Original Research

Effects of Iron Intake on Iron Stores in Elderly Men and Women: Longitudinal and Cross-Sectional Results

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Objective: For middle aged and elderly subjects there is a concern that increased iron intake, especially heme iron associated with consumption of red meat, leads to increased iron stores resulting in disturbed glucose homeostasis and risk for cardiovascular disease and certain types of cancer. The aim of this study was to investigate the influence of heme, non-heme and iron supplementation on iron stores in healthy elderly men and women.

Method: We conducted a 10 year longitudinal study (48 men and 77 women) and a one year cross-sectional study (165 men and 226 women) in healthy elderly men and women enrolled in the New Mexico Aging Process Study. Iron stores were estimated by serum ferritin concentrations and iron intake was determined by three-day food records in the longitudinal study and by a food frequency questionnaire in the cross-sectional study.

Results: We found no association between heme iron intake and iron stores in either the longitudinal or cross sectional study. In the cross-sectional study we found in women, but not in men, that age and supplemental iron intake were significantly and positively associated with increased iron stores.

Conclusion: Iron stores in elderly men are thought to reach steady state levels where iron absorption is adjusted to a level just sufficient to cover basal iron losses. In elderly women, we speculate that not enough time has elapsed for postmenopausal women to reach steady state levels of iron stores resulting in increases in iron absorption with age. Another factor is that use of hormone replacement therapy could further delay some women in reaching steady state iron levels due to continued menstrual blood losses.

INTRODUCTION

The issue of whether increased iron intake, especially heme iron intake associated with consumption of red meat, leads to increased iron stores remains controversial, even after many years of research. This issue recently gained added attention, especially for middle-aged and elderly subjects, because of reported associations between moderate increases in body iron stores and risk for some age-related chronic diseases. These include disturbed glucose homeostasis [1], the insulin resistance syndrome [2] and cardiovascular disease in carriers of the hereditary hemochromatosis gene mutations [3,4].

The association between increased body iron stores and disturbed glucose homeostasis was found in a cross-sectional study conducted in 1,013 middle-aged Finnish men after adjusting for age, family history of diabetes, body mass index (BMI), waist hip ratio (WHR), alcohol consumption, physical activity, use of oral diabetes medications and use of diuretics [1]. The Finnish study was based on the hypothesis that, while iron accumulation has to be severe to induce organ damage that leads to diabetes, a lesser accumulation of iron can alter glucose and insulin homeostasis of the body.

Fernandez-Real *et al.* [2], in a cross-sectional study of NIDDM individuals, found that insulin resistance was independently related to serum ferritin levels in individuals without evidence of iron overload. However, this cross-sectional study did not enable Fernandez-Real *et al.* [2] to infer causality between serum ferritin and insulin resistance.

Elderly individuals, especially men, found to be heterozygous for the relatively common Cys282Tyr (G845A) and the His187Asp (H63D) mutations of the human hemochromatosis associated gene (HFE) have been shown to have moderately

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increased iron stores [5]. Since increased iron stores have been noted to be an independent risk factor for acute myocardial infarction [6,7], recent studies have examined whether individuals heterozygous for the Cys282Tyr mutation are indeed at increased risk for cardiovascular disease. Two European studies reported evidence that both men and women who are carriers of the HFE Cys282Tyr mutation have a two- to three-fold increased risk of cardiovascular disease [3,4].

With new evidence that even moderate increases in iron stores may increase the risk for developing certain age related chronic diseases, there has been renewed interest in re-examining the association between dietary iron intake and iron stores. Two recent studies addressed the issue of the effects of age, non-heme iron, heme iron and supplemental iron intake on iron stores in humans. The first study was that of Hallberg *et al.* [8], who used a direct method for examining iron absorption and its association with iron stores. These authors conducted a clinical trial in which iron absorption from the whole diet was measured for five days in 31 healthy men with a mean age of 29.4±10.8 years. Non-heme iron was labeled with an extrinsic iron tracer and heme iron was labeled similarly using hemoglobin biosynthetically labeled with a different radioiron tracer. Iron absorption was examined in relation to the amount of stored iron as estimated by serum ferritin levels. They found an inverse relation between total iron absorption and concentration of serum ferritin up to 60 μg/L. In subjects with serum ferritin levels $>$60 μg/L, Hallberg *et al.* found that iron absorption decreased to a level just sufficient to cover basal iron losses, implying that, at a serum ferritin concentration $>$60 μg/L, no further accumulation of iron stores will occur by dietary iron absorption and/or supplementation. Hallberg *et al.* also compared the absorption from non-heme and heme iron and concluded that the control of heme iron absorption is also very effective. Thus, the Hallberg *et al.* findings do not support a hypothesis that a high meat intake (implying a high heme iron intake) might lead to an accumulation of iron stores in normal men by bypassing the strong regulation of iron absorption.

The second study by Fleming *et al.* [9] in 1998 reported results that were opposite of that reported by Hallberg *et al.* [8]. Fleming *et al.* [9] investigated the consumption of various dietary components and iron stores in an elderly sample of the Framingham Heart Study participants. Serum ferritin was used as a measure of body iron stores in 634 free-living elderly men and women (67 to 93 years of age). Dietary intake during the previous year was assessed by a food-frequency questionnaire. After controlling for gender, age, body mass index, total energy intake, smoking and use of aspirin and other medications known to affect blood loss, they found four dietary factors that were significant associated with iron stores. Heme iron, supplemental iron, dietary vitamin C and alcohol were positively associated with serum ferritin. Fleming *et al.* [9] concluded that dietary factors, namely heme iron intake and iron supplementation, can influence the accumulation of iron stores in elderly subjects.

Based on the report of Fleming *et al.* [9], we decided to examine the effects of iron intake on iron stores in elderly men and women enrolled in the New Mexico Aging Process Study (NMAPS) from a longitudinal and cross-sectional perspective. In the longitudinal study, we used three-day food records to assess iron intake, while in the cross-sectional study, we used a food frequency questionnaire to assess usual iron intake. Iron stores were estimated using serum ferritin concentrations.

METHODS

Subjects

In 1979, 304 elderly persons (166 women and 138 men) were recruited for a study referred in the literature as the New Mexico Aging Process Study (NMAPS). Entrance to the NMAPS is limited to persons aged 60 years or older with no known serious medical conditions; for example, persons with diagnosed cancer, aortic stenosis, diabetes or transient ischemic attack were excluded. Persons with osteoarthritis or cataracts were included if they were not taking prescription medications for these or other ailments. Once accepted into the study, subsequently diagnosed illnesses and medication use did not result in the elimination of subjects from the study. The subjects' ages ranged from 60 to 93 years in 1980 with a median age of 72 years for both males and females. Entrance was not limited to any ethnic group but all volunteers were white; 3% were of Hispanic origin. More than 40% had college degrees. For a more complete description of the population, the reader is referred to a previous publication [10]. The volunteers were seen as outpatients in the General Clinical Research Center (GCRC) at the University of New Mexico Hospital. After an overnight fast, ~50 mL of blood was obtained from each person between 0800 and 0930 hours for various immunologic and biochemical measurements. The volunteers were seen on a yearly basis at approximately the same time each year. The entire population was seen between January and November at a rate of six individuals per week. For the longitudinal analysis we used three-day food records and laboratory measurements of iron status (CBC and ferritin) collected yearly from 1984 to 1993. Subjects selected for this study had assays in 1984 or 1985 and in 1993 and fewer than five missing years of dietary records. There were 125 subjects (77 women and 48 men) that met these criteria. For the cross-sectional analysis, we used laboratory results (CBC and ferritin) from 1993 and 1996 in which a total of 165 men and 276 women were administered a food frequency questionnaire (FFQ) (one FFQ per subject).

The Human Research Review Committee of the University of New Mexico School of Medicine approved this study. Informed consent was obtained from each participant.

Laboratory Measurements

Complete blood count measurements were conducted using a Coulter "S" instrument (Coulter Electronics, Hialeah FL). Serum ferritin determinations were performed using a two-site enzyme immunoassay (Ferrizyme Kit, Abbott Laboratories, Chicago, IL).

Dietary Intake Measurements for the Longitudinal Analyses

A research nutritionist instructed the volunteers on how to keep an accurate three-day food record. Each volunteer was asked to measure all food eaten for three consecutive weekdays, Monday through Friday, and record the intake on standard coding forms. Brand names, methods of food preparation and recipes for any mixed dish eaten during the period were also recorded. Commercial plastic food models were used as instructional aids to assist the volunteers in judging portion sizes. A diet scale and an instruction booklet designed for this study were also provided. This booklet stressed the need for accuracy, completeness and recording food items prepared from a recipe.

At the end of the three-day recording period, a research nutritionist visited the volunteers' homes to review the diet records. At this time, each record was subjectively evaluated for completeness and accuracy, and the participants were asked to provide additional information about any unclear food item. If the subject used vitamin or mineral supplements, the brand name, contents and amounts of each nutrient were recorded for inclusion in determining total intakes. All food records were coded by food item and amount and then analyzed for nutrient composition. Data for 1984 through 1989 were analyzed using the Highland View Hospital-Case Western Reserve University (CWRU) Nutrient Data Base, revision 6 (1982 ed). (Case Western Reserve University, Cleveland, Ohio). Data for 1990 were analyzed by the CWRU Nutrient Data Base release 10, 1989. Data for 1991 through 1993 were analyzed using the Food Intake Analysis System (FIAS), version 2.0 (University of Texas at Houston). When we compared 31, three-day food records, randomly selected from 250 records collected in 1991, using the CWRU and FIAS, the calculated group mean dietary iron intakes were not significantly different by paired *t* test, 14.4 ± 6.8 and 14.9 ± 6.7 mg/day, respectively, $r = 0.91$.

In addition, dietary data for 1984, 1987 and 1989 were used to analyze food groups. For this analysis, the records for these years were reanalyzed using CWRU data base release 10, 1989. Food codes for meat, poultry and fish consumption were identified in existing three-day diet records as previously described [11,12]. As noted in an earlier publication [11], less than 2% of dietary energy came from foods coded as mixed dishes containing meat, poultry or fish. This 2% included the vegetable, grain, dairy and other components of the mixed dishes as well as the meat, poultry and fish. Therefore, meat, poultry and fish intakes were analyzed with mixed dishes excluded, giving only a slight underestimate of meat, poultry and fish intake. Iron from meat, poultry and fish was used to estimate heme iron intake by assuming that 40% of meat, poultry and fish iron is heme iron [13].

Dietary Intake Measurements for the Cross-Sectional Analyses

We used an adaptation of the Health Habits and History Questionnaire (HHHQ) [14–16] that was administered to each participant in the NMAPS by a trained research nutritionist. The HHHQ was modified to include southwestern regional foods. As in the longitudinal study, iron from meat, poultry and fish was used to estimate heme iron intake by assuming that 40% of meat, poultry and fish iron is heme iron [13].

Estimates of Body Iron

For the longitudinal study, we estimated iron stores by the method of Cook, *et al.* [17]. Different levels of iron status require separate calculations to estimate body iron as described in detail in our recent publication [18]. For subjects with serum ferritin concentrations above 12 μg/L, the method reduces to the following equation:

$$\text{iron stories (mg)} = 400 \times (ln\ SF - 1n\ 12),$$

where 400 is the proportionality constant, ln is the natural logarithm and SF is serum ferritin in μg/L. All of the subjects in the longitudinal study had serum ferritin concentrations above 12 μg/L. We chose to estimate iron stores in milligrams in the longitudinal study so that direct comparisons could be made with other previous studies using the formula developed by Cook *et al.* [17]. For the cross-sectional analyses, we used serum ferritin concentrations *per se* to estimate iron stores so that direct comparisons could be made with previous results reported by Fleming *et al.* [9].

Statistical Methods

The longitudinal data set consisted of up to nine yearly measurements of iron intake and iron stores over the period 1984–1993 for each of 77 women and 48 men. Serum ferritin concentrations were not determined in 1991. A regression model was fit using the generalized estimating equation approach (GEE) of Liang and Zeger [19] to allow for the serial correlation between yearly measurements of iron stores. When using this method, it is necessary to specify a working correlation structure for the data. We used an exchangeable correlation structure, which assumes that the correlation is constant between any two yearly measurements of iron stores for an individual. This correlation is the same for all individuals. Observations between individuals are assumed to be independent. The distribution of iron stores is assumed to be normal. The regression model expressed iron stores as a linear function of gender, age, total caloric intake, body mass index and dietary

and supplemental iron intake. Dietary iron intake was categorized into quartiles, and supplemental intake was categorized into three levels. We also fit a similar model using the data from years 1984, 1987 and 1989 where we could separate dietary iron into heme and non-heme components.

The GEE approach was useful for investigating the association between iron stores and iron intake at a single point in time. For each individual, iron stores and iron intake were regressed on year to provide slope parameters that could be interpreted as the average yearly changes in iron stores and iron intake over the 10 year period. Similar slopes were computed for total caloric intake and body mass index.

A normal linear regression model was fit that expressed the slope for iron stores as a linear function of gender, age in 1984, slope for total caloric intake, slope for body mass index and separate slopes for dietary and supplemental intakes of iron. The predicted value for 1988 from the individual linear regressions was used as a summary measure for each person that would represent his or her typical value for the measure over this time period. 1988 was chosen because it is near the midpoint of the time interval 1984–1993. This value was close to the observed value in 1988. The predicted value has the advantage that it is available for all persons, while the observed value for 1988 is missing for some individuals.

For the cross-sectional analysis, we employed a multiple linear regression model in which non-dietary (age, gender, BMI) and dietary factors (calories from alcohol, vitamin C, energy, total iron intake from diet (heme and non-heme iron) and supplements were examined in relationship with serum ferritin levels. In our cross-sectional analysis we attempted to analyze our data in a manner similar to that conducted in the study of Fleming *et al.* [9].

All analyses were performed using procedures of the SAS software [20]. The GEE regression was computed using a SAS macro provided by Dr. Zeger.

RESULTS

Longitudinal Study

Table 1 provides summary statistics for the 77 women and 48 men age, BMI, ferritin, and iron stores. Energy, total iron intake, dietary and supplemental vitamin C and alcohol intake were determined using the previously described three-day food records. The values shown are the predicted values for 1988. The predicted value for 1988 from the individual linear regressions was used as a summary measure for each person that would represent his or her typical value for the measure over this time period with two exceptions. Heme and non-heme iron were only available for 1984, 1987 and 1989, and mean values were determined for these years.

Fig. 1 shows the distribution of iron stores of the men and women in the study. The values shown are the predicted iron stores for 1988 based on regression analyses of iron stores on year of collection. Mean (±SD) iron stores in men were 900±340mg and in women were 831±316 mg. Iron stores, expressed as geometric mean serum ferritin concentrations, were 113 and 96 μg/L for men and women, respectively. No significant difference was noted in iron stores between the men and women (t=1.159, p=0.248). The 95% population prediction interval for serum ferritin was 30 to 611 μg/L and 20 to 450 μg/L for men and women, respectively. Apart from slight variations in mean iron stores at yearly intervals, no meaningful increases or decreases were noted in iron stores over the 10-year period for either the men or the women.

Table 1. Descriptive Statistics for Women (n=77) and Men (n=48) in the Longitudinal New Mexico Aging Process Study 1984–1993, Predicted Values for 1988

	Women Mean±SD	Men Mean±SD
Age (years)	76.8±4.6	77.6±3.7
BMI (kg/m^2)	24.6±3.5	25.3±3.2
Ferritin (μg/L)*	95.6 (20.3–449.6)	113.3 (30.4–611.3)
Iron Stores (mg)	831±316	900±340
Energy (kcal/day)	1520±229	2048±441
Total Iron Intake (mg/day)	21.6±11.0	25.4±12.1
Dietary Iron (mg/day)	12.5±2.5	15.1±3.4
Heme Iron**	0.73±0.32	0.89±0.40
Non-Heme Iron**	11.5±3.1	15.1±7.2
Supplemental Iron	9.1±10.9	10.4±11.8
% Taking Iron Supplements	39%	42%
Dietary Vitamin C (mg/day)	134±47	142±52
% Taking Supplemental Vitamin C	59%	62%
Calories from Alcohol	35±72	85±110

* Geometric mean and 95% population prediction interval.

** Heme and non-heme iron are available for 1984, 1987 and 1989 only and for this reason do not sum to total dietary iron.

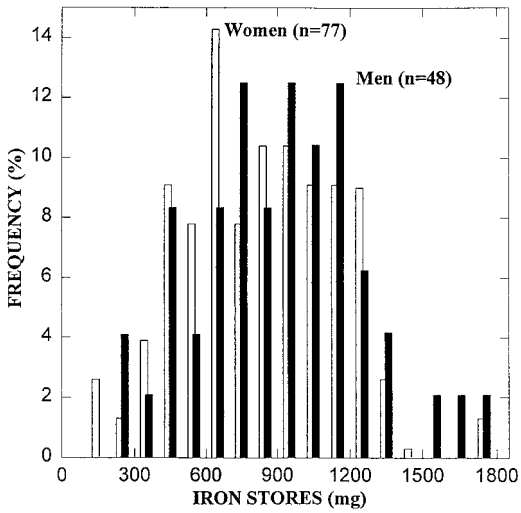

Fig. 1. Distribution of iron stores. Values shown are predicted for 1988 based on regression analyses of iron stores on year of collection.

Table 2 presents the results of the regression of iron stores on dietary and supplemental iron intakes using the GEE method. Predicted iron stores were significantly higher ($p=0.0069$) by about 50 mg for persons in the upper quartile of dietary iron intake then for persons in the lowest quartile. Persons in the second and third quartiles of iron intake had intermediate levels of predicted iron stores. Moreover, those consuming more than 18 mg of supplemental iron per day had predicted iron stores that were significantly higher ($p=0.0110$), approximately 65 mg more than those not consuming any iron supplement. Predicted iron stores did not differ significantly between men and women, by age or by total caloric intake. Body mass index had a marginally significant negative association with iron stores ($p=0.0694$).

A similar GEE regression model was computed for the years 1984, 1987 and 1989 where it was possible to separate dietary iron into heme and non-heme components. The results are given in Table 3. The results show no significant association between dietary iron intake and iron stores for either the heme or non-heme components. However, there was a significant positive association ($p=0.0428$) between supplemental iron intake and iron stores with persons consuming more than 18 mg of supplemental iron per day having 64 mg greater predicted iron stores than those not consuming supplemental iron.

Cross-Sectional Study

Table 4 provides summary statistics for the 276 women and 165 men where energy, total iron intake, dietary and supplemental vitamin C and alcohol intake were determined using the previously described food frequency questionnaire. When we grouped men and women together in a multiple regression model, we did not find any significant influence of calories from alcohol, total vitamin C intake and energy intake on serum ferritin levels. However, we found a significant age ($p=0.0180$), gender ($p=0.0002$), total iron intake ($p=0.0220$) and an interaction effect (gender by total iron intake) ($p=0.0019$) on serum ferritin levels. In the model shown (see Table 5), where we examined men and women separately and adjusted heme, non-heme and supplemental iron intake for energy intake, the only significant finding in men was a negative association of BMI on serum ferritin levels ($p=0.0444$). In women, we show a significant positive effect of age

Table 2. GEE Regression Using Iron Stores (mg) for the Years 1984–1993

Factor	Estimate	*p*-Value
Intercept*	916.4	
Female	−81.2	0.1917
Age (10 years)	−4.4	0.1193
Total caloric intake (kcal/day)	−21.2	0.3541
Body mass index (kg/m^2)	−10.8	0.0694
Dietary iron: quartiles (mg/day)		
[4.87, 10.15]	0.0	
[10.15, 12.73]	10.9	0.4179
[12.73, 15.66]	26.0	0.0941
[15.66, 32.06]	**52.4**	**0.0069**
Supplemental iron (mg/day)		
0	0.0	
[0, 18]	27.3	0.2018
[18, max]	**64.7**	**0.0110**

* Intercept is for a 65 year old male, total caloric intake of 1700 kcal/d, BMI of 25 kg/m^2, dietary iron intake of 4.87–10.15 mg/day and no supplemental iron intake.

Statistically significant findings are highlighted in bold.

Table 3. GEE Regression Using Iron Stores for the Years 1984, 1987 and 1989

Factor	Estimate	*p*-Value
Intercept*	949.4	
Female	−55.8	0.4194
Age (10 years)	**−7.8**	**0.0342**
Total caloric intake (kcal/day)	−3.0	0.9139
Body mass index (kg/m^2)	−4.9	0.4768
Heme iron: quartiles (mg/day)		
[0.00, 0.49]	0.0	
[0.49, 0.72]	−7.0	0.7870
[0.72, 1.05]	−4.8	0.8590
[1.05, 3.57]	−22.8	0.5117
Non-heme iron: quartiles (mg/day)		
[3.67, 8.48]	0.0	
[8.48, 11.21]	−0.2	0.9938
[11.21, 14.86]	47.1	0.1734
[14.86, 67.27]	40.8	0.2281
Supplemental iron (mg/day)		
0	0.0	
[0, 18]	28.9	0.4334
[18, 45]	**64.4**	**0.0428**

* Intercept is for a 65 year old male, total caloric intake of 1700 kcal/day, BMI of 25 kg/m^2, heme iron intake of 0.00–0.49 mg/day, non-heme iron intake of 3.67–8.48 mg/day and no supplemental iron intake.

Statistically significant findings are highlighted in bold.

Table 4. Descriptive Statistics for Women (n=276) and Men (n=165) in the Cross-Sectional Study

Variable	Women Mean±SD	Men Mean±SD
Age (years)	74.9±7.0	75.6±6.2
BMI (kg/m^2)	25.2±4.1	25.4±3.4
Ferritin (μg/L)*	79.3 (12.7–492.7)	100.3 (18.9–533.8)
Energy (kcal/day)	1457±443	1856±611
Total Iron Intake (mg/day)	21.0±15.6	22.7±17.7
Dietary Iron	10.9±3.3	13.9±4.3
Heme Iron	0.55±0.35	0.82±0.46
Non-Heme Iron	10.4±3.2	13.1±4.2
Supplemental Iron	10.1±14.9	8.8±17.2
% Taking Iron Supplements	51%	39%
Dietary Vitamin C (mg/day)	152±69	164±77
% Taking supplemental Vitamin C	69%	60%
Calories from alcohol	28.6±53	79±110

* Geometric mean and 95% population prediction interval.

Table 5. Regression Model* Examining Effects of Age, BMI, Heme, Non-Heme and Supplemental Iron Intake on Serum Ferritin[1] Levels

	Model					
	Men			Women		
	Estimate	SE	*p*	Estimate	SE	*p*
Non-Dietary Factors						
Age	0.0152	0.0117	0.1957	**0.0168**	**0.0073**	**0.0223**
BMI	**−0.0409**	**0.0202**	**0.0444**	0.0148	0.0121	0.2247
Dietary Factors						
Heme iron[2]	0.0071	0.1620	0.9653	0.1740	0.1611	0.2813
Non-heme iron[2]	0.0168	0.0230	0.4655	0.0105	0.0202	0.6020
Supplemental iron[3]	−0.1294	0.1436	0.3691	**0.2950**	**0.0983**	**0.0030**

[1] Serum ferritin values were log transformed.

[2] Energy adjusted heme and non-heme iron.

[3] Supplemental iron coded as yes/no.

* All results remained even when non energy adjusted values of dietary iron or heme iron are used. Very similar results are obtained when iron supplementation status is coded as a continuous variable.

Statistically significant findings are highlighted in bold.

($p=0.0223$) and supplemental iron intake ($p=0.0030$) on serum ferritin levels, but no effect of heme or non-heme iron intake.

DISCUSSION

In our longitudinal analyses, we found that iron stores were approximately normally distributed for both the men and women in this study (see Fig 1). Women had mean iron stores that were 69 mg lower than the men; however, this difference was not statistically significant. This non-significant finding, unlike the significant findings between men and women in our cross-sectional study, may reflect a lack of statistical power due to the relatively small sample size in our longitudinal study.

Iron stores were estimated from serum ferritin levels, and it might be argued that those with high iron stores had elevated ferritin levels due to the presence of inflammation, a condition known to cause an increase in serum ferritin levels. While we did not measure erythrocyte sedimentation rates (ESR) in our subjects between 1984 and 1993, a standard procedure to assess for inflammation, we did determine white blood cell counts (WBC) at yearly intervals in all of the subjects. The mean (SD) of the WBC for the men and women was 5.91 ± 1.63 and $5.77 \pm 1.17 \times 10^9$/L, respectively, and none of the subjects had a WBC$>11\times10^9$/L that would indicate acute infection [21]. In addition, the medical histories of all subjects were reviewed for indications of inflammatory diseases. One subject has a history of connective tissue disease, one subject has a history of unspecified arthropathy, two subjects have histories of cardiomyopathy and four have histories of cellulitis. No subjects have rheumatoid arthritis. However, many (55 women and 32 men) have histories of osteoarthritis. We added an indicator to our GEE models for presence of inflammatory disease, including osteoarthritis, but found no significant effect on iron stores. In a recent publication, in which we examined the impact of the mutations (HFE) associated with the development of hemochromatosis on iron stores in health elderly men and women in the NMAPS, we did have ESR data [5]. We found no association between ESR values and total iron stores, as estimated by serum ferritin concentrations. Thus, while we did not have ESR results for the present study, we feel that underlying inflammatory processes did not significantly influence ferritin levels in the longitudinal or cross-sectional study.

We used the generalized estimating equation method (GEE) to directly model the relationship between iron stores and iron intake. In the GEE model the observed level of iron stores in a given year is regressed on the observed iron intake for that same year. Data from all years are pooled. The GEE method takes into account the lack of independence of the iron stores from year to year. It can also handle the problem of missing data, although the method requires some assumptions on the form of the year-to-year correlation in iron stores. We specified that the correlation in iron stores be identical for any two years. In our data it seems likely that iron stores for two adjacent years will be more highly correlated than iron stores measured further apart in time. We have verified that this is indeed true. Correlations vary from about 0.67 for iron stores measured in 1984 and 1993 to about 0.92 for iron stores measured only one year apart. However, the GEE approach yields consistent estimators even if the correlation structure is misspecified, provided that the pattern of missing data is random [19].

The GEE regression model (Table 2) found predicted iron stores for men to be 916 mg, which would be equivalent to a geometric mean serum ferritin level of 118 μg/L. Iron stores in women were approximately 81 mg lower than for men (835 mg, equivalent to a geometric mean ferritin level of 97 μg/L), but this difference was not statistically significant. There was no relationship between age or total caloric intake and iron

stores. The marginally significant relationship between iron stores and body mass index is interesting but unexplainable at this time. Iron stores were significantly higher by approximately 52 mg for persons in the upper quartile of dietary intake (>15.66 mg/day) when compared to persons in the lowest quartile (<10.15 mg/day). This finding suggests that iron intakes in this range, i.e., less than 10.15 mg/day, may be just sufficient to replace basal losses of iron but insufficient to increase storage iron. Table 2 also shows that those individuals consuming more than 18 mg of supplemental iron per day had iron stores that were 65 mg greater than those not consuming any iron supplement ($p=0.011$).

It is generally believed that the bioavailability of heme iron is greater than non-heme iron [13], and presumably increasing the amount of heme iron in the diet will result in more iron being absorbed. We were able to separate dietary iron intake into heme and non-heme iron in 3 years that dietary intake information was collected. Table 3 shows that there was no significant association between dietary iron intake and iron stores for either heme or non-heme iron intake. Again, we found a significant effect of supplemental iron intake on iron stores in this model ($p=0.0428$).

In our cross-sectional study we found a significant interaction effect between gender by total iron intake. Because of the interaction effect, we examined men and women separately (see Table 5). For men, the only significant effect on serum ferritin in men was BMI (estimate=0.0409, $p=0.0444$). At this time we do not have an explanation for this finding. For women, significant positive effects on serum ferritin levels were noted for age ($p=0.0223$) and supplemental iron intake ($p=0.0030$) but no effect of heme or non-heme iron intake.

To summarize the combined results in the present study, the following observations are noteworthy. First, our cross-sectional results are in agreement with our longitudinal findings in that we found no effect of heme iron intake on iron stores. Supplemental iron intake had a significant effect on iron stores in women, but not in men, in the cross-sectional study as well as having a significant positive effect on iron stores in our longitudinal analysis where women and men were analyzed together. Because of the relatively small sample size in the longitudinal analyses, we did not have enough power to examine women and men separately. The positive influence of iron supplementation in the longitudinal analyses may simply reflect the fact that approximately twice as many women than men were included in this study.

The finding that age and supplemental iron had a significant effect on serum ferritin levels in women, but not men, in our cross-sectional study can possibly be explained by the following results in which we examined the impact of HFE mutations on iron stores in elderly men and women in the NMAPS [5]. We reported that the carrier frequency of the HFE 845A mutation in our population was 12.2% and the carrier frequency of the 187G mutation was 19.2% for a single mutation; 2.4% were compound heterozygous, 845A/187G, and 2.4% were homozygous for the 187G mutation. More men, 15 of 28 (54%), with estimated iron stores in the upper quartile, ≥1,050 mg, had a HFE mutation compared to 25 of 83 (30%) whose estimated iron stores were <1,050 mg ($p<0.025$). For women, 16 of 42 (38.1%) women with iron stores in the upper quartile, ≥961 mg, had a mutation and this value was not statistically different from 41 of 125 (32.8%) of women who had a mutation and had iron stores <961 mg. None of the following confounding variables, total iron intake, alcohol consumption and inflammatory processes, as determined by ESR, proved significant in explaining why men with a mutation have increased iron stores. From this study we concluded that having either a single chromosomal 845A and/or 187G mutation results in higher iron stores for men than if no HFE mutation were present. In other words, iron stores in these elderly men may have reached maximal levels as corresponding to our "setpoint" hypothesis for iron stores [22]. The setpoint theory states that healthy elderly individuals have different steady state levels of iron stores that are probably under genetic control. At steady state levels of iron stores, iron absorption is thought to be limited to amounts required to match that necessary to replace basal losses. The age at which an individual achieves his or her theoretical setpoint of iron stores has not been determined. We speculate that this may occur around age 40 in men and after menopause in women. It could be that not enough time has elapsed for our postmenopausal women to reach steady state levels of iron stores and therefore the significant increase in iron stores with age in women. Another factor to be considered is that approximately 25% of the women in the NMAPS are on hormone replacement therapy [23], and this could further delay reaching steady state iron stores due to continued loss of blood through menstruation.

We agree with Hallberg *et al.* [8] that there is probably a level of iron stores at which increased iron intake has little or no effect on increasing iron stores. However, we do not feel that the cutoff point at which this occurs corresponds to a serum ferritin concentration of ~60 μg/L. Time, age and HFE mutations need to be considered. However, the present study results were in accordance with those of Hallberg *et al.* regarding absorption of heme- and non-heme iron. We also feel that there is an effective control of both heme- and nonheme-iron and increasing heme iron in the diet will not lead to an accumulation of iron stores in normal men by bypassing the strong regulation of iron absorption.

Differences between our cross-sectional findings and those of Fleming *et al.* [9], especially the effects of heme iron intake on iron stores, could be related to a number of factors including sample size differences, interaction effects, differences in dietary and biochemical methodology and measurement error in determining dietary iron intake. It is unclear which factor or factors noted above could definitively explain the differences noted between our findings and those of Fleming *et al.* However, we believe that women and men should be examined

separately. Even though iron stores increase after menopause, the time period after menopause is critical in any analyses. In addition, the use of HRT, could also delay the achievement of maximum or steady state iron stores where increasing iron intake no longer affects the further accumulation of iron stores.

Based on our longitudinal and cross-sectional study findings, we have to conclude that increasing heme iron intake does not effectively increase iron stores in the elderly. Iron supplements, unless recommended by a physician to correct an anemic condition, may not be warranted for a healthy elderly individual consuming a balanced diet. This may be especially important for those individuals who are homozygous or heterozygous for the HFE mutations because of recent studies showing that even moderate increases in body iron may increase the risk for diabetes and cardiovascular disease.

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