assessed carotid atherosclerosis and prominent iron stores in both genders, particularly when associated with hypercholesterolemia. Regarding

all major vascular risk markers, atherosclerosis is best described as a function of serum ferritin (tissue iron) and apolipoprotein B (Table 3). The independent relation between ferritin and atherosclerosis mainly occurred in the age range of 40 to 59 years. In the elderly ( $\geq 60$  years) the predictive significance was found to decrease parallel to that of apolipoprotein B, indicating a preferential involvement of lipid peroxidation in early atherosclerosis. Nutritional deficits, a "survival bias,"<sup>13</sup> and the comparatively low variation of ferritin in the elderly may contribute to the dilution of the predictive effect with advancing age. Serum iron concentrations distribute almost equally in subjects with and without detectable carotid atherosclerosis. This finding does not contradict our hypothesis, considering that lipid peroxidation is likely to occur in the sequestered microenvironment of the vessel wall rather than in the circulation, where a strong antioxidant defense is evident and generated oxidized lipoproteins are immediately swept up by the liver.<sup>1</sup> Besides, the assessment of serum iron is susceptible to hemolysis and has the disadvantage of a comparatively high measurement variability, which may possibly mask actual differences in the iron levels of subjects with and without atherosclerosis.

Two sources of potential confounding demand further close consideration. Apoferritin takes part in the systemic response to infectious stimuli, even though quantitative changes are low compared with those of actual acute-phase proteins. To rule out the possibility that the strong relation between carotid atherosclerosis and ferritin is confounded by higher morbidity and overrepresentation of chronic infection in the atherosclerotic group, separate statistical analyses were adjusted for erythrocyte sedimentation rate, blood leukocyte count, and fibrinogen. The decrease in the odds ratio associated with serum ferritin was low at 2%. A second approach carefully checked for smokers' bronchitis, moderate and severe heart insufficiency (New York Heart Association 2 to 3), and coronary and peripheral artery disease. Again, the predictive significance of ferritin was not essentially attenuated.

Ferritin concentrations could be in part an indicator (epiphenomenon) of the chronic immune stimulation evident in atherosclerotic lesions. However, several lines of evidence argue against the relevancy of this potential bias: (1) The main increase in median ferritin levels occurs during the third (men<sup>8</sup>) and sixth (women, Table 1) decades before the development of carotid atherosclerosis, whereas in the elderly ( $\geq 60$  years) quantitative changes emerged as low. (2) Parameters of immune stimulation, including serum levels of soluble interleukin-2 receptor, interferon gamma-inducible neopterin, and antibody titers to heat-shock protein 65, were strongly correlated with carotid atherosclerosis.<sup>25,26</sup> Neither of these attributes, however, showed a consistent association with serum ferritin. (3) The addition of these immune parameters to the logistic regression model (subsample) had no influence on the predictive significance of serum ferritin.

The strong association between atherosclerosis and ferritin, the dose-response relation, the consistency with previous epidemiological studies,<sup>4</sup> and the emerging biological plausibility all advocate a possible role of body iron in early atherogenesis.<sup>27,28</sup> The interpretation of our results, however, is limited by the cross-sectional



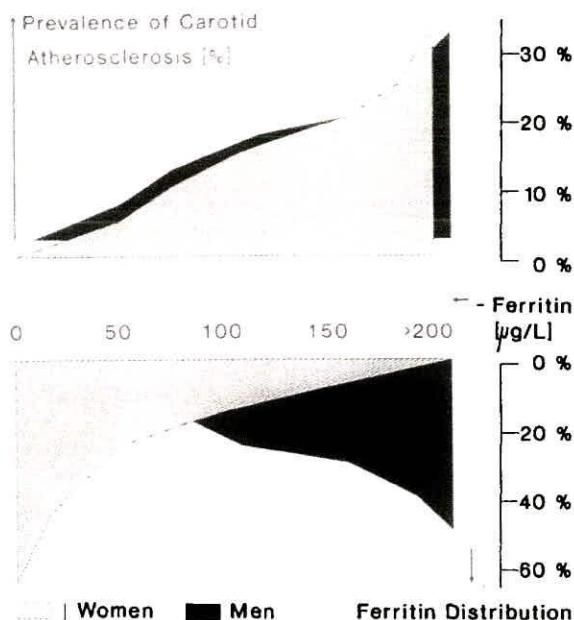


FIG 1. Graphs show prevalence of carotid atherosclerosis dependent on sex and serum ferritin concentrations.

study design, which does not permit us to draw a definite conclusion on causality.

#### Body Iron Stores: The Key for Understanding Sex Differences in Atherosclerotic Vascular Damage and Cardiovascular Diseases?

A role of body iron in atherogenesis would be particularly relevant to the hypothesis that iron deficiency protects premenopausal women from cardiovascular disease. In our population sample a comparison of the prevalence rates of carotid artery disease in both genders showed a 2:1 preponderance for men up to age 60. In the elderly a continuous narrowing in the sex differences occurred. These findings correspond considerably well with the sex-specific variations of cardiovascular diseases reported by the Framingham Study.<sup>29</sup> A convincing concept on the pathophysiological background of a protective female hormone status has not yet been devised. The effect of premenopausal and postmenopausal use of exogenous estrogen on cardiovascular disease is still controversial, with some authors reporting a disease promotion.<sup>30,31</sup> The Framingham Study revealed comparable excess incidences of cardiovascular illness in women with natural and surgical menopause, regardless of whether or not ovarian function and estrogen production were preserved. Accordingly, regular menstrual blood loss associated with a relative iron deficiency rather than endogenous hormone production may constitute the protective factor.<sup>15,16</sup>

Given the findings of the current study, at least a considerable part of the sex differences in atherosclerotic vascular disease may be explained by the variation in iron stores between genders. Indeed, men and women presenting with comparable serum ferritin levels and equal age had essentially the same prevalence of atherosclerotic lesions (Fig 1). Sex-specific differences in the overall prevalence of carotid artery disease correspond with the inverse distribution of serum ferritin concentrations in men and women (Fig 1). In a

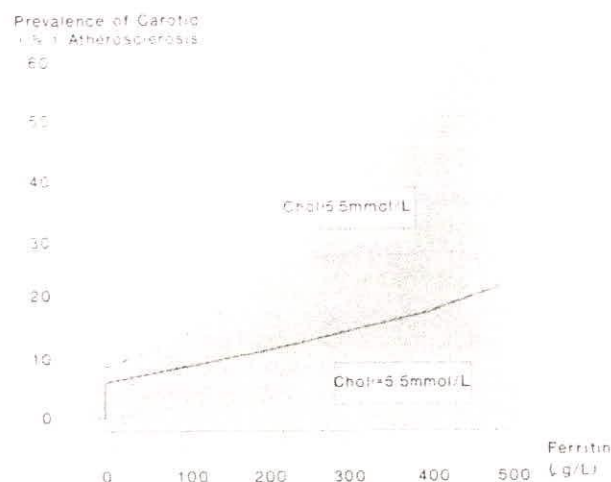


FIG 2. Graph shows prevalence of carotid atherosclerosis dependent on serum cholesterol and ferritin concentrations. Risk estimates were based on logistic regression equations fitted separately in the high-cholesterol ( $>5.5$  mmol/L) and low-cholesterol ( $\leq 5.5$  mmol/L) groups. (Age=50 years; average profile for other risk attributes.)

multivariate approach allowing for all relevant risk markers except ferritin, male sex was associated with an increased atherosclerosis risk (odds ratio, 2.4; 95% confidence interval, 1.3 to 4.3;  $P<.01$ ). When ferritin was added to the logistic regression model, sex differences nearly disappeared (odds ratio for male sex, 1.4; 95% confidence interval, 0.7 to 2.9;  $P=.4$ ).

#### Synergistic Effect of Iron Overload and Hyperlipidemia on the Manifestation of Carotid Atherosclerosis

Females with a heterozygote familial hypercholesterolemia show no excess risk of cardiovascular disease until after menopause; males, however, develop coronary artery disease soon after adolescence.<sup>32</sup> This conjecture suggests that the protective effect of the premenopausal status extends to the tolerance of hyperlipidemia. The current study showed that the atherogenicity of hypercholesterolemia depends on the tissue iron stores. This synergism fits particularly well into the hypothesis of an iron-catalyzed lipid peroxidation and is in line with a previous report on myocardial infarction<sup>4</sup> and on a synergistic effect of copper and hypercholesterolemia on the progression of carotid atherosclerosis.<sup>33</sup>

In Fig 2 the prevalence of carotid atherosclerosis is visualized as a function of cholesterol and ferritin. Several conclusions can be inferred from the logistic regression functions. (1) In the high-cholesterol group ( $>5.5$  mmol/L), ferritin imposes an "atherosclerosis risk" at levels generally regarded as normal. For example, the probability of carotid atherosclerosis for a man with a ferritin level of 156  $\mu\text{g/L}$  (median of men) is twice what it would be at a level of 21  $\mu\text{g/L}$  (median of premenopausal women). (2) Iron overload constitutes a strong indicator of carotid atherosclerosis, particularly when associated with hypercholesterolemia. (3) Subjects with a ferritin concentration at the lowest end of its observed range bear only a small "base risk" of atherosclerosis, even when cholesterol levels are extremely



high. In agreement, Murray and coworkers<sup>24</sup> found iron-deficient milk-drinking nomads uniquely free of coronary artery disease despite a fat intake that accounted for up to 70% of the total energy demand. These findings should inspire a new discussion on the definition of normal iron status and normal cholesterol levels. According to our data, both parameters are required for an accurate estimation of atherosclerosis risk. The iron status of healthy premenopausal women characterized by the absence of relevant iron stores without anemia should probably be regarded as physiologically normal.

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