

Association of Increased Ferritin with Premature Coronary Stenosis in Men

MEHRAN HAIDARI,^{1*} EBRAHIM JAVADI,² ARASHMIDOS SANATI,³ MEHRDAD HAJILOOI,¹
and JAFAR GHANBILI¹

Background: Body iron status has been implicated in atherosclerotic cardiovascular disease. The main hypothesis is that high iron status is associated with increased oxidation of LDL. We investigated the potential role of ferritin as an additional risk factor promoting atherosclerosis among a young population with coronary artery disease (CAD).

Methods: Four hundred consecutive patients (218 males, 182 females) referred for diagnostic coronary angiography were examined, and risk factors for CAD, lipids, C-reactive protein (CRP), and ferritin concentrations were recorded for all participants.

Results: Ferritin was higher in the male patients with CAD (121 $\mu\text{g/L}$; range, 56–258 $\mu\text{g/L}$) than in the men without significant CAD (73 $\mu\text{g/L}$; range, 32–138 $\mu\text{g/L}$; $P < 0.002$). Multiple logistic regression analysis, after adjustment for the established coronary risk factors, showed ferritin as an independent discriminating risk factor for CAD ($P < 0.01$). Men in the highest quartile of ferritin had an odds ratio (OR) of 1.62 [95% confidence interval (95% CI), 1.12–2.42; $P < 0.01$] compared with men in the lowest quartile of ferritin. The association between ferritin and CAD was more pronounced in male patients ≤ 50 years (OR = 2.65; 95% CI, 1.35–5.51; $P < 0.003$). Ferritin was significantly higher in diabetic male patients in comparison with nondiabetic male patients [168 $\mu\text{g/L}$ (range, 74–406 $\mu\text{g/L}$) vs 106 $\mu\text{g/L}$ (range, 44–221 $\mu\text{g/L}$), respectively; $P < 0.002$]. No association was observed between ferritin and CAD among the female patients.

Conclusion: Our data suggest that increased ferritin might be an independent predictor of premature CAD in male Iranian patients.

© 2001 American Association for Clinical Chemistry

The established risk factors of coronary artery disease (CAD)⁴, such as age, male sex, high serum cholesterol, smoking, hypertension, and glucose intolerance, do not account for the overall risk of CAD (1). Therefore, it has been hypothesized that the assessment of novel markers helps to identify persons prone to premature atherosclerosis. Age- and sex-related increases in iron stores have been linked to the pathogenesis of several common diseases, including atherosclerosis (2). Iron stores increase to concentrations above the physiologic requirements with aging, and such increased concentrations have been implicated in the pathogenesis of atherosclerosis. Interest in this hypothesis is stimulated by its capacity to explain the sex difference in atherosclerotic diseases and the option of preventive lowering of iron stores by repeated phlebotomy. Iron catalyzes the formation of reactive oxygen species through the Fenton and Haber–Weiss reactions (3). Free radicals cause lipid peroxidation, leading to the modification of LDL at the molecular level, facilitating its deposition and leading to the formation of atherosclerotic plaque (4).

Divergent information is available on the relationship between body iron stores and CAD. It has been shown that the concentrations of body iron stores are a strong predictor of CAD in eastern Finnish, men (5). Routine voluntary blood donation is associated, epidemiologically, with reduced coronary risk (6, 7). Salonen et al. (8) phlebotomized men over age 50 to reduce their body iron stores and demonstrated significantly decreased susceptibility of LDL to ex vivo oxidation. Furthermore, Kiechl et al. (9) found a highly significant correlation between

¹ Cardiovascular Research Center, and Departments of ² Biochemistry and ³ Cardiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

*Address correspondence to this author at: McMaster Bldg., Room 4011c, The Hospital for Sick Children, 555 University Ave., Toronto, Ontario, M5G 1X8 Canada. Fax 416-813-6257; e-mail mhaidari@sickkids.on.ca.

Received February 28, 2001; accepted June 15, 2001.

⁴ Nonstandard abbreviations: CAD, coronary artery disease; TC, total cholesterol; TG, triglyceride; HDL-C and LDL-C, HDL- and LDL-cholesterol; CRP, C-reactive protein; OR, odds ratio; and CI, confidence interval.

serum ferritin concentration and pathologic carotid artery wall thickening in a longitudinal cohort study. However, several epidemiologic studies investigating the association between high body iron stores and risk of cardiovascular disease in humans have not provided positive results (10–15).

The geographic distribution of CAD incidence over the last 20 years has changed, with a significant decline in industrialized and an increase in developing countries (16–18). Moreover, the characteristics of coronary risk factors among developing countries also differ in many respects from populations in developed countries (16, 19, 20). However, data regarding the distribution of CAD risk factors among these populations are very scarce in the literature.

To investigate the value of ferritin in coronary risk assessment in a population with a high prevalence of atherosclerosis (21), we evaluated the relationship between ferritin and the presence of CAD in the Iranian population.

Materials and Methods

SUBJECTS

The study population consisted of 218 men and 182 women who were undergoing coronary angiography at Shariaty Hospital of Tehran University of Medical Sciences from June to October 1999. The indications for angiography were suspicion of CAD or preoperative screening for CAD in subjects with valvular disease. All subjects had given written informed consent.

Individuals with a recent history of acute myocardial infarction or percutaneous transluminal coronary angioplasty were not included. Individuals with concomitant inflammatory diseases, cancer, or other diseases possibly associated with an acute-phase reaction, and those who took iron supplements were also excluded. Coronary angiographies were performed according to the standard Judkins technique (22). The patients were classified as CAD+ if one or more coronary arteries had a stenosis $\geq 50\%$ and as CAD- if there was no significant stenosis ($\leq 50\%$) in any artery. Coronary arteriograms were reviewed by a panel of three cardiologists with no prior knowledge of the clinical history or laboratory data for the patients. Prior medical histories and personal characteristics and habits were obtained from participants via a questionnaire. Hypertension was defined as resting systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg. The average of the last two of three seated blood pressure readings was used to detect hypertension. Cigarette smoking was defined as ever vs never smoked. Current smoking was defined as smoking cigarettes within the past month. Diabetes was defined as fasting blood glucose >7.8 mmol/L or a diagnosis of diabetes needing diet or drug therapy.

Blood samples were obtained after a 12-h fast on the day before angiographic procedure and total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), ferritin

and high-sensitivity C-reactive protein (CRP) concentrations were determined for all patients. Serum samples were separated immediately after collection by centrifugation at 2000g for 15 min and stored at -80 °C until analysis.

LABORATORY MEASUREMENTS

Cholesterol and TG concentrations were measured enzymatically (Kone Diagnosis) on a Kone specific automated analyzer. HDL-C was determined after precipitation of apolipoprotein B-containing particles by phosphotungstic acid-MgCl₂. LDL-cholesterol (LDL-C) was estimated using the Friedewald equation (23). Ferritin concentrations were measured with a RIA (Amersham International) using a multigamma Model 1261 gamma counter (LKB Wallac). The between-batch CV was 5.5% and 4.1% for ferritin concentrations of 60 and 180 $\mu\text{g/L}$, respectively ($n = 25$). Serum CRP concentrations were measured by an ultrasensitive latex-enhanced immunoturbidimetric method (Randox Laboratory Ltd). The detection range for CRP by this method was 0.0–15 mg/L. The method was standardized against the international reference preparation, CRM 470 (24). The run-to-run imprecision for CRP at concentrations of 1 and 7 mg/L was 4.8% and 3.7%, respectively. All biochemical measurements were carried out without knowledge of the angiographic findings.

STATISTICAL ANALYSIS

All data are presented as the mean \pm SD, with the exception of ferritin, which is presented as the median and 20th–80th percentiles. Statistical analyses were performed with SPSS for Windows, Ver. 10 (SPSS Inc). The Kolmogorov–Smirnov test of normality was used to test whether the distribution of variables followed a gaussian pattern. Because the ferritin frequency distribution was skewed rightward and did not follow a gaussian distribution ($P < 0.0001$), a natural logarithmic transformation was applied to normalize the data for analysis. The discrete variables were compared by the Pearson χ^2 test, and the Student *t*-test was used to compare the continuous variables. The Pearson correlation test was used to assess correlation between the continuous variables. To determine factors independently correlated with CAD, multivariate analysis was carried out by multiple logistic regression analysis using the forward stepwise likelihood ratio method. Adjusted odds ratios (ORs) for the exposure variables and CAD were obtained. All baseline variables related to CAD with a *P* value < 0.1 in univariate analysis were included in the model. All *P* values were two-tailed, and values < 0.05 were considered statistically significant. All confidence intervals (CIs) were calculated at the 95% level.

Results

Of the total 400 patients who were examined by angiography, 260 were CAD+ and 140 were free of significant CAD (CAD-). The indications for angiography in the

CAD- group were suspicion of CAD (85%) or preoperative screening for valvular diseases (15%). In the CAD- group, 16% had a 25–50% stenosis and 84% had <25% stenosis or irregularities in the coronary arteries. The indications for angiography in the CAD+ group were suspicion of CAD (99%) or preoperative screening for valvular diseases (1%).

The basic characteristics of study the participants are shown in Table 1. The analysis in the total population revealed that TC, TG, and ferritin concentrations and the ratio of TC to HDL-C (TC/HDL-C) were higher in the coronary patients, whereas CRP concentrations demonstrated no significant difference between the two groups. The study participants were stratified according to the quartiles of CRP concentrations. There was no significant difference in the percentage of individuals with CRP above the 75th percentiles among CAD- and CAD+ patients of both genders. Ferritin concentrations were higher in the men relative to the women [109 µg/L (range, 45–254 µg/L) vs 77 µg/L (32–170 µg/L), respectively; $P < 0.0001$]. Ferritin was >200 µg/L in 14% of female participants, whereas 28% of the male participants had ferritin concentrations ≥ 200 µg/L. To adjust the data for the possible effects of confounding factors, the variables were fitted to a multivariate logistic regression model using the stepwise likelihood ratio method. Age ($P < 0.0001$), sex ($P < 0.0001$), and TC ($P < 0.001$) were the only independent discriminating risk factors that showed significant association with CAD in the total population.

Among the women, no significant difference was found between the ferritin concentrations in the CAD+ and CAD- groups. The postmenopausal women showed higher ferritin relative to the premenopausal women [96 µg/L (range, 44–191 µg/L; $n = 42$) vs 37 µg/L (range, 6–82 µg/L; $n = 140$), respectively; $P < 0.0001$]. The subgroup analysis for the postmenopausal women re-

vealed no significant difference in the ferritin concentrations of the CAD+ and CAD- groups. A significant positive correlation was found between age and ferritin concentration in the women ($r = 0.391$; $P < 0.0001$).

The univariate analysis for men revealed that ferritin, TC, LDL-C, and TC/HDL-C were greater in the coronary patients relative to CAD- group. There was no significant difference in the CRP concentrations of the CAD+ and CAD- groups. When male CAD- subjects with 25–50% of stenosis ($n = 14$) were omitted from analysis, a more pronounced difference between the ferritin concentrations of CAD+ and CAD- individuals was found [CAD+, 121 µg/L (range, 56–258 µg/L; $n = 164$); CAD-, 71 µg/L (range, 26–181 µg/L; $n = 40$), respectively; $P < 0.001$]. The logistic regression model (Table 2) after controlling for age, diabetes, TC, LDL-C, TC/HDL-C, and CRP demonstrated ferritin as an independent determinant of CAD ($P < 0.01$). The OR for the highest compared with the lowest quartile of ferritin was 1.62 (95% CI, 1.12–2.42; $P < 0.01$). The subgroup analysis for male patients ≤ 50 years of age demonstrated a twofold difference in the ferritin concentrations of coronary patients relative to the CAD- group [137 µg/L (range, 75–248 µg/L; $n = 55$) vs 68 µg/L (range, 37–154 µg/L; $n = 24$), respectively; $P < 0.001$]. In this subgroup, the men in the highest quartile of ferritin had an OR of 2.65 (95% CI, 1.35–5.51) compared with the men in the lowest quartile of ferritin ($P < 0.003$). The male coronary patients ≥ 50 years of age had higher ferritin concentrations compared with the male individuals ≥ 50 years of age with no significant CAD [115 µg/L (range, 45–269 µg/L; $n = 109$) vs 77 µg/L (range, 25–222 µg/L; $n = 30$), respectively; $P = 0.049$]. However, when the predictors of angiographically significant CAD were assessed using a logistic regression model, the association between ferritin and

Table 1. Basic characteristics of study participants.

	Total population		Men		Women	
	CAD+	CAD-	CAD+	CAD-	CAD+	CAD-
n	260	140	164	54	96	86
Age, years	57 ± 10	52 ± 12 ^a	56 ± 11	50 ± 13 ^a	58 ± 9	53 ± 12 ^a
BMI, ^e kg/m ²	26.3 ± 3.8	26.7 ± 5 ^b	26.6 ± 4.7	26.8 ± 4.5 ^b	27 ± 4.3	27.2 ± 5.1 ^b
Diabetes, n (%)	56 (21)	18 (13) ^c	35 (24)	7 (13) ^c	22 (23)	12 (13.5) ^c
Hypertension, n (%)	114 (43)	45 (33) ^b	55 (34)	17 (32) ^b	57 (61)	28 (33) ^a
Smoking, n (%)	86 (33)	26 (19) ^d	74 (43)	21 (40) ^b	11 (12.5)	6 (6.5) ^b
TC, mmol/L	6.37 ± 1.28	6.03 ± 1 ^d	6.15 ± 1.15	5.5 ± 1.26 ^a	6.7 ± 1.41	6.33 ± 1.55 ^c
LDL-C, mmol/L	4.24 ± 1.09	4.13 ± 1.37 ^b	4.15 ± 1.02	3.63 ± 1.24 ^d	4.44 ± 1.18	4.40 ± 1.37 ^b
HDL-C, mmol/L	0.953 ± 0.22	0.976 ± 0.27 ^b	0.91 ± 0.19	0.90 ± 0.22 ^b	1.03 ± 0.28	1.01 ± 0.285 ^b
TC/HDL-C	7.0 ± 6.35	6.35 ± 2.13 ^c	7.05 ± 1.8	6.43 ± 2.1 ^c	6.92 ± 2.31	6.59 ± 2.17 ^b
TG, mmol/L	2.57 ± 1.51	2.26 ± 1.14 ^c	2.47 ± 1.4	2.19 ± 1.5 ^b	2.76 ± 1.69	2.28 ± 1.35 ^c
Ferritin, ^f µg/L	101 (46–230)	71 (27–162) ^a	121 (56–258)	73 (32–188) ^a	79 (38–176)	76 (26–165) ^b
CRP, mg/L	2.21 ± 1.25	2.15 ± 1.3 ^b	2.11 ± 1.25	2.05 ± 1.34 ^b	2.40 ± 1.22	2.24 ± 1.31 ^b

^{a-d} Compared with CAD+ group: ^a $P < 0.001$; ^b not significant; ^c $P < 0.05$; ^d $P < 0.01$.

^e BMI, body mass index.

^f Median (20th–80th percentiles).

Table 2. Multivariate logistic regression analysis between CAD+ and CAD- groups of men and women.

	Men		Women	
	OR	95% CI	OR	95% CI
Age	1.06 ^a	1.02–1.09	1.04 ^a	1.02–1.08
Diabetes	1.11 ^b	0.44–2.8	1.08 ^b	0.51–2.3
Hypertension	1.39 ^b	0.65–2.9	0.48 ^c	0.26–0.88
TC	1.02 ^a	1.00–1.04	1.00 ^b	0.99–1.01
LDL-C	0.96 ^b	0.85–1.08	1.00 ^b	0.93–1.08
TC/HDL-C	0.77 ^b	0.42–1.41	0.95 ^b	0.66–1.46
TG	1.00 ^b	0.99–1.00	1.00 ^b	0.99–1.00
CRP	1.09 ^b	0.82–1.46	1.06 ^b	0.82–1.37
Ferritin	1.62 ^d	1.12–2.42	0.91 ^b	0.62–1.34

^a $P < 0.001$.
^b Not significant.
^c $P < 0.01$.
^d $P < 0.05$.

significant CAD lost its statistical significance in this subgroup ($P = 0.289$).

The subgroup analysis for the male patients ≥ 60 years of age revealed no significant differences between the ferritin concentrations of the coronary patients and the CAD- group [112 $\mu\text{g/L}$ (range, 41–245 $\mu\text{g/L}$; $n = 79$) vs 79 $\mu\text{g/L}$ (range, 27–299 $\mu\text{g/L}$; $n = 17$), respectively; $P = 0.384$]. Diabetes mellitus was shown to be a potential confounder affecting the relationship between serum ferritin and CAD. Therefore, all logistic regressions were repeated omitting the data from diabetic patients. The omission of these data did not affect the observed association between ferritin and CAD. No significant correlation existed between ferritin and the measured lipid markers, CRP, and age among the male patients.

The ferritin concentrations were higher in the diabetic patients (109 $\mu\text{g/L}$; range, 45–328 $\mu\text{g/L}$; $n = 74$) in comparison with the nondiabetic individuals (92 $\mu\text{g/L}$; range, 38–190 $\mu\text{g/L}$; $n = 326$; $P < 0.01$). This difference was more pronounced among the male subpopulation [168 $\mu\text{g/L}$ (range, 74–406 $\mu\text{g/L}$; $n = 42$) vs 106 $\mu\text{g/L}$ (range, 44–221 $\mu\text{g/L}$; $n = 176$), respectively; $P < 0.01$]. The median ferritin concentration in the male diabetic patients with CAD ($n = 35$) tended to be higher relative to the male diabetic patients without CAD ($n = 7$), but the difference was not statistically significant [185 $\mu\text{g/L}$ (range, 73–421 $\mu\text{g/L}$) vs 129 $\mu\text{g/L}$ (range, 34–494 $\mu\text{g/L}$), respectively].

The percentage of individuals in the highest quartile of ferritin was almost twofold higher in the diabetic male patients relative to the nondiabetic male patients ($P < 0.003$).

Discussion

Our study demonstrated a significant association between serum ferritin and CAD in an Iranian male population with angiographically defined CAD. This association remained significant after adjustment for age, CRP, diabe-

tes, and other coronary risk factors and was more evident in the subgroup of male patients ≤ 50 years of age.

Ferritin is a high-capacity protein that serves as the body's storage site for iron, and because serum ferritin concentrations are directly proportional to intracellular ferritin concentrations, it is considered the best clinical measure of body iron stores (25). The association between high iron stores and CAD was first suggested by Sullivan (26) to explain the sex difference in CAD risk. This hypothesis offers a possible explanation for a wide range of phenomena, including not only the sex difference, but also the protection effects of aspirin, fish oil, and cholestyramine as well as the disease-promoting effect of oral contraceptives (27). Regular daily ingestion of even small doses of aspirin, if continued for months or years, may cause a substantial decrease in iron stores (28). A similar mechanism may explain the effect of fish oil consumption on the incidence of CAD. Moderate fish oil intake is associated with significantly decreased bleeding time (29). Small increases in bleeding time could lead to significant loss of stored iron from occult gastrointestinal blood loss if such increases are sustained. Cholestyramine inhibits the absorption of iron, and its prolonged feeding to rats is associated with a dose-dependent depletion of stored iron (30). It is well known that the use of oral contraceptives diminishes menstrual blood flow and increases stored iron (31).

Epidemiologic studies have found a positive relationship between body iron stores and CAD (5, 9, 32–34). Moreover, based on the experimental findings that iron concentrations in the organism are associated with accelerated production of free radicals and increased lipid peroxidation (35, 36), the hypothesis seems plausible. However, several prospective epidemiologic studies found no association between iron stores and CAD (10–15). The lack of consistency in the epidemiologic studies is probably explained by the large variability in estimates of iron stores and iron intake and by the diversity of study outcomes (2, 37). The ethnic diversity in the manifestations of CAD is another potential factor that merits consideration when interpreting the data relating ferritin and CAD. Large differences in incidence and mortality for CAD between countries have been widely documented (18, 38). At least two hypotheses can be put forward to explain this variability between countries: (a) the incidence of CAD might essentially depend on the prevalence of cardiovascular risk factors in the populations (39, 40), or (b) it might also be determined by the strength of associations between cardiovascular risk factors and CAD in countries (41). Cross-cultural prospective studies have demonstrated that at a cholesterol concentration of ~ 5.43 mmol/L, the CAD mortality rate varies from 5% in Japan to 15% in northern Europe (38). Recently, Rasmussen et al. (42), based on a prospective study, reported that individuals carrying the hemochromatosis gene (*HFE*) C282Y mutation might be at increased risk of CAD. This report is consistent with previous studies (43, 44) and

indicates that at least some inconsistency between different prospective studies for ferritin may be attributable to differences in genetic factors among different countries.

Early manifestation of CAD is a characteristic that is common among developing countries (16, 19, 45) and, according to our clinical experiences, is also frequent in the Iranian population. In the present study, 34% of the male patients with CAD were ≤ 50 years of age. The association between ferritin and CAD was more pronounced in the male patients ≤ 50 years than in the men ≥ 50 years. These data indicate that measurement of ferritin improves the risk assessment of CAD among Iranian men with premature atherosclerosis. Our study demonstrated no significant association between ferritin and CAD in the male patients ≥ 60 years of age. Aronow (10) also reported that serum ferritin is not a risk factor for CAD in men and women ≥ 62 years of age. The predictive significance of ferritin did not fully extend to elderly populations, possibly because of high rates of interfering diseases and "survival bias" in this subpopulation.

In evaluating the relationship between iron status and the risk of CAD, it is important to rule out non-iron-related factors that can influence the biochemical measures of iron status. One important factor of this type is inflammation. Serum ferritin increases with inflammation; consequently, the association between serum ferritin and CAD could be confounded by inflammation. This may explain the failure of some previous studies to find an association between ferritin and CAD (46). In the present study, the adjustment for high-sensitivity CRP, a sensitive marker of inflammation, did not affect the results.

The analysis for women revealed no significant association between ferritin and CAD. Further investigations are required to confirm this observation. The lack of association may indicate that a higher ferritin concentration is required to contribute to the progression of atherosclerotic lesions in women. Salonen et al. (5) demonstrated that a ferritin concentration ≥ 200 $\mu\text{g/L}$ was associated with a 2.2-fold increase in the risk of acute myocardial infarction in men. This ferritin concentration was observed in only 14% of the female participants, whereas 28% of the men had ferritin concentrations ≥ 200 $\mu\text{g/L}$. In addition, the effects of other postulated factors associated with the female gender might contribute to the low ferritin concentrations in women. The cardioprotective effect of estrogens in women is well established from epidemiologic and clinical studies (47). The increase in lipid peroxide concentrations in the serum and liver of female mice after ovariectomy provided evidence for antioxidant activities of female hormones. This increase was abolished by the administration of female hormones (48). However, the Heart and Estrogen/Progestin Replacement Study (HERS) trial, a prospective secondary prevention study, did not show a beneficial influence of estrogen replacement therapy on cardiovascular mortality in postmenopausal women (49). This may indicate that

alternative treatment strategies for women at coronary risk after menopause need to be evaluated, especially in the light of recent findings of Wassmann et al. (50), which indicate that estrogen deficiency in spontaneously hypertensive rats leads to increased vascular free-radical production via increased vascular angiotensin type 1 receptor overexpression and produces endothelial dysfunction.

The major cause of morbidity and mortality of patients with diabetes is macrovascular disease (51). The mechanisms by which diabetes accelerates atherosclerosis are not well understood. Diabetic patients often have several cardiovascular risk factors, including obesity, high blood pressure, microalbuminuria, and dyslipidemia. However, these risk factors are assumed to account for only $\sim 50\%$ of coronary heart disease in diabetic patients (52, 53). Diabetic patients may be exposed to increased oxidative stress. Higher concentrations of lipid peroxides have been detected in the plasma and lipoprotein fractions of diabetic patients (54, 55). LDL isolated from diabetic patients appears to be more susceptible to in vitro oxidation (56, 57). Furthermore, antioxidant mechanisms may also be compromised. A variety of defects in plasma antioxidant status have been described in diabetes, including reduced ascorbate (58), urate (59), and vitamin E (60). Our data indicated that diabetic male patients had higher ferritin concentrations relative to nondiabetic individuals. This finding is consistent with previous studies (61–63) and supports the hypothesis that an imbalance between oxidative stress and antioxidant status exists in diabetic patients.

The potential limitations of this study merit consideration. Our results share the limitations of cross-sectional, observational studies. We evaluated association, not prospective prediction or causation. The study of CAD using CAD- patients referred for angiography for clinical reasons is attractive because a large difference in the extent of coronary atherosclerosis is identified. However, this design has special limitations. The CAD- individuals who were referred for angiography because of suspected CAD were likely to have more risk factors and differ in unknown ways from free-living CAD- populations; for example, contrary to findings of many other studies, CRP, HDL-C, and hypertension are unrelated to CAD in these patients. Therefore, caution should be exercised in interpreting the findings of this study. The definition of CAD patients as having a $>50\%$ obstruction in any coronary artery remains arbitrary. The absence of $>50\%$ stenosis in the coronary arteries does not preclude controls from having atherosclerosis. In spite of having normal angiograms, some individuals in the CAD- group may have had hemodynamically insignificant atherosclerotic plaques that might be detected by intravascular ultrasound.

These considerations would more likely underestimate than overestimate the strength of ferritin as a risk factor. However, the findings from the current study may be applied to individuals who are referred for angiography,

but cannot be extrapolated directly to the general population.

In conclusion, the present study suggests that a high stored iron concentration, as assessed by serum ferritin, is a strong and independent risk factor for premature CAD in the male Iranian population.

We gratefully acknowledge the excellent editorial assistance of Taryne M. Chong.

References

- Braunwald E. Shattuck lecture—cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N Engl J Med* 1997;337:1360–9.
- Sempos CT, Looker AC, Gillum RF. Iron and heart disease: the epidemiologic data. *Nutr Rev* 1996;54:73–84.
- Wardman P, Candeias LP. Fenton chemistry: an introduction. *Radiat Res* 1996;145:523–31.
- Navab M, Fogelman AM, Berliner JA, Territo MC, Demer LL, Frank JS, et al. Pathogenesis of atherosclerosis. *Am J Cardiol* 1995;76:18C–23C.
- Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 1992;86:803–11.
- Meyers DG, Strickland D, Maloley PA, Seburg JK, Wilson JE, McManus BF. Possible association of a reduction in cardiovascular events with blood donation. *Heart* 1997;78:188–93.
- Tuomainen TP, Salonen R, Nyyssonen K, Salonen JT. Cohort study of relation between donating blood and risk of myocardial infarction in 2682 men in eastern Finland. *BMJ* 1997;314:793–4.
- Salonen JT, Korpela H, Nyyssonen K, Porkkala E, Tuomainen TP, Belcher JD, et al. Lowering of body iron stores by blood letting and oxidation resistance of serum lipoproteins: a randomized crossover trial in male smokers. *J Intern Med* 1995;237:161–8.
- Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F. Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. *Circulation* 1997;96:3300–7.
- Aronow WS. Serum ferritin is not a risk factor for coronary artery disease in men and women aged > or = 62 years. *Am J Cardiol* 1993;72:347–8.
- Sempos CT, Looker AC, Gillum RF, Makuc DM. Body iron stores and the risk of coronary heart disease. *N Engl J Med* 1994;330:1119–24.
- Moore M, Folsom AR, Barnes RW, Eckfeldt JH. No association between serum ferritin and asymptomatic carotid atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. *Am J Epidemiol* 1995;141:719–23.
- Manttari M, Manninen V, Huttunen JK, Palosuo T, Ehnholm C, Heinonen OP, Frick MH. Serum ferritin and ceruloplasmin as coronary risk factors. *Eur Heart J* 1994;15:1599–603.
- Rauramaa R, Vaisanen S, Mercuri M, Rankinen T, Penttila I, Bond MG. Association of risk factors and body iron status to carotid atherosclerosis in middle-aged eastern Finnish men. *Eur Heart J* 1994;15:1020–7.
- Solymoss BC, Marcil M, Gilfix BM, Gelinac F, Poitras AM, Campeau L. The place of ferritin among risk factors associated with coronary artery disease. *Coron Artery Dis* 1994;5:231–5.
- Reddy KS, Yusuf S. Emerging epidemic of cardiovascular disease in developing countries. *Circulation* 1998;97:596–601.
- Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* 1997;349:1269–76.
- Tunstall-Pedoe H, Kuulasmaa K, Amouyel P, Arveiler D, Rajakangas AM, Pajak A. Myocardial infarction and coronary deaths in the World Health Organization MONICA Project. Registration procedures, event rates, and case-fatality rates in 38 populations from 21 countries in four continents. *Circulation* 1994;90:583–612.
- Enas EA, Yusuf S, Mehta JL. Prevalence of coronary artery disease in Asian Indians. *Am J Cardiol* 1992;70:945–9.
- Kamath SK, Hussain EA, Amin D, Mortillaro E, West B, Peterson CT, et al. Cardiovascular disease risk factors in 2 distinct ethnic groups: Indian and Pakistani compared with American premenopausal women. *Am J Clin Nutr* 1999;69:621–31.
- Sarraf-Zadegan N, Sayed-Tabatabaei FA, Bashardoost N, Maleki A, Totonchi M, Habibi HR, et al. The prevalence of coronary artery disease in an urban population in Isfahan, Iran. *Acta Cardiol* 1999;54:257–63.
- Judkins MP. Selective coronary arteriography. I. A percutaneous transfemoral technic. *Radiology* 1967;89:815–24.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- Whicher JT, Ritchie RF, Johnson AM, Baudner S, Bienvenu J, Bliurup-Jensen S, et al. New international reference preparation for proteins in human serum (RPPHS). *Clin Chem* 1994;40:934–8.
- Cook JD, Lipschitz DA, Miles LE, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 1974;27:681–7.
- Sullivan JL. Iron and the sex difference in heart disease risk. *Lancet* 1981;1:1293–4.
- Sullivan JL. The iron paradigm of ischemic heart disease. *Am Heart J* 1989;117:1177–88.
- Graham DY, Smith JL. Aspirin and the stomach. *Ann Intern Med* 1986;104:390–8.
- von Houwelingen R, Nordoy A, van der Beek E, Houtsmuller U, de Metz M, Hornstra G. Effect of a moderate fish intake on blood pressure, bleeding time, hematology, and clinical chemistry in healthy males. *Am J Clin Nutr* 1987;46:424–36.
- Thomas FB, McCullough FS, Greenberger NJ. Inhibition of the intestinal absorption of inorganic and hemoglobin iron by cholestyramine. *J Lab Clin Med* 1971;78:70–80.
- Frassinelli-Gunderson EP, Margen S, Brown JR. Iron stores in users of oral contraceptive agents. *Am J Clin Nutr* 1985;41:703–12.
- Morrison HI, Semenciw RM, Mao Y, Wigle DT. Serum iron and risk of fatal acute myocardial infarction. *Epidemiology* 1994;5:243–6.
- Lauffer RB. Iron stores and the international variation in mortality from coronary artery disease. *Med Hypotheses* 1991;35:96–102.
- Tuomainen TP, Punnonen K, Nyyssonen K, Salonen JT. Association between body iron stores and the risk of acute myocardial infarction in men. *Circulation* 1998;97:1461–6.
- Balla G, Jacob HS, Eaton JW, Belcher JD, Vercellotti GM. Hemin: a possible physiological mediator of low density lipoprotein oxidation and endothelial injury. *Arterioscler Thromb* 1991;11:1700–11.
- Cozzi A, Santambrogio P, Levi S, Arosio P. Iron detoxifying activity of ferritin. Effects of H and L human apoferritins on lipid peroxidation in vitro. *FEBS Lett* 1990;277:119–22.
- Corti MC, Gaziano M, Hennekens CH. Iron status and risk of cardiovascular disease. *Ann Epidemiol* 1997;7:62–8.
- Verschuren WM, Jacobs DR, Bloemberg BP, Kromhout D, Menotti A, Aravanis C, et al. Serum total cholesterol and long-term coronary heart disease mortality in different cultures. Twenty-five-

- year follow-up of the seven countries study. *JAMA* 1995;274:131–6.
39. Whitty CJ, Brunner EJ, Shipley MJ, Hemingway H, Marmot MG. Differences in biological risk factors for cardiovascular disease between three ethnic groups in the Whitehall II study. *Atherosclerosis* 1999;142:279–86.
 40. Simon A, Dimberg L, Levenson J, Lanoiselee C, Massonneau M, Eriksson B, et al. Comparison of cardiovascular risk profile between male employees of two automotives companies in France and Sweden. The Coeur Project Group. *Eur J Epidemiol* 1997;13:885–91.
 41. Jee SH, Beaty TH, Suh I, Yoon Y, Appel LJ. The methylenetetrahydrofolate reductase gene is associated with increased cardiovascular risk in Japan, but not in other populations. *Atherosclerosis* 2000;153:161–8.
 42. Rasmussen ML, Folsom AR, Catellier DJ, Tsai MY, Garg U, Eckfeldt JH. A prospective study of coronary heart disease and the hemochromatosis gene (HFE) C282Y mutation: the Atherosclerosis Risk in Communities (ARIC) study. *Atherosclerosis* 2001;154:739–46.
 43. Roest M, van der Schouw YT, de Valk B, Marx JJ, Tempelman MJ, de Groot PG, et al. Heterozygosity for a hereditary hemochromatosis gene is associated with cardiovascular death in women. *Circulation* 1999;100:1268–73.
 44. Tuomainen TP, Kontula K, Nyyssonen K, Lakka TA, Helio T, Salonen JT. Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation: a prospective cohort study in men in eastern Finland. *Circulation* 1999;100:1274–9.
 45. Pay S, Ozcan N, Tokgozoglu SL. Elevated Lp(a) is the most frequent familial lipoprotein disorder leading to premature myocardial infarction in a country with low cholesterol levels. *Int J Cardiol* 1997;60:301–5.
 46. Manfroi WC, Zago AJ, Caramori PR, Cruz R, Oliveira J, Kirschnick LS, et al. Does serum ferritin correlate with coronary angiography findings? *Int J Cardiol* 1999;69:149–53.
 47. Barrett-Connor E, Bush TL. Estrogen and coronary heart disease in women. *JAMA* 1991;265:1861–7.
 48. Yagi K. Female hormones act as natural antioxidants—a survey of our research. *Acta Biochim Pol* 1997;44:701–9.
 49. Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/Progestin Replacement Study (HERS) Research Group. *JAMA* 1998;280:605–13.
 50. Wassmann S, Baumer AT, Strehlow K, van Eickels M, Grohe C, Ahlbory K, et al. Endothelial dysfunction and oxidative stress during estrogen deficiency in spontaneously hypertensive rats. *Circulation* 2001;103:435–41.
 51. Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham Study. *JAMA* 1979;241:2035–8.
 52. Pyorala K, Laakso M, Uusitupa M. Diabetes and atherosclerosis: an epidemiologic view. *Diabetes Metab Rev* 1987;3:463–524.
 53. Uusitupa M, Siitonen O, Pyorala K, Aro A, Hersio K, Penttila I, Voutilainen E. The relationship of cardiovascular risk factors to the prevalence of coronary heart disease in newly diagnosed type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1985;28:653–9.
 54. Stringer MD, Gorog PG, Freeman A, Kakkar VV. Lipid peroxides and atherosclerosis. *BMJ* 1989;298:281–4.
 55. Nishigaki I, Hagihara M, Tsunekawa H, Maseki M, Yagi K. Lipid peroxide levels of serum lipoprotein fractions of diabetic patients. *Biochem Med* 1981;25:373–8.
 56. Bowie A, Owens D, Collins P, Johnson A, Tomkin GH. Glycosylated low density lipoprotein is more sensitive to oxidation: implications for the diabetic patient? *Atherosclerosis* 1993;102:63–7.
 57. Rabini RA, Fumelli P, Galassi R, Dousset N, Taus M, Ferretti G, et al. Increased susceptibility to lipid oxidation of low-density lipoproteins and erythrocyte membranes from diabetic patients. *Metabolism* 1994;43:1470–4.
 58. Som S, Basu S, Mukherjee D, Deb S, Choudhury PR, Mukherjee S, et al. Ascorbic acid metabolism in diabetes mellitus. *Metabolism* 1981;30:572–7.
 59. Herman JB, Medalie JH, Goldbourt U. Diabetes, prediabetes and uricaemia. *Diabetologia* 1976;12:47–52.
 60. Karpen CW, Cataland S, O'Dorisio TM, Panganamala RV. Production of 12-hydroxyeicosatetraenoic acid and vitamin E status in platelets from type I human diabetic subjects. *Diabetes* 1985;34:526–31.
 61. Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapay T, Guyader D, et al. Insulin resistance-associated hepatic iron overload. *Gastroenterology* 1999;117:1155–63.
 62. Ford ES, Cogswell ME. Diabetes and serum ferritin concentration among U.S. adults. *Diabetes Care* 1999;22:1978–83.
 63. Hughes K, Choo M, Kuperan P, Ong CN, Aw TC. Cardiovascular risk factors in non-insulin-dependent diabetics compared to non-diabetic controls: a population-based survey among Asians in Singapore. *Atherosclerosis* 1998;136:25–31.