Aspirin intake and the use of serum ferritin as a measure of iron status1–4

Diana J Fleming, Paul F Jacques, Joseph M Massaro, Ralph B D’Agostino Sr, Peter WF Wilson, and Richard J Wood

ABSTRACT

Background: Atherosclerosis, a primary cause of myocardial infarction (MI), is an inflammatory disease. Aspirin use lowers risk of MI, probably through antithrombotic and antiinflammatory effects. Because serum ferritin (SF) can be elevated spuriously by inflammation, reported associations between elevated SF, used as an indicator of iron stores, and heart disease could be confounded by occult inflammation and aspirin use if they affect SF independently of iron status.

Objective: We tested the hypothesis that aspirin use is associated with reduced SF.

Design: We used analysis of covariance to investigate the relation between SF and categories of aspirin use in 913 elderly participants aged 67–96 y in the Framingham Heart Study.

Results: After adjustment for sex, age, body mass index, smoking, alcohol use, concentrations of C-reactive protein and liver enzymes, white blood cell count, and use of nonaspirin nonsteroidal antiinflammatory drugs and other medications, subjects who took >7 aspirins/wk had a significantly lower (by 25%) geometric mean SF than did nonusers, who took <1 aspirin/wk (71 compared with 95 μg/L, respectively; \( P \) for trend = 0.004). This effect of aspirin on SF was more marked in diseased subjects than in healthy subjects (mean SF was 50% lower compared with 21% lower, respectively).

Conclusions: Aspirin use is associated with lower SF. We suggest this effect results from possible increased occult blood loss and a cytokine-mediated effect on SF in subjects with inflammation, infection, or liver disease. The relations between aspirin, inflammation, and SF may confound epidemiologic associations between elevated SF, as an indicator of iron stores, and heart disease risk.

KEY WORDS Aspirin, serum ferritin, myocardial infarction, elderly, cytokine, C-reactive protein, inflammation, iron stores, epidemiology, atherosclerosis, antiinflammatory

INTRODUCTION

It was suggested that elevated body iron stores are a risk factor for heart disease (1, 2). This idea was supported by the results of 2 prospective studies showing that subjects with moderate serum ferritin (SF) concentrations (≥200 μg/L) had an ≈2-fold greater factor-adjusted risk of acute myocardial infarction (MI) than did subjects with SF <200 μg/L (3, 4). However, this association between elevated iron stores and heart disease is controversial because it was not found in 4 other prospective studies that also used SF as a measure of iron stores (5–9). SF is a commonly used measure of iron status that accurately reflects total body iron stores under most circumstances (10–13). However, a major drawback to its use in estimating body iron stores is that ferritin synthesis and secretion by hepatic cells can be increased by inflammatory cytokines (14), which could result in increased blood SF concentrations that do not reflect actual increases in iron stores.

It is currently believed that aspirin plays a pivotal role in the pathogenesis of heart disease (15, 16). Aspirin is a commonly used over-the-counter medication that lowered the risk of a first MI in subjects enrolled in a large clinical trial (17). Independent of its action in lowering heart disease risk, aspirin may also reduce body iron stores because of increased risk of bleeding, a well-known adverse effect associated with its use (18–23). Three studies investigated changes in hemoglobin concentrations in conjunction with aspirin use, but the results of these studies were inconclusive (24–26). Although 2 of these studies (24, 25) found an apparent decrease in hemoglobin concentration in aspirin-treated subjects, mean hemoglobin concentrations remained within the normal reference range during the limited period of observation in these iron-replete subjects. Furthermore, hemoglobin concentration is the last iron index to change in uncomplicated iron deficiency, and thus it may not provide information about early stages of iron stor-
age depletion, which instead is reflected by decreased SF concentration. A possible adverse effect of aspirin use on body iron stores was suggested recently by a study of Danish men aged 40–70 y; aspirin users had significantly lower SF than did nonusers (27). However, in that study, other factors that might influence SF concentrations were not addressed.

Thus, we undertook the analyses described in this article to test the hypothesis that regular aspirin users among the elderly men and women in the Framingham Heart Study cohort have significantly lower SF concentrations than do nonusers of aspirin. In this analysis we controlled for potential confounding influences of chronic diseases and use of other medications known to affect blood measures of iron status.

SUBJECTS AND METHODS

Study population

The Framingham Heart Study is a longitudinal study of heart disease risk factors that was begun in 1948–1950 and is described in detail elsewhere (28). The procedures and protocols of the study were approved by the Institutional Review Board for Human Research at Boston Medical Center. The study population originally consisted of 5127 men and women aged 30–62 y who were selected largely at random from residents of Framingham, MA. Data collected in the study have included demographics, an extensive medical history including personal habits, a detailed physical examination, and various clinical and biochemical indexes. Subjects were invited for follow-up examinations every 2 y to screen for the development of disease and to measure changes in clinical, biochemical, and behavioral variables.

A total of 1401 surviving members of the original cohort, now aged 67–96 y, participated in cycle 20 of data collection between February 1988 and January 1990. All materials and data used for the present analysis were collected at the cycle 20 examination. Serum samples were not sufficient for measuring either C reactive protein (CRP) or iron indexes for 385 subjects, and 55 persons were missing information on aspirin use, so the sample size was reduced to 961. The rationale and sequence for exclusions are described below and resulted in a final reduction of the sample to 913 subjects.

Biochemical indexes

Blood samples were collected from nonfasting subjects by venipuncture into evacuated EDTA-coated tubes. One day after collection, the samples were received at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, where clinical chemistry analyses were performed. Serum aliquots were stored in trace-mineral-free Nunc vials (Nalge Nunc International Corp, Naperville, IL) at −20°C.

SF concentration was measured by using the Magic Ferritin 125I radioimmunoassay (Ciba Corning, Norwood, MA). In our laboratory, assay of the World Health Organization International Ferritin Standard 80/578 with this radioimmunoassay yielded mean ferritin values that were within 5–10% of the stated concentrations of this quality control. The rate of degradation of ferritin in specimens stored at −20°C is <0.3%/y (29). Because the Framingham cycle 20 serum samples were stored at −20°C for 3–5 y before we assayed for ferritin, this translates into a small practical effect (0.9–1.5%) on the original values.

CRP, which is used as an inflammatory index (30, 31), was measured by using an immunoturbidimetric method with a CRP SPQ Test System Antibody Reagent Set II (INSCSTAR, Stillwater, MN) on a Cobas Fara II Centrifugal Analyzer (Roche, Nutley, NJ). Because the detection limit of our CRP assay was 6 mg/L, we defined inflammation as a CRP concentration ≥6 mg/L. Recently, ultrasensitive CRP assays with detection limits as low as 0.05 mg/L were developed; these assays can detect low-grade inflammation (30, 32). Because our detection limit was 6 mg CRP/L, we clearly missed identifying those subjects with mild inflammation.

Concentrations of the liver enzymes alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase were measured by using an in vitro diagnostic reagent system (Roche) on a Cobas Fara II Centrifugal Analyzer (Roche). A System 9000 Diff Model Automated Cell Counter (Serono-Baker Diagnostics Inc, Allentown, PA) was used to measure white blood cell count in whole blood samples.

Exclusions

Blood ferritin concentrations can be elevated in conditions of pathologic iron overload. The most common inherited form is hereditary hemochromatosis, an autosomal recessive disorder characterized by increased iron absorption that affects ≈1 in 300 persons in populations of northern European descent (33). We identified those subjects with the highest probability of being homozygous for hemochromatosis as those with all 3 of the following abnormal iron indexes: SF >300 μg/L, serum iron >32 μmol/L (180 μg/dL), and transferrin saturation >0.50 (50%) (34, 35). Three individuals met these criteria and were excluded from our analyses. Various heterozygous genotypes for the HFE gene can influence iron stores (36–38). Because we did not analyze the genotypes of the subjects, the effect of these heterozygous genotypes on SF in this population is unknown. An additional female subject with a SF concentration of 934 μg/L was excluded because she had unusually high values for hemoglobin (203 g/L), hematocrit (0.59, or 59%), and red blood cell count (6.7 × 1012/L, or 6.7 × 10¹²/mm³). She may have had polycythemia vera, a neoplastic stem cell disorder of unknown cause. Associated primarily with excessive proliferation of red blood cell precursors, it is typically characterized by elevated hemoglobin and hematocrit with or without an abnormal red blood cell count (39).

Anemia of chronic disease (ACD) is a mild to moderate form of anemia that is hypochromic and often microcytic. ACD accompanies most acute and chronic conditions involving inflammation, infection, liver disease, and malignancy (30, 31, 40–43). It is characterized by both impaired iron metabolism and impaired erythropoiesis. Although the precise mechanisms underlying ACD are still unknown, research suggests that both aspects of its pathogenesis are cytokine mediated (40–43). ACD generally mimics the hematologic profile of iron deficiency anemia, except that it involves a normal or elevated SF concentration in contrast with the decreased SF observed in uncomplicated iron deficiency (40–44). The increased SF concentration results from the fact that ferritin is a positive acute-phase protein whose synthesis and secretion by hepatic cells are increased by inflammatory cytokines (14). Thus, although SF typically reflects body iron stores (10–13, 45), during various diseases and conditions including malignancy (30, 31) it becomes an unreliable indicator of iron status because its blood concentrations become disproportionately elevated compared with actual iron stores (42–44).
46). Because of the many different types of cancer and the potentially varying, unknown effects of different malignancies on SF concentrations, we excluded subjects with active cancer at exam 20 (n = 53; 5%) from our analyses.

Data analysis

To collect data about aspirin use, we asked subjects the question “Number of aspirins per week?” Their responses were classified into 4 categories: nonusers (<1 aspirin/wk; n = 609; 66.7%), 1–6 aspirins/wk (n = 90; 9.9%), 7 aspirins/wk (n = 131; 14.3%), and >7 aspirins/wk (n = 83; 9.1%). Information about the dose of aspirin used was not obtained. We assumed that our elderly subjects used the commonly available standard-dose adult aspirin containing ~325 mg acetylsalicylic acid/tablet.

Our analysis included several covariates previously shown to be associated with SF. Ferritin concentrations are higher in women in this population were elderly, with a mean age of 56. It is also well known that use of both estrogen and progestosterone may cause breakthrough bleeding in postmenopausal women, and thus may be a potential confounder of SF values in our elderly female subjects. We consider this to be of small importance varying, unknown effects of different malignancies on SF concentrations, we excluded subjects with active cancer at exam 20 (n = 53; 5%) from our analyses.

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Iron stores are also influenced by excess blood loss (54). Therefore, we adjusted for the potential confounding effects of various medications other than aspirin that are known to increase the possibility of bleeding, although these potential effects have not been shown to be associated with ferritin concentrations. We created a yes-or-no variable for use of medications, within the past year, that are in our model because previously we found that SF concentrations were positively correlated with body size as measured by BMI in this population (53). Smoking status during the past year was included in the model because of its possible influence on iron stores (49). The smoking variable was created as a yes-or-no variable and included cigarette, pipe, and cigar smokers.

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TABLE 1
Characteristics of subjects in the normal and diseased groups

<table>
<thead>
<tr>
<th></th>
<th>Total population (n = 913)</th>
<th>Normal group (n = 790)</th>
<th>Diseased group (n = 123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (%)</td>
<td>40</td>
<td>40</td>
<td>39.8</td>
</tr>
<tr>
<td>Age (y)</td>
<td>76.1 ± 0.19^2</td>
<td>76.0 ± 0.02</td>
<td>76.8 ± 0.57</td>
</tr>
<tr>
<td>BMI (in kg/m^2)</td>
<td>26.7 ± 0.16</td>
<td>26.6 ± 0.17</td>
<td>27.5 ± 0.54</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>13.0</td>
<td>12.8</td>
<td>14.6</td>
</tr>
<tr>
<td>Alcohol users (%)</td>
<td>51.0</td>
<td>51.7</td>
<td>48.8</td>
</tr>
<tr>
<td>Aspirin use (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median intake</td>
<td>33.3</td>
<td>33.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Range of aspirins/wk</td>
<td>1–84</td>
<td>1–56</td>
<td>1–84</td>
</tr>
<tr>
<td>NSAID (%)</td>
<td>14.5</td>
<td>15.4</td>
<td>9.0</td>
</tr>
<tr>
<td>Antiplatelet drugs</td>
<td>7.7</td>
<td>7.1</td>
<td>11.4</td>
</tr>
<tr>
<td>Antiulcer drugs (%)</td>
<td>3.4</td>
<td>3.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Anticoagulants (%)</td>
<td>2.6</td>
<td>2.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Serum ferritin (μg/L)</td>
<td>88 (82.7, 93.7)^3</td>
<td>87 (81.1, 92.7)</td>
<td>97 (82.0, 115.1)</td>
</tr>
</tbody>
</table>

^1 There were no significant differences between the normal and diseased groups for any of the variables shown. Means and prevalence estimates were determined on the basis of nonmissing data. Missing values include 73 for BMI (56 and 17 in the normal and diseased groups, respectively), 2 for alcohol use in the normal group, 4 for antiplatelet medication (3 and 1 in the normal and diseased groups, respectively), 3 for NSAID (2 and 1 in the normal and diseased groups, respectively), and 6 for anticoagulant agents (4 and 2 in the normal and diseased groups, respectively). We used chi-square analysis to compare proportions between groups (and Fisher’s exact test when ≥25% of expected cell counts were <5) or Student’s t test to compare means between groups. NSAID, nonsteroidal antiinflammatory drugs.

^2 x ± SE.

^3 Geometric mean with 95% CI in parentheses.

RESULTS

Characteristics of the total sample

Characteristics of our study population are shown in Table 1. Fifty-one percent of subjects used medications associated with risk of increased blood loss (aspirin, nonaspirin NSAID, antiplatelet drugs, and anticoagulants) or medications used to treat conditions involving risk of blood loss (antiulcer agents). Of these subjects, 81.5% used a medication from 1 of the drug categories, 16.7% used medications from 2 categories, 1.5% used medications from 3 categories, and 1 subject used medications from 4 categories. In our population, 6.5% (n = 59 of 913) had an inflammatory condition, 3.7% (n = 34 of 913) had abnormally elevated liver enzymes, and 4.2% (n = 38 of 903) had an infection. Of these 131 subjects, 88.5% (n = 116) had 1 condition, 4.5% (n = 6) had 2 conditions, and 0.8% (n = 1) had 3 conditions. Thus, 13.5% (n = 123) of the total sample composed the diseased group. The SF concentrations of these subjects were likely to be elevated, disproportionate to actual iron stores, as a result of underlying disease conditions. In the total sample, men had a significantly higher geometric mean SF (112.7 μg/L; 95% CI: 102.3, 124.1) than did women (74.7 μg/L; 95% CI: 69.0, 80.8; P = 0.0001).

Aspirin use and mean SF concentrations in the total population

The results of the analysis of covariance for aspirin use and SF in the total population are shown in Table 2. After adjustment for sex, age, BMI, smoking status, alcohol intake, use of nonaspirin NSAID and other medications associated with blood loss, inflammation, infection, and possible liver disease, those who used >7 aspirins/wk had a significantly lower (by 25%) mean SF (71 μg/L; 95% CI: 57, 87) than did nonusers of aspirin (95 μg/L; 95% CI: 88, 103). There was a highly significant, inverse linear trend between aspirin use and SF (P = 0.004). Adjustment for covariates had little effect on the association between aspirin use and SF (data without adjustment for covariates are not shown). The 3 possible interactions between aspirin use and 1) inflammation, 2) infection and liver disease combined, and 3) inflammation, infection, and liver disease combined (ie, the diseased group) were not significant. However, the interaction between the diseased group and aspirin use was marginally significant (P = 0.07). This prompted us to examine the relation between SF and aspirin use in the normal and diseased groups separately.

Comparison of characteristics between the normal and diseased groups

As shown in Table 1, there were no significant differences between the normal and diseased groups in the proportions of men and women or the percentages of subjects who were smokers, alcohol users, or users of aspirin, nonaspirin NSAID, antulcer medications, antiplatelet drugs, or anticoagulants. Furthermore, mean age and BMI did not differ significantly between the groups. The range of aspirin intake was 1–56 aspirins/wk in the normal group and 1–84/wk in the disease group. Median aspirin intake in the category of subjects who took >7 aspirins/wk was the same for both groups: 14 aspirins/wk, or 2/d. The diseased group had a higher geometric mean SF concentration than did the normal group (97 and 87 μg/L, respectively), but this difference was not significant.

Aspirin use and mean SF concentrations in the normal and diseased groups

There was an inverse association between SF concentration and aspirin intake in both the normal group and the diseased group; the relation was stronger in the diseased group (Figure 1, A and B; Table 2). In the normal group, there was a marginally significant difference (P = 0.06) between the adjusted mean SF
values of 65 μg/L in the >7 aspirins/wk category and 82 μg/L in the nonusers category; the former mean value is 21% lower than the latter. Also in the normal group, the adjusted mean SF concentrations in the 1–6 aspirins/wk and 7 aspirins/wk categories (78 and 75 μg/L, respectively) were not significantly different from the mean for nonusers. In the diseased group, those using >7 aspirins/wk had a significantly lower (by 50%) adjusted mean SF concentration than did the nonusers (59 compared with 119 μg/L, respectively; \( P = 0.02 \)). In addition, there was a marginally significant difference (\( P = 0.06 \)) between the mean SF of subjects using 7 aspirins/wk (74 μg/L) and that of the nonusers.

Differences in adjusted mean SF concentrations between the normal and diseased groups for each aspirin-use category are shown in Table 2; for nonusers, this difference was –37 μg/L (normal – diseased).

DISCUSSION

These findings clearly show an inverse association between aspirin use and SF concentrations in the free-living, elderly Framingham Heart Study cohort. Our results are consistent with those of Milman et al (27), who found that among 1146 Danish men aged 40–70 y who were not blood donors, those taking aspirin (\( n = 170 \)) had significantly lower median SF concentrations than did aspirin nonusers (136 and 169 μg/L, respectively). However, 57% of the aspirin users took the standard dose of aspirin used in Denmark, 1–3 g acetylsalicylic acid/d, which is 3–9 times the standard US dose of 325 mg. Because it is well known that aspirin’s adverse effects with regard to blood loss are dose dependent, the lower SF observed in Danish aspirin users could be a result of increased blood loss associated with use of high doses of aspirin, and thus may not be observed in other populations. Moreover, no attempt was made by the investigators to account for potential influences on SF other than aspirin. Our data from this US population extend the observations of Milman et al (27) by examining the association between SF and categories of aspirin intake in elderly men and women while controlling for potential confounding factors, such as age (47), BMI

**TABLE 2**

<table>
<thead>
<tr>
<th>Aspirin use</th>
<th>Total population (( n = 913 ))</th>
<th>Normal group (( n = 790 ))</th>
<th>Diseased group (( n = 123 ))</th>
<th>Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1/wk (nonusers)</td>
<td>95 (88, 103)</td>
<td>82 (75, 89)</td>
<td>119 (96, 146)</td>
<td>–37</td>
</tr>
<tr>
<td>1–6/wk</td>
<td>86 (71, 105)</td>
<td>78 (63, 97)</td>
<td>77 (47, 126)</td>
<td>–1</td>
</tr>
<tr>
<td>7/wk</td>
<td>82 (70, 97)</td>
<td>75 (62, 89)</td>
<td>74 (48, 114)</td>
<td>–1</td>
</tr>
<tr>
<td>&gt; 7/wk</td>
<td>71 (57, 87)</td>
<td>65 (51, 81)</td>
<td>59 (35, 99)</td>
<td>–6</td>
</tr>
</tbody>
</table>

*In the normal and diseased groups, serum ferritin concentrations were adjusted for sex, age, BMI, smoking status, alcohol use, and use of antiulcer, antiplatelet, anticoagulant, and nonaspirin nonsteroidal antiinflammatory medications. In the total population, we adjusted for these 9 factors plus inflammation, infection, and possible liver disease.

†Difference in adjusted geometric mean serum ferritin concentrations between the normal and diseased groups (normal – diseased).

‡Adjusted geometric mean with 95% CI in parentheses.

FIGURE 1. Adjusted geometric mean serum ferritin concentrations (with 95% CI) by category of usual aspirin intake for the normal group (\( n = 790 \); A) and the diseased group (\( n = 123 \); B). The means were adjusted for sex, age, BMI, smoking status, alcohol use, and use of antiulcer, antiplatelet, anticoagulant, and nonaspirin nonsteroidal antinflammatory medications. The median number of aspirins used by the subjects in each aspirin-intake category are plotted on the x axis for each group. These medians are the same for the normal and diseased groups in all aspirin-intake categories except for the category of 1–6 aspirins/wk. The medians are as follows: for nonusers (<1 aspirin/wk), 0 aspirins; for 1–6 aspirins/wk, 3 aspirins for normal group and 3.5 aspirins for diseased group; for 7 aspirins/wk, 7 aspirins; and for >7 aspirins/wk, 14 aspirins.
An obvious explanation for the observed inverse association between aspirin use and SF concentration could be lower body iron stores resulting from increased occult blood loss (not measured in this study) associated with aspirin use. The increased risk of blood loss in aspirin users results from the 2 well-known, major side effects of aspirin: gastrointestinal irritation and an increased bleeding tendency (18, 20–22, 58–60). The inverse association between aspirin intake and SF concentration that we observed in the total population was much stronger in the subgroup of diseased elderly subjects with underlying inflammation, infection, liver disease, or more than one of these conditions than in the rest of the population, or normal group (Figure 1, B compared with A, respectively). A simple explanation for the stronger inverse association in the diseased group than in the normal group cannot be found in either differences in subject characteristics or patterns of aspirin use. We propose a cytokine-mediated mechanism that does not involve blood loss to explain this relation between aspirin use and SF. First, in those elderly subjects who did not use aspirin, the 45% higher adjusted mean SF concentration in the diseased group than in the normal group most likely reflects spuriously elevated SF concentrations resulting from underlying pathology. That is, because of the presence of disease, blood ferritin concentrations were likely increased as a result of cytokine-mediated synthesis and secretion of ferritin from the liver, and thus it is unlikely that these SF concentrations reflected higher iron stores. Second, the minimal difference in adjusted mean SF concentrations between the diseased and normal groups among aspirin users is consistent with a lowering of cytokine-elevated SF concentrations in the diseased group subsequent to the antiinflammatory effect of aspirin. We speculate that in the diseased group, aspirin intake was sufficient to decrease the presence of inflammation and inflammatory cytokines in the body, reducing blood ferritin concentrations that had been elevated previously in an iron-independent manner via cytokine-induced ferritin gene expression (14). In our cohort, this idea was supported by additional analyses (data not shown) indicating that among those individuals in the diseased group who had a detectable CRP concentration (≥6 mg/L), the geometric mean CRP of aspirin users was significantly lower than that of subjects who did not use aspirin (8.9 compared with 12 mg/L; P = 0.05, Student’s t test). Furthermore, of all the elderly subjects with an elevated CRP concentration (n = 59), those individuals with CRP values ≥10 mg/L, the median value for the group, had a nonsignificantly higher geometric mean SF (117 μg/L) than did those subjects with CRP values below the median (86 μg/L). Although these limited observations provide only weakly suggestive evidence of an antiinflammatory effect of aspirin that subsequently might reduce cytokine-induced elevations in SF concentrations, one recently published report from a clinical trial supports this notion. Ikonomidis et al (61) investigated the effects of a 300-mg daily dose of aspirin on cytokines and CRP in a 6-wk randomized, double-blind, placebo-controlled crossover trial in 40 patients (mean age 55 y) with atherosclerotic disease and clinically stable angina. The authors observed that after wk 6 of aspirin administration, there were significant reductions in proinflammatory cytokine (interleukin 6, or IL-6) and CRP concentrations in plasma. Interestingly, the authors speculated that “the reduction of CRP by aspirin is likely secondary to the reduction in IL-6, because IL-6 is known to stimulate the synthesis of acute phase proteins by the liver.” Because ferritin is also a positive acute phase protein (31), we suggest that the reduction of circulating proinflammatory cytokines by aspirin would also result in a reduction in plasma ferritin concentrations that had been elevated previously because of underlying inflammatory processes rather than higher iron stores.

Thus, we believe that the observed inverse association between SF concentration and aspirin use is likely a result of the combined effects of 2 processes: 1) a probable lowering of body iron stores resulting from increased occult blood loss in chronic aspirin users, and 2) a less obvious mechanism involving a differential reduction in SF concentrations in diseased subjects because of the antiinflammatory action of aspirin on cytokine-induced ferritin production.

We believe that the inverse association between aspirin intake and SF concentration has implications for epidemiologic studies investigating the relation between elevated body iron stores and risk of heart disease, and possibly other chronic diseases for which underlying chronic inflammation is a pathogenic factor. First, as illustrated in Figure 2A, we suggest that inflammation is likely a true confounder of the association between elevated iron stores and risk of MI. It is now widely accepted that atherosclerosis, the principal contributor to the pathogenesis of MI, is an inflammatory disease—a response to injury of the vascular endothelium (16). Independent of its involvement in MI risk,
inflammation also increases the blood concentration of ferritin as a positive acute phase protein, as previously discussed (14). Because the chronic, low-grade inflammation of atherosclerosis may be present throughout a person’s lifetime (16), and therefore years before symptomatic coronary heart disease, we believe that the SF concentrations in epidemiologic studies investigating the association between elevated iron stores and risk of MI could be confounded by being spuriously elevated because of the chronic, mild inflammation of atherosclerosis, other inflammatory diseases, or both. This could occur even if the studies excluded prevalent cases of heart disease or individuals with a history of cardiovascular disease.

Second, the inverse association that we observed between aspirin intake and SF concentration suggests that in study populations in which aspirin use is common, aspirin may be an important confounder of the association between elevated iron stores, as measured by SF, and risk of MI (Figure 2B) because aspirin use is associated independently with both decreased SF (Figure 1) and decreased risk of MI (17, 62). Aspirin may lower SF concentrations through both blood loss and its antiinflammatory action. Thus, because aspirin users are more likely to have lower SF concentrations and a lower risk of heart disease, SF concentrations and risk of MI would be noncausally linked through the complex effects of aspirin on SF. Consequently, an association between moderately elevated SF concentrations and increased risk of MI could be observed in some study populations because of spuriously elevated SF concentrations as a result of underlying inflammation (from atherosclerosis or other processes); these SF values would not truly reflect higher iron stores.

In conclusion, our study clearly shows that aspirin use in elderly Americans is associated with lower SF concentrations. It is uncertain whether these reduced SF concentrations actually indicate lower body iron stores because of the antiinflammatory effects of aspirin and the fact that ferritin is an acute phase protein whose blood concentration is influenced by proinflammatory cytokines. We suggest that one possible way to eliminate potential confounding by aspirin use in epidemiologic studies of SF and chronic disease is to include only those subjects with an undetectable CRP as determined by an ultrasensitive CRP assay. To our knowledge, this has not been done in any of the prospective studies examining the association between elevated SF concentrations and risk of MI (3–9).

We thank Irwin Rosenberg and Gerard E Dallal of Tufts University for their inspiration and encouragement in investigating this topic. We also thank the Nutrition Evaluation Laboratory at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University for their invaluable biochemical analyses.

REFERENCES
