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Background—Epidemiological studies aimed at correlating coronary heart disease (CHD) with serum ferritin levels have thus far yielded inconsistent results. We hypothesized that a labile iron component associated with non–transferrin-bound iron (NTBI) that appears in individuals with overt or cryptic iron overload might be more suitable for establishing correlations with CHD.

Methods and Results—We investigated the relation of NTBI, serum iron, transferrin saturation, and serum ferritin with risk of CHD and acute myocardial infarction (AMI). The cohort used comprised a population-based sample of 11 471 postmenopausal women aged 49 to 70 years at enrollment in 1993 to 1997. During a median follow-up of 4.3 years (quartile limits Q1 to Q3: 3.3 to 5.4), 185 CHD events were identified, including 66 AMI events. We conducted a case-cohort study using all CHD cases and a random sample from the baseline cohort (n=1134). A weighted Cox proportional hazards model was used to estimate hazard ratios for tertiles of iron variables in relation to CHD and AMI. Adjusted hazard ratios of women in the highest NTBI tertile (range 0.38 to 3.51) compared with the lowest (range −2.06 to −0.32) were 0.84 (95% confidence interval 0.61 to 1.16) for CHD and 0.47 (95% confidence interval 0.31 to 0.71) for AMI. The results were similar for serum iron, transferrin saturation, and serum ferritin.

Conclusions—Our results show no excess risk of CHD or AMI within the highest NTBI tertile compared with the lowest but rather seem to demonstrate a decreased risk. Additional studies are warranted to confirm our findings. **(*Circulation*. 2006;113:1942-1949.)**

Key Words: iron ■ cardiovascular diseases ■ epidemiology ■ follow-up studies ■ myocardial infarction

The concept that iron depletion could protect against ischemic heart disease was largely based on 2 clinical findings with regard to women after menopause: (1) that cardiovascular disease incidence increases gradually, largely narrowing the gender gap, and (2) that there is a parallel increase in body iron stores.[1] That concept, better known as the "iron hypothesis," gained additional support when correlations between cardiovascular disease and other variables of body iron status began to be established. Experimental studies provided strong evidence for the involvement of labile iron in oxidative stress and the latter in the pathogenesis of atherosclerosis[2,3] and cardiac ischemia/reperfusion injury.[4,5] Iron can be potentially toxic owing to its catalytic role in the formation of extremely reactive hydroxyl radicals via Fenton chemistry; however, epidemiological studies that attempted to establish a relationship between coronary heart disease (CHD) and iron status variables have been inconsistent,[6] with several large studies that did find an effect,[7–10] whereas others did not.[11–13]

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An outstanding issue in clinical studies has been the identification of variables associated with iron that best reflect or represent the potentially toxic role of iron in diseases.[14] Serum ferritin, which has been used in most of the aforementioned studies, has been considered the best clinical marker for body iron stores[15] and, indirectly, an indicator that reflects body iron overload; however, serum ferritin levels also increase in a variety of inflammatory and stressful conditions not associated with iron metabolism per se. Moreover, ferritin itself neither carries forms of iron that are redox active and chelatable (that is, labile) nor is involved in iron delivery to cells. In addition, both serum iron and transferrin concentration, which are markers of iron release into the

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plasma and utilization for iron incorporation, primarily in hemoglobin, are less informative than serum ferritin when it comes to body iron status. On the other hand, plasma non–transferrin-bound iron (NTBI), first detected in sera of patients with thalassemia major[16,17] and subsequently in patients with hemochromatosis[18,19] and patients undergoing dialysis,[20,21] might contain potentially toxic forms of iron that lead to iron overload and/or ensuing oxidative stress. Preliminary studies indicated that NTBI can be detected in individuals possessing less than fully saturated transferrin levels, in subjects with transferrin saturation <45%, such as hemochromatosis heterozygotes,[22] and in some alcoholics.[23] These findings suggest that in certain situations related to a disturbed iron metabolism, NTBI might exist in the general population. Given these facts, we hypothesized a possible role of NTBI, through its catalytic role in the formation of harmful oxygen radicals, in CHD. The purpose of this population-based cohort study was to investigate the relation of NTBI, serum iron, transferrin saturation, and serum ferritin with coronary heart disease in postmenopausal women.

Methods

Cohort

A group of 17 357 women aged 49 to 70 years among breast cancer–screening participants were enrolled between 1993 and 1997 in the Prospect-EPIC study, which is 1 of the 2 Dutch contributions to the European Prospective Investigation into Cancer and Nutrition (EPIC).[24,25] At recruitment, all women underwent a physical examination, filled out a food frequency questionnaire and a general questionnaire relating to lifestyle and medical factors, and donated a 30-mL nonfasting blood sample. Samples were fractionated into serum, citrated plasma, buffy coat, and erythrocyte aliquots of 0.5 mL each and stored under liquid nitrogen at −196°C for future research. All women signed an informed consent form before study inclusion. The study complies with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Medical Center Utrecht.

For the purpose of this study, we excluded 362 women who did not consent to linkage with vital status registries or who were not traceable, 774 women who had missing questionnaires or blood samples, and 134 women who reported a daily energy intake less than 500 kcal/d. Furthermore, we excluded 1047 women who reported a history of cardiovascular disease (*International Classification of Diseases,* 9th Revision [*ICD*-9] codes 390 to 459) at baseline. We restricted our cohort to women for whom information on menopausal status was known and who reported absence of menstrual bleeding periods, either natural or hormone-induced, in the 12 months before enrollment in the study. This restriction was performed to minimize the large variations in iron variables due to differences in menopausal status. In total, there were 11 471 women remaining in the analyses. We used a case-cohort design as introduced by Prentice.[26] The case-cohort design consists of a subcohort randomly sampled from the full cohort at the beginning of the study and a case sample that consists of all cases that are ascertained during follow-up. With this sampling strategy, the subcohort may include incident cases of CHD that will contribute person-time as controls until the moment they experience the event. We selected a random sample of ≈10% (n=1134) from the baseline cohort to serve as the subcohort. The advantage of this design is that it enables the performance of survival analyses without the need to collect expensive laboratory data for the entire cohort.

Follow-Up and End Points

Data on morbidity were obtained from the Dutch Centre for Health Care Information, which maintains a standardized computerized register of hospital discharge diagnoses. Admission files are filed continuously from all general and university hospitals in the Netherlands since 1990. Whenever a patient is discharged from a hospital, data on sex, date of birth, dates of admission and discharge, 1 mandatory principal diagnosis, and up to 9 optional additional diagnoses are recorded. All diagnoses are coded according to the *ICD*-9. Follow-up was complete until January 1, 2000. The database was linked to the cohort on the basis of birth date, sex, postal code, and general practitioner with a validated probabilistic method.[27] Information on vital status was gained through linkage with the municipal administration registries. Causes of death were obtained from the women's general practitioners.

Using the *ICD*-9 codes, we categorized cardiovascular disease events (codes 390 to 459) as CHD (codes 410 to 414), including acute myocardial infarction (AMI; code 410), or other cardiovascular disease. Whenever multiple events occurred, the first diagnosis was taken as an end point. For the present analyses, coronary events were the end points of interest.

For all women who had a cardiovascular event, follow-up ended at the date of diagnosis or, when hospital admission had not occurred, at the date of death. Moving out of the Netherlands (n=1) and death due to causes other than cardiovascular disease (n=15) were considered censoring events. All others (n=1046) were censored on January 1, 2000.

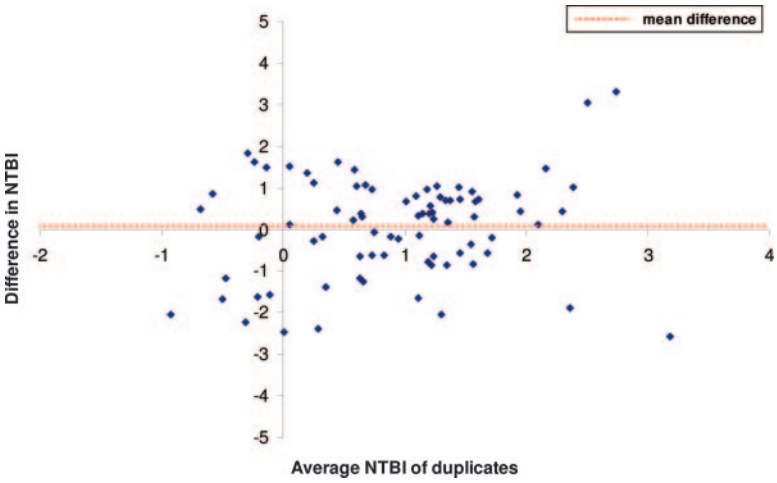
Baseline Measurements

Women were classified according to their smoking habits as current smokers, past smokers, or never smoked. Alcohol intake was divided into 5 categories: <1, 1 to 5, 5 to 15, 15 to 30, and >30 g/d. Systolic and diastolic blood pressures were measured in duplicate, and the mean value was calculated. Furthermore, height and weight were measured in subjects without shoes wearing light indoor clothing to compute body mass index, defined as weight divided by height squared (kg/m^2). Hypercholesterolemia and diabetes mellitus were defined as a self-reported physician diagnosis. Hypertension was defined as measured systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or self-reported physician diagnosis.

Biochemical measurements were performed for all subcohort members and CHD cases by standard laboratory procedures. Because of overlap between the cases and the subcohort, the total number of analyzed samples was 1297. Sera of cases were randomly distributed among those of the subcohort, and all biochemical analyses were performed without knowledge of disease status. Total cholesterol and glucose were determined by an automated enzymatic procedure on a Vitros 250 (Johnson & Johnson, Rochester, NY). Serum iron, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol were estimated with a colorimetric assay on a Hitachi 904 (Johnson & Johnson, Rochester, NY). As a marker for inflammation, high-sensitivity C-reactive protein (hsCRP) was measured in citrated plasma by the Behring BNII nephelometric method (Dade Behring, Deerfield, Ill). hsCRP values below the detection limit of 0.2 mg/L (n=50) were set to 0.1 mg/L. Serum ferritin was assessed with an automated immunometric assay on the Immulite (Diagnostic Products Corp, Los Angeles, Calif). Serum transferrin values were obtained by immunochemical turbidimetry on a Hitachi 904. Total iron-binding capacity (in micromoles per liter) was calculated as serum transferrin (in grams per liter)×25.14, and transferrin saturation was calculated as the ratio of serum iron to total iron-binding capacity.

NTBI was measured by a fluorescence-based 1-step assay as introduced by Breuer and Cabantchik.[28] To assess agreement between duplicate measurements of 80 samples, Bland-Altman plotting was performed (Figure). Mean NTBI levels are not related to the difference.

The interassay and intra-assay coefficients of variation (CVs) for serum ferritin are 7.1% (concentration of 101 μg/L) and 7.1% (concentration of 130 μg/L), respectively. For serum iron, the overall CV was 2.9%, and for transferrin, it was 6.85%. We have no information on the reproducibility of the NTBI measurement; however, we used the technique developed by Breuer and Cabantchik[28] and performed this assay in close cooperation with this group. The same technique was incorporated in an international round robin, and



Bland-Altman plot showing difference in measurements of NTBI.

the results were published recently.[29] In that report, among others, CVs of 3 different laboratories that performed this specific assay were presented. Between-sample CVs ranged from 49.3 to 213.1, and within-sample CVs ranged from 4.4 to 29.5.

Data Analysis

Baseline characteristics for the women of the subcohort were summarized according to the tertile distribution of NTBI. Means and SDs were computed for normally distributed variables and medians and quartile limits (quartiles 1 to 3 [Q1-Q3]) for variables that showed skewed distributions. Categorical variables were expressed as frequencies. For further analyses, hsCRP and ferritin concentrations were logarithmically transformed to produce approximately normal distributions. Tests for linear trends were conducted by adding the tertiles of each iron variable as a continuous variable in the model.

To assess the relationship between the iron variables (ie, NTBI, serum iron, transferrin saturation, and serum ferritin) and heart disease, we used a Cox proportional hazards model with an estimation procedure adapted for case-cohort designs. We used the unweighted method by Prentice,[26] which is incorporated in the macro ROBPHREG made by Barlow and Ichikawa. This macro is available at http://lib.stat.cmu.edu/general/robphreg and can be implemented in the SAS statistical software package version 8.2. It computes weighted estimates together with a robust standard error, from which we calculated 95% confidence intervals (CIs). Tertiles were calculated for each iron variable on the basis of their distribution in the random sample. In addition, we investigated whether a dose-response relation exists between serum ferritin and CHD by dividing serum ferritin concentration into tertiles based on the distribution in the random sample or whether a high-threshold relation exists by dichotomizing serum ferritin concentration as above or below 200 μg/L.[7]

Age (continuous), body mass index (continuous), alcohol intake (categorical), smoking status (current/past/never), hypertension (yes/no), diagnosis of hypercholesterolemia (yes/no), LDL cholesterol (continuous), HDL cholesterol (continuous), diagnosis of diabetes (yes/no), glucose (continuous), and hsCRP (continuous) were evaluated for confounding. The fit of the proportional hazards model was evaluated by examining the log-minus-log plots in SPSS version 11.5. The assumptions of proportionality were met.

Effect modification of NTBI by alcohol intake was examined by performing stratified analyses. Alcohol intake was dichotomized at the cutoff value of 10 g of alcohol per day, which corresponds to the consumption of 1 glass of any alcoholic beverage.

Apart from the case-cohort analyses, all statistical analyses were performed with the statistical package SPSS (SPSS for Windows, release 11.5.0, 2002; Chicago, Ill, SPSS Inc). A probability value of <0.05 was considered statistically significant.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

During a median follow-up of 4.3 years (Q1-Q3, 3.3 to 5.4), 185 first fatal and nonfatal CHD events occurred, of which 66 were AMIs, among the 11 471 women of the baseline cohort. In the subcohort of 1134 women, 22 first CHD events were documented. In total, 11 of the 185 first events were fatal.

Table 1 presents the baseline characteristics of the subcohort according to tertiles of NTBI. It shows that women with the highest NTBI values (third tertile) consumed on average slightly more alcohol, had a lower body mass index, and were less often hypertensive than women with the lowest NTBI values (first tertile). Higher values of NTBI were associated with higher levels of serum iron, transferrin saturation, serum ferritin, and HDL cholesterol and lower levels of hsCRP.

In Table 2, unadjusted and adjusted hazard ratios (HRs) and their 95% CIs for CHD events are presented for the second and third tertiles of NTBI, serum iron, transferrin saturation, and serum ferritin compared with the lowest tertiles. When estimates were fully adjusted for age, body mass index, alcohol intake, smoking status, hypertension, diabetes mellitus, hypercholesterolemia, glucose, HDL cholesterol, LDL cholesterol, and hsCRP, there was no association between NTBI levels and CHD events. Neither was there an association between serum iron, transferrin saturation, or serum ferritin and CHD events.

Table 3 presents the HRs for the risk of AMI. When fully adjusted, women within the highest NTBI tertile had a lower risk of AMI than women in the lowest NTBI tertile (HR 0.47, 95% CI 0.31 to 0.72). Similarly, compared with the lowest tertiles, the risk of AMI was 0.49 (95% CI 0.25 to 0.94) for the highest tertile of serum iron and 0.50 (95% CI 0.24 to 1.01) for the highest tertile of transferrin saturation. Women in the highest serum ferritin tertile (indicating a concentration >136 μg/L) were not at an increased risk of AMI compared with women in the lowest tertile (HR 0.55, 95% CI 0.23 to 1.31). Also, when the cutoff value of ≥ 200 μg/L was used, no association with the risk of AMI was observed (HR 0.82, 95% CI 0.35 to 1.95).

TABLE 1. Baseline Characteristics of the Subcohort (n=1132) According to NTBI Tertiles

	Tertiles		
	1 (Lowest)	2	3 (Highest)
No. of subjects	378	378	378
NTBI range, μmol/L	−2.06 to −0.32	−0.32 to 0.38	0.38 to 3.51
Age at intake, y*	59 (55–63)	59 (54–64)	59 (54–64)
Body mass index, kg/m²†	26.3 (4.2)	26.2 (3.8)	25.6 (4.0)
Alcohol intake, g/d*	2.6 (0.1–9.6)	2.9 (0.3–10.9)	4.1 (0.4–15.3)
Hypertension, n (%)‡	183 (48)	171 (45)	155 (41)
Diagnosis of hypercholesterolemia, n (%)§	17 (5)	16 (4)	21 (6)
Diagnosis of diabetes mellitus, n (%)§	9 (2)	14 (4)	7 (2)
Smoking status, n (%)			
Current	70 (19)	87 (23)	97 (26)
Past	135 (36)	118 (31)	116 (31)
Never	173 (46)	173 (46)	165 (44)
Serum iron, μmol/L†	12.8 (3.4)	16.2 (3.4)	20.4 (4.1)
Transferrin saturation, n (%)†	18.2 (5.2)	22.6 (4.8)	28.7 (6.2)
TIBC, μmol/L†	71.7 (9.6)	72.2 (8.7)	71.7 (8.5)
Serum ferritin, μg/L*	101 (56–144)	101 (65–152)	115 (73–176)
Total cholesterol, mmol/L†	6.01 (1.10)	6.05 (0.90)	5.92 (0.93)
HDL cholesterol, mmol/L†	1.49 (0.38)	1.58 (0.40)	1.64 (0.43)
LDL cholesterol, mmol/L†	4.04 (0.99)	4.06 (0.89)	3.99 (0.91)
Serum glucose, mmol/L†	4.5 (1.3)	4.6 (1.5)	4.6 (1.5)
hsCRP, mg/L*	1.6 (0.7–3.6)	1.2 (0.6–2.5)	0.9 (0.5–1.8)

TIBC indicates total iron binding capacity.
*Median (interquartile range).
†Mean (SD).
‡Systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, and/or self-reported physician diagnosis.
§Self-reported physician diagnosis.

We have adjusted the associations for hsCRP, an indicator for low-level inflammation, but this did not change our results fundamentally. Correction for hsCRP may have accounted only for the acute-phase inflammatory response and not for the effects of chronic inflammation as seen in anemia of chronic diseases. However, when we excluded subjects who had a serum iron concentration below 10.7 μmol/L in combination with a serum ferritin concentration above 50 μg/L, cutoff values that are often used as guidelines for the diagnosis of anemia of chronic diseases, the inverse relation was sustained.

Alcohol consumption did not modify the association between NTBI and CHD risk (data not shown).

Discussion

In this study, in a normal population, we were not able to demonstrate an association between the highest tertile of circulating NTBI, serum iron, transferrin saturation, or serum ferritin and risk of CHD. Contrary to the initial assumption of a positive relation between iron status and CHD, we observed a statistically significant inverse association of NTBI and serum iron with risk of AMI. Although not statistically significant, transferrin saturation and serum ferritin were also inversely related with risk of AMI.

One advantage of a prospective over a retrospective study design is that exposure data are collected at the beginning of the study and that subjects are followed up over time for the occurrence of disease. The possibility that estimates are biased by the fact that emerging diseases cause a change in the exposure variable is thereby reduced to a minimum. However, in view of the iron hypothesis, the observed inverse association was unexpected. A possible explanation for these findings may be the presence of unidentified chronic conditions of inflammation, infection, or malignancy.[30] Chronic disease is reflected by moderate anemia, low serum iron levels, and decreased transferrin saturation despite adequate iron stores, and it is generally accompanied by higher morbidity and mortality rates in older people.[31]

In this population of healthy individuals, negative NTBI values were measured. NTBI values in sera are derived from an Fe-calibration curve. To prevent scavenging of NTBI in sera by free iron–binding sites on transferrin, gallium chloride ($GaCl_3$) was added to block the free iron–binding sites on transferrin; however, because $GaCl_3$ is not fully capable of doing this, some free iron-binding sites will still exist, and this may yield negative NTBI values. Although the NTBI values cannot be considered as absolute concentrations, the

TABLE 2. HRs (95% CIs) for Risk of CHD

		HR (95% CI)			
	Cases*	Unadjusted	Age Adjusted	Multivariable 1 Adjusted†	Multivariable 2 Adjusted‡
NTBI, μmol/L					
Tertiles§					
1 (Lowest)‖	70	1.0	1.0	1.0	1.0
2 (Middle)	64	0.93 (0.66–1.32)	0.90 (0.61–1.34)	1.01 (0.65–1.56)	1.03 (0.62–1.70)
3 (Highest)	51	0.74 (0.57–0.95)	0.72 (0.55–0.95)	0.85 (0.63–1.16)	0.84 (0.61–1.16)
P_{trend}		0.025	0.032	0.359	0.373
Serum iron, μmol/L					
Tertiles¶					
1 (Lowest)‖	66	1.0	1.0	1.0	1.0
2 (Middle)	67	0.93 (0.70–1.25)	0.97 (0.72–1.31)	1.08 (0.80–1.46)	0.94 (0.65–1.35)
3 (Highest)	52	0.78 (0.54–1.12)	0.82 (0.57–1.17)	1.00 (0.72–1.39)	0.89 (0.65–1.23)
P_{trend}		0.185	0.285	0.963	0.491
Transferrin saturation, %					
Tertiles#					
1 (Lowest)‖	69	1.0	1.0	1.0	1.0
2 (Middle)	71	1.04 (0.76–1.43)	1.08 (0.78–1.50)	1.19 (0.86–1.63)	1.16 (0.85–1.60)
3 (Highest)	45	0.65 (0.44–0.96)	0.68 (0.46–1.00)	0.83 (0.56–1.23)	0.87 (0.59–1.30)
P_{trend}		0.046	0.076	0.441	0.591
Serum ferritin, μg/L					
Tertiles**					
1 (Lowest)‖	69	1.0	1.0	1.0	1.0
2 (Middle)	71	1.13 (0.83–1.55)	1.07 (0.78–1.46)	1.13 (0.81–1.58)	1.08 (0.75–1.56)
3 (Highest)	45	1.04 (0.70–1.54)	0.93 (0.59–1.47)	0.90 (0.53–1.51)	0.79 (0.46–1.35)
P_{trend}		0.841	0.746	0.667	0.317
<200 μg/L	156	1.0	1.0	1.0	1.0
≥200 μg/L	29	1.02 (0.72–1.43)	0.95 (0.67–1.37)	0.84 (0.54–1.31)	0.73 (0.43–1.23)

*Because of missing data on the covariates of the multivariable models, it is possible a subject was excluded from the analyses.

†Adjusted for age (continuous), body mass index (continuous), alcohol intake (5 categories), and hsCRP (continuous).

‡Adjusted for age (continuous), body mass index (continuous), alcohol intake (5 categories), hsCRP (continuous), smoking (never/past/current), hypertension (yes/no), diagnosis of hypercholesterolemia (yes/no), diagnosis of diabetes mellitus (yes/no), glucose (continuous), LDL cholesterol (continuous) and HDL cholesterol (continuous).

§Ranges: first tertile −2.06 to −0.32 μmol/L; second tertile −0.32 to −0.38 μmol/L; third tertile −0.38 to 3.51 μmol/L.

‖Reference category.

¶Ranges: first tertile 2.5–14.2 μmol/L; second tertile 14.2–18.3 μmol/L; third tertile 18.3–39.4 μmol/L.

#Ranges: first tertile 4.3%–19.8%; second tertile 19.8%–25.5%; third tertile 25.5–57.1%.

**Ranges: first tertile 3.2–75.7 μg/L; second tertile 75.7–136 μg/L; third tertile 137–1158 μg/L.

values, even those below 0 μmol/L, do reflect some combination of actual NTBI present and the iron-binding capacity of the serum. The observation of negative NTBI values in previous reports has caused skepticism about the NTBI measurement.[32] Some have cast doubt on the existence of NTBI in normal situations and assert that when NTBI appears in the circulation, it is scavenged almost immediately by transferrin and other ligands. NTBI could be the reactive component in areas of oxidative stress, even in healthy individuals without iron overload. NTBI appears to be inappropriate as a marker for the hazardous effect of iron among healthy individuals. Whereas in subjects with iron overload, such as those with thalassemia major or hemochromatosis, NTBI concentrations are high and can be measured systemically, in normal subjects without iron overload, NTBI levels might be too low to persist systemically, because NTBI will be scavenged almost immediately by proteins such as transferrin. However, NTBI may still arise very locally in situations of oxidative stress and exert its effects.

The present study is limited by the relatively short period of follow-up and the low number of fatal events due to CHD. This might have reduced the power to show an effect, and we cannot exclude the possibility of a false-negative finding. Other studies are certainly needed to confirm or reject this finding. We performed a post hoc power analysis based on a case-control design as the most conservative approach for the highest versus the lowest tertile of NTBI; for CHD (185 cases/949 controls), we had

TABLE 3. HRs (95% CIs) for Risk of AMI

		HR (95% CI)			
	Cases*	Unadjusted	Age Adjusted	Multivariable 1 Adjusted†	Multivariable 2 Adjusted‡
NTBI, μmol/L					
Tertiles§					
1 (Lowest)‖	27	1.0	1.0	1.0	1.0
2 (Middle)	24	0.91 (0.53–1.54)	0.87 (0.47–1.61)	0.93 (0.49–1.77)	0.82 (0.38–1.81)
3 (Highest)	15	0.56 (0.38–0.83)	0.55 (0.38–0.79)	0.61 (0.42–0.89)	0.47 (0.31–0.72)
P_{trend}		<0.001	<0.001	0.009	<0.001
Serum iron, μmol/L					
Tertiles¶					
1 (Lowest)‖	28	1.0	1.0	1.0	1.0
2 (Middle)	21	0.69 (0.42–1.13)	0.73 (0.45–1.18)	0.78 (0.48–1.27)	0.65 (0.40–1.07)
3 (Highest)	17	0.60 (0.37–0.96)	0.64 (0.39–1.06)	0.70 (0.41–1.20)	0.49 (0.25–0.94)
P_{trend}		0.026	0.064	0.168	0.021
Transferrin saturation, %					
Tertiles#					
1 (Lowest)‖	27	1.0	1.0	1.0	1.0
2 (Middle)	26	0.97 (0.60–1.59)	1.03 (0.63–1.70)	1.08 (0.63–1.86)	1.03 (0.62–1.73)
3 (Highest)	13	0.48 (0.24–0.98)	0.51 (0.24–1.07)	0.57 (0.27–1.21)	0.50 (0.24–1.01)
P_{trend}		0.043	0.080	0.161	0.063
Serum ferritin, μg/L					
Tertiles**					
1 (Lowest)‖	27	1.0	1.0	1.0	1.0
2 (Middle)	23	0.88 (0.54–1.43)	0.82 (0.50–1.32)	0.86 (0.53–1.41)	0.88 (0.50–1.54)
3 (Highest)	16	0.60 (0.26–1.38)	0.52 (0.21–1.28)	0.53 (0.21–1.33)	0.55 (0.23–1.31)
P_{trend}		0.198	0.127	0.150	0.142
<200 μg/L	57	1.0	1.0	1.0	1.0
≥ 200 μg/L	9	0.86 (0.41–1.83)	0.80 (0.36–1.76)	0.79 (0.34–1.85)	0.82 (0.35–1.95)

*Because of missing data on the covariates of the multivariable models, it is possible a subject was excluded from the analyses.

†Adjusted for age (continuous), body mass index (continuous), alcohol intake (5 categories), and hsCRP (continuous).

‡Adjusted for age (continuous), body mass index (continuous), alcohol intake (5 categories), hsCRP (continuous), smoking (never/past/current), hypertension (yes/no), diagnosis of hypercholesterolemia (yes/no), diagnosis of diabetes mellitus (yes/no), glucose (continuous), LDL cholesterol (continuous), and HDL cholesterol (continuous).

§Ranges: first tertile −2.06 to −0.32 μmol/L; second tertile −0.32 to −0.38 μmol/L; third tertile −0.38 to −3.51 μmol/L.

‖Reference category.

¶Ranges: first tertile 4.7–14.2 μmol/L; second tertile 14.2–18.3 μmol/L; third tertile 18.3–39.4 μmol/L.

#Ranges: first tertile 4.7%–19.8%; second tertile 19.8%–25.5%; third tertile 25.5%–37.1%.

**Ranges: first tertile 3.2–75.7 μg/L; second tertile 75.7–136 μg/L; third tertile 137–1158 μg/L.

80% power to detect an OR of 1.61 (or 0.62), whereas for AMI (66 cases/949 controls), we had 80% power to detect an OR of 2.10 (or 0.42).

Even if we assume that one important pathway by which iron could exert its hazardous effects on CHD is through aggravation of tissue injury during reperfusion after an ischemic event, it may still take many years to fully manifest the effects of other established cardiovascular risk factors leading to the event. Although the present study population consisted of postmenopausal women aged 49 to 70 years, this group still might have been too young to detect a possible effect on case fatality.

Another proposed mechanism of action for iron in cardiovascular disease risk may involve the formation of oxidized LDL particles, a key step in the atherogenic process.[33] At present, substantial evidence of this comes from experimental and observational studies,[34–36] whereas the majority of human epidemiological studies have been much less conclusive.[37–41]

Finally, it has been demonstrated that Fe(II) could also play a role by impairing endothelium-dependent vasoreactivity and inducing platelet activation, leading to accelerated thrombosis.[42,43] This iron-induced platelet activation is mediated by hydroxyl radical production and involves protein kinase C activity.

A downward trend of CHD risk with increasing levels of serum iron and transferrin saturation is in accordance with findings from previously published reports. Results from the National Health and Nutrition Examination Survey I (NHANES 1) epidemiological follow-up study showed that serum iron was inversely associated with the risk of myocar-

dial infarction in women but not in men.[44] In a multivariable-adjusted model, the investigators found a relative risk of 0.61 (95% CI 0.40 to 0.93) for myocardial infarction in the highest quartile of serum iron versus the lowest quartile. This relative risk is comparable to the relative risk of 0.49 (95% CI 0.25 to 0.94) for myocardial infarction in the present study when the third tertile of serum iron was compared with the first. Likewise, a statistically significant inverse relation between transferrin saturation and CHD was reported in women (relative risk 0.65, 95% CI 0.48 to 0.89) but not in men. In 1999, Danesh and Appleby[45] published a meta-analysis, which also included the abovementioned NHANES study, of published prospective studies on iron status and CHD. In this meta-analysis, individuals in the top third of serum iron and transferrin saturation were compared with those in the bottom third. The results showed a combined relative risk of 0.8 (95% CI 0.7 to 1.0) and 0.9 (95% CI 0.7 to 1.1) for serum iron and transferrin saturation, respectively. For serum ferritin, a combined risk ratio of 1.03 (95% CI 0.83 to 1.29) was estimated for subjects with concentrations $\geq$200 μg/L versus $<$200 μg/L. Thereafter, other studies have been performed that reported either no relationship or strong inverse relationships.[8,13,46]

It might be that in the normal range, plasma iron, and even NTBI, has a beneficial effect. After all, iron is an essential nutrient that is needed for numerous biological functions of the body. In extreme situations of iron deficiency and iron overload, the effects may well be different, but such subjects were hardly represented in the present study. Slightly increased iron levels could increase survival in early life, whereas at older ages, relatively high levels may have a potentially hazardous risk effect. The widespread distribution of the C282Y mutation in the HFE gene among populations of northern European descent also indicates some form of selective advantage.[47,48] It is said that in former times, the C282Y mutation could have conferred protection against certain pathogens early in life and has compensated for limited dietary iron intake.[48,49]

To the best of our knowledge, this is the first prospective study that investigates the relation between NTBI and CHD events. Recently, Derstine et al[50] presented data from 3 controlled feeding studies in healthy subjects with normal iron status. They found no evidence for a relation between NTBI or any other measure of iron status and LDL oxidative susceptibility, a possible risk factor for cardiovascular disease.

In summary, the findings in the present study do not support the presence of an excess risk of CHD or myocardial infarction in normal postmenopausal women due to relatively higher levels of NTBI, serum iron, transferrin saturation, and serum ferritin. If anything, there is evidence that relatively low iron levels may be a risk factor for coronary events. Future studies are needed to exclude or reject this finding.

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Disclosures

None.

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CLINICAL PERSPECTIVE

It has been hypothesized that high blood levels of iron are detrimental to the vasculature and are a risk factor for coronary heart disease. In blood, most of the iron is bound to the transport protein transferrin. Serum concentrations of ferritin, a large iron storage protein, are directly proportional to intracellular ferritin concentrations. These measures have traditionally been used to study the relationship between iron and heart disease. Epidemiological studies have not been able to firmly establish such a relationship. However, transferrin and ferritin may not be a good reflection of the iron that is unbound and able to exert harmful effects. This study measured non–transferrin-bound iron (NTBI) and studied whether this labile iron component is associated with coronary heart disease or acute myocardial infarction risk in a population of middle-aged women who were followed up to 4 years after enrollment in the study. Women who had NTBI levels in the top third of the population distribution had a 0.8 times lower risk of coronary heart disease, which was not statistically significant, and a 0.5 times lower risk of acute myocardial infarction, which was statistically significant. Similar results were found for transferrin and serum iron levels. Although at the moment we cannot fully explain whether higher NTBI levels are actually protective against acute myocardial infarction, they do not appear to confer an increased risk.

Correction

In the article "Non–Transferrin-Bound Iron and Risk of Coronary Heart Disease in Postmenopausal Women" by van der A et al that appeared in the April 25, 2006, issue (*Circulation*. 2006;113:1942–1949), the number of the grant from the European Commission was incorrectly listed as QLRT-2001-004. The correct grant number is QLRT-2002-00444. This error has been corrected in the current PDF (http://circ.ahajournals.org/cgi/reprint/113/16/1942).

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