Body Iron Stores and the Risk of Carotid Atherosclerosis

Prospective Results From the Bruneck Study

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Abstract

Background Iron release from tissue iron stores may accelerate lipid peroxidation by virtue of its pro-oxidant properties and thus promote early atherogenesis.

Methods and Results The present prospective survey addresses the potential association between serum ferritin concentrations and the 5-year progression of carotid atherosclerosis as assessed by ultrasonographic follow-up evaluations. The study population comprises a random sample of 826 men and women 40 to 79 years old. Serum ferritin was one of the strongest risk predictors of overall progression of atherosclerosis. The main part of this association appeared to act through modification of the atherogenic potential of LDL cholesterol (OR [95% CI]) for a 1-SD unit increase in ferritin at LDL levels of 2.5, 3.5, and 4.9 mmol/L. 1.55 [1.30 to 1.85], 1.77 [1.40 to 2.24], and 2.05 [1.50 to 2.80], P=0.0012 for effect modification. Changes in iron stores during the follow-up period modified atherosclerosis risk, in that lowering was beneficial and further iron accumulation exerted unfavorable effects. All these findings applied equally to incident atherosclerosis and the extension of preexisting atherosclerotic lesions. The significance of prominent iron stores in the development of carotid stenosis was clearly less pronounced. Finally, ferritin and LDL cholesterol showed a synergistic association with incident cardiovascular disease and death (n=59).

Conclusions The present study provided strong epidemiological evidence for a role of iron stores in early atherogenesis and suggests promotion of lipid peroxidation as the main underlying pathomechanism. This hypothesis could in part explain the sex difference in atherosclerotic vascular disease.

Key Words: atherosclerosis • myocardial infarction • follow-up studies • lipoproteins • population

Introduction

The possibility that iron overload plays a role in CVD was postulated by J.L. Sullivan in 1981.1 2 Prominent iron stores may facilitate ischemic illness by enhanced reperfusion injury and atherogenic properties. The latter mechanism, although possibly of outstanding significance, has not yet attracted adequate attention in epidemiological research. Actually, the postsecretory oxidative modifications of lipoproteins constitute a crucial step in lipid-induced atherogenesis.2 3 4 5 and tissue iron may be crucially involved in this process by virtue of its outstanding pro-oxidant properties.6 Interest in this hypothesis is stimulated by its capacity to explain the sex difference in atherosclerotic disease7 7 and the option of a preventive lowering of iron stores by repeated phlebotomy. The present prospective survey addresses the potential effects of iron stores on 5-year changes of carotid atherosclerosis in a large, randomly selected population.

Methods

Study Population

Features of the study design and survey area have been detailed previously.6 In brief, at the 1990 baseline, our study population comprised an age- and sex-stratified random sample of all inhabitants of Bruneck (Bolzano Province, Italy) 40 to 79 years old (125 women and 125 men in the fifth to eighth decades). A total of 93.6% participated, with data assessment completed in 919 subjects. During the follow-up period between summer 1990 and summer 1995, 62 individuals died (annual mortality, 1.3%). Population mobility within the Bruneck area was low, at 0.2% per year. One subject moved away and could not be traced (0.1%). The follow-up was 99.9% complete for clinical end points (n=918) and 96.5% complete for sonographic reassessment of survivors (n=826). An extensive clinical and laboratory screening identified 46 subjects with no adequate indirect estimates of body iron stores available, namely those with neoplastic, inflammatory, and liver diseases. The screening procedure has been detailed previously.7 All participants gave informed consent before entering the study.

Scanning Protocol and Definition of Ultrasound End Points

The complete set of pertinent information concerning the definition of sonographic end points has been published previously.6
The ultrasound protocol involves the scanning of the internal (bulbous and distal segments) and common (proximal and distal segments) carotid arteries on either side with a 10-MHz imaging probe and a 5-MHz Doppler probe. Atherosclerotic lesions were defined by two ultrasound criteria: (1) wall surface (protrusion into the lumen or roughness of the arterial boundary) and (2) wall texture (echoenhency). The maximum axial diameter of plaques, the vessel diameter in diastole, and Doppler frequency spectra were assessed in each of eight vessel segments (see above). An atherosclerosis summing score was calculated by addition of the maximum plaque diameters. Scanning was performed twice, namely in 1990 and 1995, by the same experienced sonographer, who was unaware of the subjects’ clinical and laboratory characteristics. Based on the follow-up evaluation, we assessed 5-year changes in the vascular status (overall progression) and differentiated three epidemiologically and etiologically different steps: (1) Incidence of atherosclerosis was defined by the occurrence of new plaques in previously normal segments. (2) Extension of atherosclerosis was coded present when the relative increase in the maximum plaque diameter between 1990 and 1995 exceeded the double measurement error of the method (distal internal carotid artery, 35%; bulbous, 30%; common carotid artery, 20%). (3) Development of stenosis between 1990 and 1995 was assumed whenever the extension criterion was met and a narrowing of the lumen >40% was achieved in the follow-up examination. The cutoff of 40% appeared to be a biological threshold in our population, at which marked changes in the growth kinetics of plaques (acceleration), in the risk profiles, and in the vascular remodeling process occurred, indicating a shift in the underlying pathomechanisms from continuous step-by-step mechanisms toward occasional disease progression by plaque thrombosis. In 4 subjects who underwent carotid endarterectomy, sonographic evaluations performed 10 days after surgery served as new baseline records.

8-mode and Doppler ultrasound are validated methods for quantifying vessel stenosis and the size of atherosclerotic plaques. To assess the reproducibility of these techniques in our survey, rescannings were performed in subsamples by the same sonographer (n=100) and by an independent (blinded) sonographer (n=50). When the assessment of plaque diameter was focused on, relative measurement errors that describe the interobserver error as a percentage of the pooled mean were generally low, at 10% (common carotid artery), 15% (bulbous), and 17.5% (distal internal carotid artery), as was intraobserver variability 12%, 14%, and 19%, respectively. The measurement error of estimates for diameter stenosis was assessed by the quadratic addition of the relative errors for the plaque and lumen diameter and amounted to 17.5% (bulbous). Finally, reproducibility of the ultrasound outcome categories used in the present analysis was excellent, as indicated by (weighted) k coefficients of >0.8.

Clinical Evaluation and End Points
All participants underwent a complete clinical examination with cardiological and neurological priority. Systolic and diastolic blood pressures were taken in a sitting position after ≥10 minutes of rest (mean of three independent measurements). The average number of cigarettes smoked per day was noted for each smoker and ex-smoker. A standardized oral glucose tolerance test (75 g glucose in 10% solution) was performed in all subjects except those on insulin therapy. Diabetes mellitus was coded present for subjects with fasting glucose levels >7.8 mmol/L (140 mg/dL) and/or a 2-hour value >11.1 mmol/L (200 mg/dL). Regular alcohol consumption was quantified in terms of grams per day. Fatal and nonfatal myocardial infarction were deemed confirmed when World Health Organization criteria for definite disease status were met. Bypass surgery and percutaneous transluminal coronary angioplasty were each performed in one subject. Both had experienced definite myocardial infarction between 1990 and 1995. Ischemic stroke and transient ischemic attack were classified according to the criteria of the National Survey of Stroke. The diagnosis of incident peripheral artery disease required a positive response to the Rose questionnaire, with the vascular nature of complaints documented by standard diagnostic procedures. Finally, angina pectoris was suspected on the basis of a positive Rose questionnaire or ECG and confirmed by exercise ECG or coronary angiography, if applicable. Self-reported data were verified from hospital records, death certificates, and information from general practitioners and supplemented by a thorough screening of the hospital database for diseases of interest to minimize recall bias and selective nonresponding.

Laboratory Methods
Blood samples were taken from the antecubital vein after subjects had fasted and abstained from smoking for at least 12 hours. In subjects with acute infectious disease, samples were drawn at an interval of at least 5 weeks. Serum ferritin was assessed with a fluorometric enzyme immunoassay (“sandwich assay”) with 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 μg/L, respectively (n=30). Serum iron was measured with a centrifugal analyzer with Ferrozine as chromogen. TS was calculated as the ratio of iron to transferrin. Apolipoproteins were measured with a nephelometric fixed-time method (apolipoprotein A-1, CV=5.7%; apolipoprotein B, CV=2.4%). HDL cholesterol was determined enzymatically (CHOD-PAP method, Merck, CV=2.2% to 2.4%). LDL cholesterol was calculated with the Friedewald formula except for subjects with triglyceride concentrations >4.52 mmol/L. Antithrombin III, fibrinogen, and other parameters were measured with standard procedures.

Statistics
Incidence rates of atherosclerosis were expressed as events per 100 person-years (incidence density). Strength and type of association between ferritin, LDL, and overall progression of atherosclerosis were assessed by logistic regression analysis. To assess effects of iron stores on various phases of atherosclerosis, separate equations were fitted for subjects without carotid atherosclerosis at the 1990 baseline (no atherosclerosis versus incident atherosclerosis during follow-up) and in those with preexisting lesions (no change versus extension of atherosclerosis versus occurrence of stenosis). In an attempt to obtain the most statistically significant scale in the logit, quintiles of given variables were modeled with indicator variables in separate analyses. Trends were estimated by visual inspection of plots of the logit (log odds) against the midpoints of quintiles and by application of orthogonal polynomials. Because these procedures indicated linearity in the logit for ferritin, LDL cholesterol, and Apob (1990 to 1995), continuously scaled variables were used. The dose-response type of association between ferritin and incident atherosclerosis was further visualized in a graph presenting crude and regression-standardized risks by ferritin quintiles (Fig 1A). The marginal method of the regression adjustment technique was used, because it does not rely on the rare-disease assumption. The logistic regression models were supplemented and confirmed by linear regression analyses that used the difference in the atherosclerosis summing score as a continuous outcome variable. Finally, crude and adjusted hazard ratios of incident cardiovascular disease and mortality were calculated by Cox models. The proportional-hazard assumptions were satisfied.

Figure 1. Crude and regression-standardized risks of incident atherosclerosis (1990 to 1995) according to ferritin quintiles. Figure documents dose-response relation between ferritin measurements and risk of early atherogenesis. n=476.
Results

Median and range of ferritin concentrations were 164 μg/L (6 to 1600 μg/L) in men, 22 μg/L (2 to 177 μg/L) in premenopausal women, and 73 μg/L (4 to 795 μg/L) in postmenopausal women. Body iron stores emerged as consistent from age 40 to 79 years in men but gradually increased in the decade after menopause in women at an average annual rate of 0.7 mg/kg body wt, corresponding to a Δferritin of 45 μg/L. A full description of the iron status was given elsewhere. Demographic features and levels of selected risk items are given in Table 1.

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Serum ferritin emerged as one of the strongest risk indicators of overall progression of carotid atherosclerosis when the analysis was adjusted for baseline vascular status (atherosclerosis score), age, sex, risk attributes, and alcohol consumption (OR, 1.50/SD unit; \( P = .0002 \)). Exclusion of subjects with spurious estimates of iron stores further increased the predictive significance of ferritin measurements (OR, 1.78/SD unit; \( P < .0001 \)). Fig 1 documents the dose–response type of association.

The present study permitted a differentiation of etiologically and epidemiologically distinct steps in atherogenesis: the predictive significance of ferritin measurements applied equally to the incidence of atherosclerotic lesions in subjects with previously normal vessels and to the extension of plaque size in individuals with preexisting lesions (Table 2). Both processes may be subsumed as early carotid artery disease and were a domain of lipid-induced atherogenesis. The main part of the relation between ferritin and these early steps in atherogenesis appeared to act through modification of the atherogenic potential of LDL (\( P < .01 \) and \( P < .05 \) for effect modification; Table 2). In contrast, occurrence of stenosis (advanced atherogenesis) did not rely to the same extent on iron stores or on hyperlipidemia. Instead, it emerged as a domain of procoagulant and hemodynamic factors. Results did not differ between sexes. Adjusted ORs of incident atherosclerosis in women were 1.82, 2.25, and 2.84 per SD unit increase in ferritin at LDL levels of 2.5, 3.6, and 4.9 mmol/L and in men, 1.80, 2.21, and 2.79, respectively (\( P < .8 \) for sex difference at given LDL levels). Corresponding ORs for the extension of preexisting atherosclerotic lesions in women and men amounted to 1.46, 1.65, and 1.89 and 1.66, 1.88, and 2.16, respectively (\( P < .3 \) for sex difference). Finally, analyses that excluded subjects with prevalent CVD and/or substituted apolipoprotein B for LDL cholesterol yielded nearly identical results.

<table>
<thead>
<tr>
<th>Table 2. Synergistic Association of Ferritin and LDL Cholesterol With Ultrasound and Clinical Measures in Subjects Free of Neoplastic, Inflammatory, Liver, and Autoimmune Disease (Follow-up 1990–1995)</th>
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</table>

For confirmation purposes, logistic regression models (individual-based approach) were supplemented with linear regression equations that used the difference in the atherosclerosis summing score as a continuous outcome variable (mean difference 1990 to 1995, 1.51 mm). This procedure did not rely on any categorization and offers a higher statistical power but ignores differential effects of risk factors in various stages of atherogenesis. It accords perfectly with the logistic regression models in that ferritin emerges as a strong and independent predictor of 5-year changes in atherosclerosis (\( P < .0001 \)) when the analysis was adjusted for the same set of covariates and restricted to subjects free of neoplastic, inflammatory, and liver diseases. The slope of the regression lines did not differ between sexes (regression coefficients for men and women, 0.0025 [95% CI, 0.0012 to 0.0038]) and 0.0027 [95% CI, 0.0022 to 0.0052], respectively. The association was more pronounced in subjects with high LDL cholesterol levels (>3.6 mmol/L [regression coefficient, 0.0030 [95% CI, 0.0012 to 0.0048]]) than in those with low or normal concentrations (<3.6 mmol/L [regression coefficient, 0.0016 [95% CI, 0.00 to 0.0032]]). A conventional level of significance for effect modification, however, was obtained only after exclusion of subjects with advanced atherogenesis (regression coefficients, 0.0033 versus 0.0015; \( P < .05 \) for effect modification). The latter group did not show a significant relation with LDL cholesterol and ferritin but had a considerable weight in the linear regression model for the usually prominent increase in the atherosclerosis summing score.

Serum iron was definitely unrelated to all ultrasound end points. TS was a weak risk predictor for the extension of preexisting plaques, regardless of whether it was treated as a continuous variable (OR, 1.18/SD unit; \( P = .11 \)) or categorized (TS > 60% versus TS < 60%; OR, 3.02, \( P = .09 \)).

A total of 28 subjects received statin therapy for primary preventive purposes. The risk benefit in this subgroup clearly exceeded that expected for the pure lipid-lowering effect (expected rate, -24% observed rate -57%), as reported previously. Effects of iron stores on atherogenesis were more pronounced in smokers than in nonsmokers or former smokers (\( P = .09 \) for effect modification). Other types of medication, vitamin supplements, and dietary factors were also investigated.
risk conditions did not modify the ferritin-atherosclerosis relation, or the number of given subjects was too low for meaningful analysis.

Next, we attempted to estimate the potential effects of 5-year changes in iron stores on the progression of atherosclerosis. For this purpose, subjects with infectious, neoplastic, and liver disease in either evaluation were excluded (n=95), because they clustered at the extreme margins of the ferritin scatter (1990 versus 1995) (Fig 2•). In the remaining population, changes in ferritin modified the risk of overall progression of carotid atherosclerosis, in that a lowering of serum concentrations was beneficial (OR [95% CI] for a 1-SD unit decrease in Δferritin at LDL levels of 2.5, 3.6, and 4.9 mmol/L: 0.85 [0.63 to 1.14], 0.75 [0.56 to 0.99], and 0.67 [0.48 to 0.94], respectively, P=.021 for effect modification). The merits of iron depletion applied equally to blood donors (n=42), subjects who experienced chronic or acute bleeding events (n=58), and the remainder (P=.65 for effect modification). Conversely, ongoing iron accumulation further enhanced risk estimates. This risk burden and the benefit afforded by iron depletion were of similar magnitude provided that (opposite) changes in ferritin levels are comparable.

During 4241 person-years of follow-up, we documented 59 clinical end points. Ferritin emerged as a risk predictor of cardiovascular death and nonfatal CVD, especially in subjects with hyperlipidemia (Table 2•). The study had insufficient power to settle the issue for various types of CVD. Further inclusion of subjects with incident angina pectoris in the clinical outcome group did not affect strength or type of association between ferritin and atherosclerosis.

Finally, we assessed age-adjusted incidence rates of carotid atherosclerosis by sex and menopausal status. We focused on the age range of 40 to 59 years and excluded subjects with no adequate indirect estimate of iron stores available, i.e., those with neoplastic, inflammatory, and liver disease. The incidence of carotid atherosclerosis in men (4.4/100 person-years) was more than three times that of premenopausal women (1.3), with postmenopausal women in between (natural menopause, 3.4; surgical menopause, 4.0). Notably, an elevated atherosclerosis risk after surgical menopause was observed in women with hysterectomy and oophorectomy (2.9) and slightly more pronounced in those with pure hysterectomy (5.5). The above sex and menopause differences almost disappeared when study subjects were further stratified according to their ferritin concentrations. Individuals with levels <50 μg/L (1990 and 1995) faced a generally low and those with levels ≥50 μg/L a high risk of incident atherosclerosis (Fig 3•): men (0.3 versus 4.8/100 person-years), premenopausal women (0.2 versus 4.5), postmenopausal women with natural menopause (1.6 versus 4.4), and those with surgical menopause (1.2 versus 5.1).

Discussion

Experimental and Biological Basis of Iron-Induced Lipid Peroxidation in Atherogenesis

Active ferrous iron (Fe^{2+}) is a highly effective promoter of lipid peroxidation in cell-free medium and amplifies the pro-oxidant capacity of vascular cells. In the subintimal space, the compartment in which lipid peroxidation may primarily occur, iron is available from various sources, including ubiquitous ferritin, heme deposits, iron compounds complexed with proteoglycans, receding macrophages (exocytosis of lysosomal iron), and damaged vascular cells. Fe^{2+} is released from ferritin and other scavengers in rough proportion to the amounts of iron stores by leukocyte-derived oxygen radicals or by oxygen-free redox systems such as hydroquinones in cigarette smoke. This process is stimulated by disturbances of oxygen supply in atherosclerotic lesions. Indeed, from active segments of human atherosclerotic lesions, ferrous iron was identified.
Biosaturation of oxygen supply in atheroelastic lesions results in the production of reactive oxygen species (ROS). Current evidence suggests that ROS are key players in the development of atherosclerosis.

Epidemiological Evidence of Iron–Catalyzed Lipid Peroxidation in Human Atherogenesis

In 1993, we were the first to demonstrate a synergistic association of hyperlipidemia and serum ferritin with prevalent atherosclerosis in the carotid arteries. The present evaluation confirmed and extended this observation in a prospective setting. Serum ferritin emerged as one of the strongest risk predictors of 5-year progression of carotid atherosclerosis. The main part of this association appeared to act through modification of the atherogenic potential of LDL (Table 2). Cigarette smoking has been reported to amplify lipid peroxidation by ferritin iron release and tend to enhance synergistic effects of iron and LDL cholesterol on atherogenesis in our survey (P<0.09 for effect modification). All these findings applied preferentially to early stages in carotid artery disease (initiation and extension of plaques), which is a domain of lipid–induced atherogenesis, and were of only minor relevance in the final chain of events leading to stenotic or occlusive vessel disease (plaque thrombosis). Serum ferritin emerged as a significant risk predictor for early atherogenesis even at low LDL cholesterol levels. Thus, further atherogenic properties may be proposed that act independently of a lipid pathway. As a potential clue, high tissue iron levels may promote smooth muscle cell proliferation (mitogenic properties).

Advantages of our study include the differentiation of epidemiologically distinct phases of atherogenesis, the accurate identification of subjects with spurious estimates of iron stores (namely, those with neoplastic, inflammatory, and liver disease), and the use of serum ferritin as a superior indicator of tissue iron. Comparatively, TS has a high analytic variability due to hemolysis, high measurement error, and extensive day-to-day variations (30% to 50%) and was clearly inferior to ferritin for the purpose of analyzing effects of iron stores on atherogenesis in our survey. Theoretically, ferritin could be a noncausal indicator of nutritional risk factors or an epiphenomenon of chronic immune stimulation evident in atherosclerosis, rather than a surrogate of body iron stores. Several lines of evidence, however, argue against this interpretation: (1) When the analysis was adjusted for meat and alcohol intake as the main source of dietary iron in our population and restricted to subjects not receiving iron supplements (98.5% of the population sample), results did not change appreciably. Changes in iron stores that did not originate from dietary measures (postmenopausal iron accumulation, blood donation, etc) prominently affected atherogenesis risk. (2) A variety of acute-phase reactants (C-reactive protein, fibrinogen, α1-antitrypsin, ceruloplasmin, etc) and markers of T-cell and macrophage activation were strongly correlated with carotid atherosclerosis, as was antibody titer to heat–shock protein 65. Neither of these attributes, however, showed a consistent relation with ferritin in subjects free of neoplastic, inflammatory, and liver disease.

Two recent cross-sectional surveys investigated the relation between ferritin and intima-media thickening (precursor of atherosclerosis) and reported negative findings. These studies do not necessarily contradict our results, because body iron stores may promote atherosclerosis at a stage beyond intima-media thickening.

Iron Stores and Cardiovascular Disease

A growing number of studies in this field have accumulated, but as yet no consensus has emerged. Most surveys were not designed primarily to analyze the key issues. Application of less appropriate indicators of iron stores and/or failure to exclude subjects with disease status, however, tend to bias evidential relations toward unity. Negative or inconsistent findings from these studies do not refute the hypotheses that prominent iron stores facilitate CVD or that iron depletion protects against CVD.

Most surveys that measured serum ferritin or attempted to quantify dietary heme-iron provided support for a risk factor status of prominent iron stores, including the present evaluation and surveys from Kuopio and Boston. A few exceptions exist. The predictive significance of ferritin did not fully extend to elderly populations, possibly because of high rates of interferring diseases, “survival bias,” and shifts in the relative significance of early toward advanced atherosclerosis.

Iron Stores and Sex Difference in Atherosclerosis

In premenopausal women, the incidence of atherosclerosis and CVD is less than half that of age-matched men. The female advantage is evident in severe hypercholesterolemia, which does not affect cardiovascular risk until after menopause. Depletion of iron stores by regular menstrual blood loss may be one source of protection in premenopausal subjects. Indeed, from a purely mathematical point of view, variation of iron stores between sexes could account for the sex difference in incident atherosclerosis observed in our survey. Iron–deficient men and women constitute a low-risk group, whereas subjects with prominent iron stores face a high-risk burden independent of sex and menopausal status (Fig 3). Likewise, the gradual increase in the incidence of atherosclerosis after menopause was best described as a function of iron accumulation. Notably, iron–deficient postmenopausal women partly retained a protective status against atherosclerosis (Fig 3), whereas women with pure hysterectomy (n=28), which caused accelerated iron accumulation but preserves hormone production, did not. Our survey does not rule out some kind of additional hormonal protection.

Modification of Atherosclerosis Risk After Changes in Iron Stores

Fluctuations in iron stores during follow-up, in terms of both iron depletion and accumulation, significantly affect atherosclerosis risk. The benefit afforded by a lowering of body iron was especially apparent in subjects with hyperlipidemia. Even though this kind of analysis cannot substitute for a controlled intervention study, it may be useful in optimizing the design of such a trial. Middle-aged healthy subjects without severe stenotic atherosclerosis at baseline may constitute an appropriate target group. Ultrasound end points are preferable for their high accuracy to monitor early stages of coronary artery disease.
Study Limitations
Several limitations of the present study afford further consideration: (1) Our survey enrolled an entirely white population and covered the age range between 40 and 84 years. Inference of results to other race or age groups demands caution. (2) As anticipated, ferritin could theoretically be a noncausal marker of the yet unknown actual risk condition. (3) Population surveys are subject to selective nonresponding and measurement error. Owing to the high participation and follow-up rates and the low amount of missing data in our study, relevancy of selection bias may be low, although not negligible. The ultrasound outcome categories in our study emerged as highly reproducible (k coefficients for remeasurements, >0.8). Unavoidable measurement errors of independent variables in turn may be expected to bias evident relations toward unity rather than to create spurious findings.

Conclusions
The present results are compatible with the hypothesis that iron-induced lipid peroxidation is crucially involved in the early steps of human atherogenesis. Both ferritin and LDL cholesterol levels are necessary to accurately estimate the risk of progressive atherosclerosis in given subjects. The iron status of premenopausal women characterized by iron depletion without anemia may possibly be regarded as physiologically normal for humans. General recommendations on lowering of iron stores for prevention of CVD and identification of target groups for such therapy await further results from intervention trials.

Selected Abbreviations and Acronyms
CI = confidence interval
CV = coefficient of variation
CVD = cardiovascular disease
OR = odds ratio
TS = transferrin saturation

Footnotes
1 Members of the Bruneck Study Group are listed in the "Appendix." ☑

Appendix 1
The Bruneck Study Group included Martin Oberhollenzer, Stefan Brandt, Paula Eder, Klaus Oberlechner, Harald Steiner, and Arno Gasperi, Department of Internal Medicine, Bruneck Hospital, Italy; Agnes Mair and Peter Santer, Department of Laboratory Medicine, Bruneck Hospital, Italy; Gregor Rungger and Franz Spogler, Department of Neurology, Innsbruck University Hospital, Austria; Elmar Jarosch and Maria Schober, Department of Laboratory Medicine, Innsbruck University Hospital, Austria; Christian Wiedermann, Department of Internal Medicine, Innsbruck University Hospital, Austria; and Enzo Bonora and Michele Muggeo, Department of Endocrinology and Metabolism, Verona University Hospital, Italy.

Received March 10, 1997; revision received July 17, 1997; accepted July 20, 1997.

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