# Iron status in association with cardiovascular disease risk in 3 controlled feeding studies[1–3]

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**ABSTRACT**

**Background:** The role of body iron stores in free radical–induced peroxidation and cardiovascular disease risk has been debated, but controlled feeding studies using measurements of non-transferrin-bound iron (NTBI) and LDL oxidation have not been conducted.

**Objective:** We tested the hypothesis that NTBI and other measures of iron status do not affect oxidative susceptibility in healthy subjects with normal iron status.

**Design:** Plasma samples were analyzed from 77 healthy men and women aged 20–65 y who participated in 3 controlled feeding studies in which the type and amount of dietary fat were controlled. Iron status and in vitro LDL oxidation were assessed at baseline and at the end of each feeding period (4–8 wk).

**Results:** No significant relations were found between any measure of iron status (ferritin: 83 ± 8.9 μg/L; iron: 20.9 ± 5.4 μmol/L; TIBC: 74.4 ± 11.0 μmol/L; NTBI: 0.184 ± 0.15 μmol/L) and the in vitro measures of LDL oxidation (total dienes: 485 ± 55 μmol/mg LDL protein; lag time: 51.7 ± 15.9 min; and rate of oxidation: 25.4 ± 6.8 μmol dienes · $min^{-1}$ · g LDL $protein^{-1}$). Equal-iron peanut butter–based diets were associated with higher plasma iron in men (22.4 ± 3.8 μmol/L) than was the olive oil diet (17.7 ± 4.5 μmol/L) ($P = 0.02$), but this slight elevation did not alter LDL oxidation.

**Conclusions:** Diet composition may affect plasma iron in men, but LDL oxidative susceptibility is unaffected by the subtle variation in iron status. Thus, the results do not support a relation between iron status and LDL oxidative susceptibility, a possible risk factor for cardiovascular disease. *Am J Clin Nutr* 2003;77:56–62.

**KEY WORDS** Cardiovascular disease, iron, ferritin, total-iron-binding capacity, TIBC, conjugated dienes, lag time, lipids, LDL oxidation

## INTRODUCTION

Before menopause, women are one-half as likely as similarly aged men to experience cardiovascular disease (CVD) mortality, with an estimated 10-y difference in age-related rates between the sexes (1, 2). In addition, mean plasma ferritin concentrations in men are estimated to be twice those of women, and the difference is greatest during the childbearing years (3). Sullivan (4, 5) first proposed the iron-heart hypothesis, which suggests an interaction between body iron pools and lipid peroxidation. However, recent studies have not consistently shown a causal relation between iron and atherosclerosis, and one study implicating iron in lipid peroxidation was unable to show a dose-response relation (6).

Various markers of iron status have been proposed to alter LDL oxidative susceptibility. The transition metal ions, particularly non-transferrin-bound iron (NTBI), are implicated in free radical–induced lipid peroxidation (4, 7–11). In an unbound form, iron easily participates in the Fenton reaction, resulting in the production of harmful oxy and peroxy free radicals (12). Plasma NTBI rarely exists in healthy persons, but its presence has been correlated with oxidative injury (13, 14). Measures of iron status such as total-iron-binding capacity (TIBC), ferritin, transferrin saturation, dietary iron intake, and total body iron also have been evaluated as potential markers of oxidative stress. However, these measures of iron status were not associated with CVD "severity, incidence, or overall mortality" in various large cohort studies (15–19). Last, the lipid composition of the diet also may contribute to the susceptibility of LDL to undergo oxidation. Several studies indicate that diets rich in saturated and polyunsaturated fatty acids increase the oxidation of LDL compared with diets high in monounsaturated fatty acids (20–26). Therefore, the composition of dietary fat may mask the detection of iron's effects on CVD risk status.

Many studies have not carefully controlled dietary lipids, antioxidants, and iron. Thus, the present study was conducted to clarify the relations between serum ferritin, plasma iron, TIBC, transferrin saturation, NTBI, and in vitro LDL oxidation in subjects who had participated in well-controlled feeding studies. A second objective was to explore whether the type and amount of dietary fat could change the oxidative susceptibility of LDL to iron.

## SUBJECTS AND METHODS

### Experimental design and subjects

This report assayed archived serum and plasma samples for ferritin, iron, TIBC, and NTBI from each of 3 diet studies [DELTA (Dietary Effects on Lipoproteins and Thrombogenic Activity),

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Superspread, and Peanut] to evaluate the potential role of iron status on in vitro LDL oxidative status. The controlled feeding studies were conducted in a metabolic kitchen, and the subjects were randomly assigned to treatment diets according to a randomized, balanced-order study design. The experimental design and lipid and lipoprotein results were described previously (27–29; RL Morgan, TD Etherton, TA Pearson, PM Kris-Etherton, unpublished observations, 1998).

Briefly, protocol 1 of the DELTA Study was conducted to evaluate the effects of reducing total dietary fat and saturated fat on lipids, lipoproteins, and hemostatic factors in different population groups. The DELTA Study used a controlled multicenter study design. During protocol 1, 18 healthy men and 9 healthy women aged 24–65 y were studied at the Pennsylvania State Research Center. The study used a randomized, double-blind, 3-period crossover design. All meals and snacks were provided, and the subjects committed to eating only food that was provided by the Research Center. The 3 diets were as follows: *1*) the average American diet (AAD): 34% of energy from fat and 15% from saturated fat; *2*) a Step I diet: 29% of energy from fat and 9% from saturated fat; and *3*) a very-low-saturated-fat diet: 25% of energy from fat and 6% from saturated fat. In addition, all diets provided 7% of energy from polyunsaturated fatty acids, 13% of energy from monounsaturated fatty acids, and ≈300 mg cholesterol/d. The AAD and Step I diets contained ≈17 mg Fe/d, and the very-low-saturated-fat diet contained 18 mg Fe/d. Each diet period lasted 8 wk and was followed by a 4–6 wk break during which the subjects consumed their habitual diet before another feeding trial period began. Blood samples were collected from fasted subjects at baseline and during the seventh week in each diet period. The effects of the experimental diets on plasma lipids and lipoproteins were reported by Ginsberg (27).

The Superspread Study was designed to examine blood lipid responses to the addition of plant sterols into the diet (28). Men ($n = 18$) and women ($n = 10$) between the ages of 20 and 67 y with LDL-cholesterol concentrations between the 50th and 95th percentiles, plasma cholesterol concentrations between the 10th and 90th percentiles, and triacylglycerol concentrations between the 5th and 95th percentiles for sex, age, and race were studied. This study used a randomized crossover design. Two experimental diets were evaluated. The diets consisted of an AAD with an additional 3.3 mg Fe/d and 3 g stanols/d (nonesterified from soybean oil) incorporated in margarine (30 g/d) or a similar quantity of stanol-free margarine (control diet). Each feeding period lasted 4 wk. Blood samples from fasted subjects were collected at baseline and during the last week of each diet period.

The Peanut Study was designed to compare the effects of plasma lipids on 4 blood-cholesterol-lowering diets with an AAD (29; RL Morgan et al, unpublished observations, 1998). The cholesterol-lowering diets included one that was high in peanuts or peanut butter (peanut diet), a second that was high in peanut oil, a third that was high in olive oil, and a fourth that was low in total fat (Step II diet). We evaluated samples from 9 healthy men and 11 healthy women aged 20–60 y with total cholesterol concentrations between the 15th and 90th percentiles and HDL and LDL concentrations between the 10th and 90th percentiles. The Peanut Study used a 5-period crossover design; each period lasted 4 wk and was followed by a 4-d break. The AAD provided 34% of energy from fat: 16% from saturated fat, 11% from monounsaturated fat, and 7% from polyunsaturated fat. The olive oil diet provided 34% of energy from fat (7% from saturated fat, 21% from monounsaturated fat, and 6% from polyunsaturated fat). The peanut oil diet provided 34% of energy from fat (7% from saturated fat, 18% from monounsaturated fat, and 9% from polyunsaturated fat). The peanut butter diet provided 36% of energy from fat (8% from saturated fat, 18% from monounsaturated fat, and 10% from polyunsaturated fat). The low-fat (Step II) diet provided 26% of energy from fat (7% from saturated fat, 12% from monounsaturated fat, and 7% from polyunsaturated fat). All diets in this study contained ≈200 mg cholesterol, except for the AAD—which contained 400 mg cholesterol. Subjects committed to eating all and only the food provided by the Metabolic Diet Study Center. Blood samples from fasted subjects were collected at the end of each diet period.

The procedures followed were in accord with the ethical standards of and were approved by the Institutional Review Board of The Pennsylvania State University.

### Laboratory measurements

All blood samples (≈40 mL each) were collected according to standardized protocols reported previously, and plasma aliquots were stored at −80 °C until all samples were analyzed. The 3 studies originally evaluated relations between in vitro LDL oxidation in individuals consuming various experimental diets in a controlled feeding setting. CVD risk factors evaluated in response to the different test diets studied were LDL cholesterol, HDL cholesterol, total cholesterol, triacylglycerol, total dienes produced, rate of LDL oxidation, and lag time for oxidation. In the current study, we analyzed serum ferritin, plasma iron, TIBC, and some measurements of NTBI.

LDL oxidation was determined by measuring conjugated dienes (30) and lipid peroxides (31). Measurements of LDL oxidation were completed within 18 h of LDL dialysis. Conjugated diene formation was determined according to the method of Esterbauer et al (30) by frequently monitoring the change in absorbance at 234 nm. After the dilution of 100 μg LDL protein with 1 mL phosphate-buffered saline and the addition of copper chloride (final concentration: 0.01 mmol/L), absorbance was recorded at 3-min intervals at 37 °C for 2.5 h with a model 50 UV Spectrophotometer (SmithKline Beckman, Los Angeles). The rate of oxidation was determined by measuring the slope of the linear portion of the curve (ΔA234/min). Conjugated dienes were determined by calculating the difference in maximum absorbance between the decomposition phase and absorbance at the beginning of the lag phase. The amount of conjugated dienes formed was determined by using the molar absorptivity of $2.95 \times 10^4$ $(\text{mol/L})^{-1} \times \text{cm}^{-1}$.

Lipid peroxides were determined after $Cu^{2+}$-induced oxidation of the LDL fraction based on the peroxide-mediated oxidation of $Fe^{2+}$ to $Fe^{3+}$. This occurs at an acidic pH with the ferric iron complex measured at 560 nm (31). After incubation of 200 μg LDL protein with $Cu^{2+}$ (10 μL, 1 mmol/L) for 3–6 h, the quantity of lipid peroxide formed was determined by using the molar absorptivity of $4.52 \times 10^4$ $(\text{mol/L})^{-1} \times \text{cm}^{-1}$. Conjugated dienes were determined by calculating the difference in maximum absorbance between the decomposition phase and absorbance at the beginning of the lag phase.

Iron-status analyses included the measurement of serum ferritin (Diagnostics Products Corporation, Los Angeles), TIBC, and plasma iron concentrations (32). NTBI was analyzed by using a modified version of the method developed by Zhang et al (33). After microfiltration of the sample and nitrotriacetic acid disodium salt solution, samples were analyzed by atomic absorption

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spectrophotometry to measure the total free iron concentration. The CV for the NTBI determination was 5.1%, for ferritin was 4.7%, for plasma iron was 6.3%, and for TIBC was 3.8%. Quality-control standards were used for each assay.

### Statistical procedures

The iron measures obtained were used in conjunction with data obtained from the previous diet studies (28, 29; RL Morgan et al, unpublished observations, 1998) to evaluate the potential role of iron in LDL oxidation when experimental diets are well controlled. The sample sizes for each study were previously determined to be adequate to detect an effect of diet on LDL oxidation within a 3-wk time period and not necessarily to detect a response in variation in dietary iron intake on LDL oxidation. Data were tested for normality of distribution and log transformed if necessary (ferritin). Repeated-measures analysis of variance was used to test for the main effects of diet and feeding period on iron status. The failure of feeding period within a study to reach statistical significance was taken as evidence for the lack of effect of order, or repeated measurements, on our outcome measures. Stepwise regression analysis was used to examine the strength of the relation of iron status to LDL oxidation within and between studies. A significant increase in $R^2$ values ($P < 0.05$) with the addition of a variable was deemed to have statistical meaning. These analyses were performed with and without diet period as an initial factor. Tukey-Kramer adjusted $P$ values were used to determine statistical differences in post hoc comparisons when the $F$ ratio was significant. $P$ values $< 0.05$ were considered statistically significant. The data are expressed as means ± SEs. The analyses were performed with EXCEL (version 2000; Microsoft Inc, Seattle) and SAS (version 8.0; SAS Institute, Cary, NC) software.

## RESULTS

The plasma lipid and iron-status indexes for all 3 studies are reported in **Table 1** and represent the values obtained at the end of each of the feeding trial periods. The length of individual feeding periods varied between 4 and 8 wk, depending on the study. As expected, changes in the type and amount of dietary fat were associated with significant changes in blood lipids and indexes of LDL oxidation, as previously reported (27–29; RL Morgan et al, unpublished observations, 1998). In contrast, the consumption of controlled diets with similar iron contents (17–21 mg/d) resulted in a significant change only in plasma iron concentrations in men in the Peanut Study (**Figure 1**). Plasma iron was significantly higher when men consumed the peanut butter diet (22.4 μmol Fe/L) than when they consumed the olive oil (17.7 μmol Fe/L) and peanut oil (15.9 μmol Fe/L) diets. Transferrin saturation did vary by diet, as would be expected from the changes in plasma iron. No effect of these dietary manipulations on plasma iron in women ($P = 0.43$) was observed, and the effect of diet on other measures of iron status in women was also not significant. Importantly, there was no effect of the order in which diets were given on measures of iron status in men or women. Although the amount of blood taken over the course of the longer study (the Peanut Study) removed a considerable amount of iron from the body pool, the balanced assignment of subjects to dietary order prevented a significant effect of within-subject change in ferritin. That is, although an individual may have had a significant change in iron status from period 1 to period 4 in the Peanut Study, the diet that subject was consuming during period 4 was not always the same and thus we could test the strength of the relation of diet to NTBI and LDL oxidation without the concern of repeated phlebotomy causing bias.

As reported previously (27–29; RL Morgan et al, unpublished observations, 1998), low-saturated-fat diets (the low-fat and the Step II diets) and diets high in monounsaturated fats, such as the olive oil diet, resulted in lower plasma total and LDL cholesterol than did the AAD. These diets also tended to result in longer lag times, although there was some specificity of effect. Importantly, these effects of dietary lipids on LDL oxidation occurred within a time period of several weeks and there was no carryover from one dietary period to another. These dietary effects on LDL oxidation were not related to the iron status of the subjects, as evidenced by a lack of a statistically significant relation between any measures of iron status (plasma iron, TIBC, ferritin, transferrin saturation, and NTBI) and lipid peroxidation measures (LDL oxidation lag time, LDL oxidation rate, and LDL dienes) (**Table 2**). That is, individuals at the higher end of the distribution of LDL oxidation did not have a higher iron status, nor did individuals with a higher iron status have more LDL oxidation. We tested these relations with both log-transformed and non-log-transformed data.

There was sufficient sample for 36 of the Peanut Study aliquots (19 samples from women and 17 samples from men) to determine NTBI and to relate that proposed prooxidant variety of iron to measures of LDL oxidation and ferritin concentrations. The relation between the NTBI and dienes, lag time, or rate of LDL oxidation was not statistically significant ($r$ = 0.040, 0.026, and 0.119, respectively; $P > 0.05$; scatter plot data not shown), nor was there a significant relation between NTBI and ferritin concentrations (**Figure 2**). Again, log transformation of the data did not change this lack of relation.

## DISCUSSION

Some in vitro and animal studies and iron reduction through phlebotomy have given some support to the iron-heart hypothesis, indicating that available iron may participate in free radical reactions and that decreased iron stores or iron chelation may diminish lipid peroxidation (9, 34–36). However, larger cohort and in vivo studies supporting a role of iron in the development of CVD have not consistently shown a causal relation with atherosclerosis (15, 17, 19, 37–39). In concordance with the studies with negative findings, the present study found no association between iron status and LDL oxidation.

Contrary to the iron-heart hypothesis, a meta-analysis of 570 coronary heart disease (CHD) cases in 5 studies that compared subjects with baseline ferritin concentrations of ≥200 with those <200 μg/L resulted in a combined risk ratio of 1.03 (95% CI: 0.83, 1.29) after control for confounding factors (40). Concerning transferrin saturation, 5 other prospective studies ($n$ = 6194 cases, baseline weighted mean age of 56 y) resulted in a combined risk ratio for CHD risk of 0.92 (95% CI: 0.74, 1.14) when comparing the upper and lower one-third (40). Risk ratios for TIBC ($n$ = 2755 cases, baseline weighted mean age of 58 y) and CHD risk yielded 0.98 (95% CI: 0.66, 1.46), and risk ratios for plasma iron (total of 2848 cases, baseline weighted mean age of 58 y) and CHD yielded a risk ratio of 0.83 (95% CI: 0.67, 1.03) (40). Despite possible weak associations, this systematic evaluation of the current prospective studies does not provide convincing evidence for an association between iron status and CVD risk. Similarly, in the current study, iron status was not significantly different in

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**TABLE 1**
Lipids, lipoproteins, LDL oxidation, and iron-status results from 3 controlled feeding trials[1]

| | TC | LDL-C | HDL-C | TG | Lag time | Rate | Dienes | Ferritin | TIBC | Iron | Transferrin saturation |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mmol/L* | *mmol/L* | *mmol/L* | *mmol/L* | *min* | *$\mu mol \cdot min^{-1} \cdot mg$ protein$^{-1}$* | *μmol/mg* | *μg/L* | *μmol/L* | *μmol/L* | *%* |
| Delta Study ($n$ = 27) | | | | | | | | | | | |
| Average American diet | 5.52 ± 0.67[a] | 3.67 ± 0.66[a] | 1.28 ± 0.28[a] | 1.17 ± 0.11 | 53.9 ± 1.3 | 25.5 ± 1.0[a] | 524.0 ± 7.8[a] | 95.9 ± 26.2 | 82.2 ± 3.3 | 18.1 ± 1.0 | 22.0 ± 1.3 |
| Step I diet | 5.23 ± 0.62[a,b] | 3.42 ± 0.60[a,b] | 1.19 ± 0.28[a,b] | 1.31 ± 0.16 | 52.1 ± 1.6 | 23.1 ± 1.2[b] | 490.3 ± 5.2[b] | 95.4 ± 26.1 | 80.8 ± 3.9 | 18.7 ± 0.9 | 22.4 ± 2.4 |
| Low-fat, Step II diet | 5.02 ± 0.65[b] | 3.29 ± 0.60[b] | 1.11 ± 0.26[b] | 1.30 ± 0.16 | 52.5 ± 1.3 | 23.3 ± 1.5[a,b] | 493.1 ±5.4[b] | 85.3 ± 20.0 | 79.8 ± 2.7 | 18.3 ± 0.9 | 23.6 ± 2.1 |
| Superspread Study ($n$ = 28) | | | | | | | | | | | |
| Average American diet with stanols | 5.18 ± 0.09 | 3.16 ± 0.07 | 1.35 ± 0.07 | 1.45 ± 0.11 | 49.0 ± 4.6 | 27.6 ± 1.4 | 456.9 ± 12.3 | 70.2 ± 10.7 | 71.0 ±1.7 | 21.6 ± 0.9 | 31.1 ± 1.5 |
| Average American diet without stanols | 5.34 ± 0.11 | 3.57 ± 0.09 | 1.21 ± 0.04 | 1.18 ± 0.09 | 45.3 ± 4.2 | 25.2 ± 1.4 | 453.0 ± 13.3 | 65.8 ± 10.5 | 76.7 ± 2.4 | 22.7 ± 1.1 | 29.8 ± 1.3 |
| Peanut Study ($n$ = 20) | | | | | | | | | | | |
| Average American diet | 5.41 ±0.21[a] | 3.52 ± 0.25[a] | 1.29 ± 0.15[a] | 1.33 ± 0.11[a] | 57.3 ± 2.6[a] | 27.7 ± 1.2[a] | 509.6 ± 13.9 | 52.6 ± 9.7 | 79.1 ± 3.1 | 18.4 ± 1.1[a] | 23.8 ± 1.8[a,b] |
| Low-fat, Step II diet | 4.92 ± 0.23[b] | 3.01 ± 0.20[b] | 1.24 ± 0.10[b] | 1.48 ± 0.12[b] | 66.3 ± 2.9[b] | 26.2 ± 1.2[a,b] | 496.3 ± 13.8 | 42.2 ± 8.0 | 76.6 ± 2.8 | 19.3 ± 1.4[a] | 26.0 ± 2.4[a,b] |
| Olive oil diet | 4.79 ± 0.26[b] | 2.98 ± 0.22[b] | 1.28 ± 0.11[a,b] | 1.15 ± 0.12[b] | 66.0 ± 2.2[b] | 23.9 ± 1.0[b] | 501.6 ± 14.9 | 48.0 ± 8.7 | 76.8 ± 2.7 | 16.4 ± 1.0[b] | 21.3 ± 1.2[b] |
| Peanut oil diet | 4.93 ± 0.20[b] | 3.13 ± 0.18[b] | 1.26 ± 0.14[a,b] | 1.18 ± 0.14[b] | 63.0 ± 2.9[a,b] | 28.4 ± 1.1[a] | 518.1 ± 14.0 | 49.0 ± 11.1 | 75.2 ± 3.0 | 18.0 ± 1.4[a] | 24.9 ± 1.8[a,b] |
| Peanut butter diet | 4.82 ± 0.24[b] | 3.03 ± 0.21[b] | 1.26 ± 0.10[a,b] | 1.16 ± 0.11[b] | 66.2 ± 3.6[b] | 29.0 ± 1.32[a] | 511.9 ± 15.1 | 51.8 ± 10.8 | 77.6 ± 3.3 | 20.4 ± 1.0[a] | 27.3 ± 2.2[a] |

[1] $\bar{x}$ ± SE. TC, total cholesterol; LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triacylglycerol; TIBC, total-iron-binding capacity. Values within a column with different superscript letters are significantly different, $P < 0.05$ (ANOVA).

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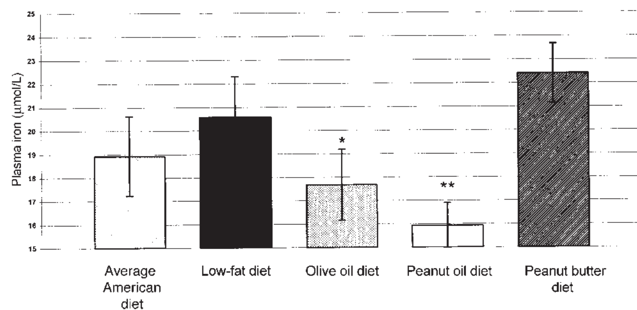


**FIGURE 1.** Mean (± SE) plasma iron concentrations in men ($n$ = 9) during 5 dietary periods in the Peanut Study. *,**Significantly different from the peanut butter diet (Tukey-Kramer test): $^{*}P < 0.05$, $^{**}P < 0.01$.

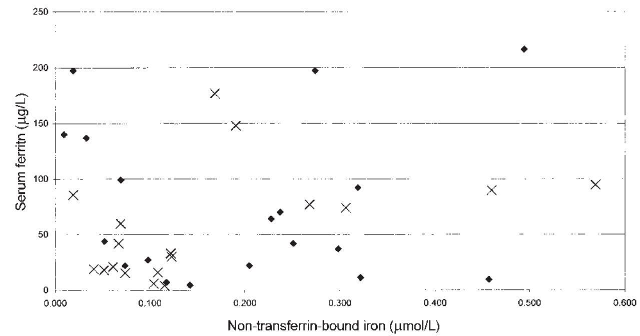


**FIGURE 2.** Scatter plot of non-transferrin-bound iron against serum ferritin from the Peanut Study ($n$ = 18 for each diet period plotted). ◆, Data collected in the first feeding trial; ×, data collected in the second feeding trial. There was no significant relation between these 2 variables when either feeding period was considered separately or when both were considered together ($P > 0.05$).

individuals with higher levels of LDL oxidative susceptibility, nor were higher concentrations of iron associated with increased oxidative risk.

Although little conclusive evidence exists to support a role of ferritin or plasma iron in CVD risk, it seems plausible that free iron would be increased with elevated ferritin and potentially contribute to oxidative stress. In normal healthy cells, NTBI exists in very low concentrations. Iron is both transported by transferrin [Ka (binding affinity constant) = $10^{15}$] and stored in a ferric state that is difficult to reduce. However, the superoxide radical is capable of reducing ferritin iron to the reactive ferrous state (41). On reduction, superoxide or hydrogen peroxide is capable of releasing the metal ions to a free form that may participate in forming highly reactive hydroxyl radicals (42). Studies have correlated elevated body iron and ferritin with increased amounts of NTBI (43, 44). In contrast, we found no such relation between the NTBI concentration and serum ferritin in a limited range. This correlation may only exist in disease states of iron overload such as hemochromatosis or iron release in reperfusion after ischemia. In fact, all of the studies reporting a correlation between NTBI and ferritin were conducted in subjects with β-thalassemia and idiopathic hemochromatosis. Thus, elevated NTBI in diseases of iron overload may not necessarily represent the effect of elevated body iron in the general population. Our feeding studies did not include large numbers of people with high ferritin concentrations (> 200 μg/L), nor did we determine the amount of iron contained within the serum ferritin molecules. In nondiseased situations, the amount of iron within the core of plasma ferritin is very low and unlikely to contribute to iron-based lipid peroxidation.

In contrast with still unclear roles of dietary iron and iron status in lipid peroxidation, the type and amount of dietary fat are important determinants of LDL oxidative susceptibility (25, 26, 45–47). The DELTA, Peanut, and Superspread studies were originally designed, in part, to evaluate whether the composition or quantity of dietary fats affects LDL oxidative susceptibility. Studies indicate that reducing dietary fat and substituting saturated and polyunsaturated fatty acids for monounsaturated fatty acids may effectively reduce LDL oxidation and CVD risk (25). All 3 studies reported an increased LDL oxidative susceptibility with diets high in saturated fat as opposed to lower-fat and high-monounsaturated-fat diets. These effects, however, did not appear to be affected by body iron stores. In the Peanut Study, a significant difference between plasma iron concentrations in men was seen between the 5 dietary periods, suggesting that diet may have some effect on body iron homeostasis. However, the highest concentrations of plasma iron noted in the peanut butter diet had a significantly longer lag time than did concentrations in the AAD (29; RL Morgan et al, unpublished observations, 1998). If iron did augment oxidative stress, as proposed by the iron-heart hypothesis, we would expect that the diet with the greatest mean iron concentration would correspond to a higher degree of LDL oxidation. However, the AAD, which had the greatest oxidative susceptibility, did not have significantly different plasma iron or serum ferritin concentrations from those seen with the other diets. Moreover, men who consumed the peanut butter diet had the highest concentrations of plasma iron, yet there was no effect on LDL oxidative susceptibility. Thus, our data suggest that there is no relation of iron status to LDL oxidative susceptibility. It is possible that longer time periods are necessary for dietary lipids to interact with circulating iron and alter the potential for oxidation of LDLs. However, considering that plasma iron turnover occurs in minutes and that the Fenton reaction is also very rapid, it is difficult to imagine that a feeding period longer than those used in the current study would have altered our findings.

In conclusion, this study does not support the hypothesis that iron affects LDL oxidative susceptibility in vivo. The well-controlled feeding studies discussed showed no relation between iron intake, free iron concentrations, and a measure of lipid peroxidation. In addition, no

**TABLE 2**
Standardized correlation coefficients (β weights) between measures of iron status and indexes of LDL oxidation in 3 controlled feeding trials[1]

| | Ferritin | Transferrin saturation | Plasma iron |
|---|---|---|---|
| LDL oxidation lag | 0.631 | 5.63 | 0.593 |
| LDL oxidation rate | 1.393 | 1.061 | 0.432 |
| LDL dienes | −0.076 | 1.52 | −0.487 |

[1] $n$ = 237 observations (samples from all subjects and from all diet periods of the 3 studies are combined). β Weights were determined after entry of diet and sex into the prediction equation; none were significant ($P <$ 0.05). Separate analysis of each of these relations of LDL oxidation indexes with iron-status measurements were performed individually in age and study cohorts, and no significant relations were observed. Maximum $R^2$ = 0.073; minimum $R^2$ = 0.0003.

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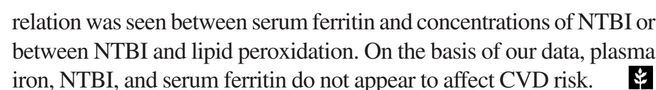

relation was seen between serum ferritin and concentrations of NTBI or between NTBI and lipid peroxidation. On the basis of our data, plasma iron, NTBI, and serum ferritin do not appear to affect CVD risk.

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