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Iron stores and coronary artery disease: A clinical application of a method to incorporate measurement error of the exposure in a logistic regression model

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Abstract

Rates of coronary artery disease (CAD) increase sharply after menopause. We examined the hypotheses that high iron stores, as measured by plasma ferritin levels, are a risk factor for CAD and that the increase in iron stores after menopause is at least in part responsible for the rise in CAD in women. We also investigated measurement error of plasma ferritin using a Bayesian conditional independence model and incorporated it into the estimation of the odds ratio (OR) for males. Cases had ⩾1 coronary artery stenosis ⩾70%. Controls had no visible coronary lesions on angiography. The median plasma ferritin level was 48 mg/L (interquartile range: 28 to 86) among 244 cases and 45 mg/l (24 to 85) among 140 controls. The multivariate analyses among females, males, and females and males combined did not support an association between plasma ferritin levels and CAD (OR for one unit change in log ferritin 1.01, 95% CI 0.71–1.44, OR 0.95, 95% CI 0.66–1.37 and OR 0.95, 95% CI 0.75–1.21, respectively). Accounting for the measurement error of ferritin in males slightly improved the precision of the estimate of the OR but did not unmask an association (OR: 0.94, 95% CI 0.69–1.30). We conclude that high ferritin levels before or after menopause are not associated with CAD. Measurement error might be considered in situations where a one-time measurement is assumed to be representative of long-term exposure. © 2000 Elsevier Science Inc. All rights reserved.

Keywords: Ferritin; Coronary artery disease; Menopause; Measurement error

1. Introduction

Well-established risk factors for coronary artery disease (CAD) account for no more than 50% of the variation in CAD risk. In addition, differences in these cardiac risk factors between men and women do not explain entirely why men have a higher CAD risk than women. Thus, other risk factors clearly must be operative. Recently, it has been suggested that one of the final pathways for the development of atherosclerosis is the oxidation of low-density lipoprotein (LDL) [1]. In fact, there is growing evidence that LDL must be modified before it is pathogenic. Iron may contribute to this process, since iron can catalyze the reactions leading to highly reactive hydroxyl radicals, which in turn oxidize LDL [2]. Therefore, iron-mediated oxidation of LDL is the proposed mechanism explaining the link between iron and CAD [3]. Women have lower iron stores than men before menopause due to menstrual loss. At menopause, iron stores gradually increase in parallel with CAD incidence [4]. This increase in CAD rate has been usually attributed to hormonal changes but also could be partly related to changes in iron stores. Thus, our primary hypothesis is that high iron stores as reflected in the circulating ferritin level is a risk factor for CAD and our secondary hypothesis is that the lower CAD risk of women compared to men is related, in part, to iron stores.

Several studies have investigated a role for iron in the development of CAD. The results of these studies were inconsistent for at least two potential reasons. First, different measures of iron metabolism were used. Consequently, the results were not comparable. Second, in all previous studies, a one-time measurement of iron metabolism was used and assumed to be representative of the average level for any given individual.

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Ferritin levels, as with many other cardiac risk factors, may vary over time and/or be measured imprecisely. Therefore, a one-time measurement of such a potential risk factor could be an imprecise estimate of the true average within-subject exposure. This problem of measurement error is frequently encountered in cardiovascular epidemiology, for example, smoking habits, and the presence of hyperlipidemia, hypertension, and diabetes may all vary over time. It is well known that bias can occur in the estimation of the effects of risk factors on disease occurrence when one or more risk factors are measured with error [5–7]. Measurement error could be related to the biochemical test used to measure ferritin or to the within-subject variability that occurs over time. It is the latter source of measurement error that is accounted for in the present study, since errors in the biochemical test are negligible. A wide variety of techniques have been suggested to correct problems caused by measurement errors [8,9]. In this paper we use a Bayesian conditional independence model to adjust for measurement error [10,11]. A Bayesian approach is selected because it allows the combination of information contained in two different data sets needed to adjust for measurement error in our study. The first data set provides information about the disease/exposure relationship, while the second provides information about the long-term variability of ferritin measures.

2. Materials and methods

Two different populations are investigated in this study. The first population consists of a group of individuals from a case-control study designed to investigate the potential association between plasma ferritin and CAD in both men and women. The second population consists of healthy males in whom serial measurements of plasma ferritin, at least one year apart, were measured. The latter data set was used to assess the biological stability of plasma ferritin over time and to incorporate measurement error adjustments into the estimation of the odds ratio (OR) for ferritin among males in the case-control study.

2.1. Case-control study

2.1.1. Setting and population

Patients who underwent an elective coronary angiography at the Cleveland Clinic Foundation between May 12, 1993 and June 12, 1995 and who were referred to the cardiology outpatient department were invited to participate in this case-control study. At the time of the outpatient evaluation, before coronary angiography, those who consented had an additional 10 cc's of blood drawn along with the routinely drawn blood for clinical purposes. The additional blood sample was the only non-routine intervention. It was frozen for subsequent analysis. We excluded patients with an insufficient quantity of blood for measurement of ferritin. After angiography, cases and controls were identified based on the results of the angiograms. Angiography reading was blinded to the ferritin level.

2.1.2. Case definition

The diagnosis of CAD was based on the presence of at least one stenosis obstructing 70% or more of any epicardial coronary artery lumen determined in a standard manner during the coronary angiograms [12].

2.1.3. Control definition

The control group comprised a consecutive sample of individuals who underwent coronary angiography and who had no visible CAD. The usual clinical definition for absence of CAD is absence of lesions $\geqslant$70%. Some definitions use a 50% cut-off [13]. In this study, to avoid potential misclassification of disease status, only individuals without any visually detectable lesions were included as controls. Since it is rare to find normal coronaries in this population, subject accrual was limited by the identification of controls.

Ideally, controls should be a random sample from the same cohort (secondary base) from which the cases were selected. Thus, our strategy for control selection was to take the patients with normal coronary angiograms at the coronary angiography laboratory at the Cleveland Clinic Foundation during our study period. Similarly to the cases, these individuals had been referred from the outpatient department. Thus, the secondary base is very likely to be the same for cases and controls. As a result of this process, cases and controls had a similar cardiac risk factor profile because physicians referred patients for angiography based on their pre-test probability of disease.

2.1.4. Exposure

Iron stores were assessed by measurement of plasma ferritin concentrations. Ferritin levels are thought to correlate better with iron stores than any other measure of iron metabolism [14]. Plasma ferritin was measured by a Microparticle Enzyme Immunoassay using an IMX Analyzer (Abbott Laboratories, Abbott Park, IL, USA).

2.1.5. Cardiac risk factors

Smoking was defined as never, current, and prior smoking. Hypertension was defined as a history of hypertension or the use of anti-hypertensive medication. Hypercholesterolemia was defined as a fasting level of total cholesterol greater than 200 mg/dl or the use of a lipid-lowering drug. Diabetes was defined as a fasting glucose level greater than 140 mg/100 ml or a reported history of diabetes.

2.1.6. Data collection

Data were obtained from four sources: a chart review for information on cardiac risk factors and evidence of liver disease and ongoing infectious disease; results from the coronary angiograms to identify cases and controls; a venous blood sample drawn for plasma ferritin measurements; and a phone survey of female subjects only for information on menopause.

2.2. Measurement error of plasma ferritin

For the second part of the study, we performed serial measurements of ferritin on the frozen plasma of 259 males.

The frozen plasma of males who were undergoing yearly routine medical examination was available from a blood bank at the Cleveland Clinic Foundation. We measured ferritin in males to assess the extent of measurement error in men, since they would be expected to have lower within-subject variability than women. Using the same immunoassay as for the case-control study to measure ferritin levels, we first tested whether ferritin measurements are reproducible when measured on plasma that has been frozen for a period of up to 9 years. Measurements of the ferritin level obtained on a fresh specimen 9 years previously were the same as measurements obtained on the same samples 9 years later. We then performed 870 serial measurements of samples collected 1 year apart for an average of approximately 3 measurements per individual.

2.3. Statistical analysis

2.3.1. Case-control study

Descriptive statistics (such as means, standard deviations, frequencies (%), median and interquartile ranges) were used to compare the case and control groups with respect to demographic characteristics, risk factor profiles and factors that potentially affect the measurement of plasma ferritin. Levels of the exposure (plasma ferritin), expressed as median and interquartile ranges, were compared between all cases and controls and in subgroups: men and women, and women before and after menopause.

We performed a logistic regression with disease status (presence or absence of CAD) as the dependent variable and the natural logarithm (log) of plasma ferritin as a continuous independent variable. The log of ferritin was used to better model the measurement error, as described below. To evaluate potential confounding factors, we obtained the ORs of the disease exposure association that were adjusted for cardiac risk factors (age, HDL, LDL, hypertension, family history of cardiac disease, hypercholesterolemia, smoking, diabetes) and for factors that potentially affect the measurement of ferritin, which include ongoing infection (white blood cell count), liver disease (total protein, albumin, bilirubin and liver enzymes) and renal dysfunction (urea and creatinine). The ORs and 95% confidence intervals (CI) were obtained from the regression coefficients.

To address the role of gender, all analyses were performed separately for men and women and in a combined regression model that included both genders. Approximate Bayes Factors as calculated through the Bayesian Information Criterion (BIC) were used to select final models [15]. The BIC has been shown to have better out-of-sample predictive properties compared to the usual “backwards” or “forwards” stepwise selection procedures. These tend to overfit the model to the data, which often leads to poor generalizability.

2.4. Measurement error analysis

We used a conditional independence model as described by Richardson and Gilks [10,11,16] to investigate the possible effects of measurement error of ferritin on the estimated OR for men only. Our model can be described in four stages (see Appendix for full details).

The first stage relates the outcome (presence or absence of CAD) to the unobserved true ferritin value for each subject and other covariates, the latter assumed to be measured without error. At this stage, we used a standard logistic regression model with terms for true log ferritin, age, and HDL cholesterol. These variables were selected as the best candidates based on the BIC criterion except for ferritin, which we included as the variable of primary interest in this study. An interaction term for log ferritin $\times$ HDL cholesterol also was used in a preliminary model, but eliminated due to lack of evidence for this interaction. We also examined the effects of other potential confounders for the effect of ferritin, but none were found to change the coefficient for log ferritin in any important way. As is standard in conditional independence models, we assumed that conditional on true ferritin values, the logistic regression coefficients were not affected by the distribution of observed ferritin values.

At the second stage, we modeled the observed log ferritin value as a function of the true log ferritin value within each subject. We assumed that the observed log ferritin values would be normally distributed about the true mean within-subject value, with a variance that was estimated from the data set of serial ferritin measurements. Using the same variance for true ferritin in these two data sets provided a link between them in our model, which enables the combination of information in each data set to contribute to the final parameter estimates. We used log ferritin throughout, since within-subject ferritin had a skewed (non-normal) distribution in our serial measurement data, such that subjects with higher values of ferritin had larger measurement errors than lower values of ferritin. This was corrected by taking the logarithm, after which a normal measurement error model across the range of log ferritin values fit the data well.

The third stage of the model describes the distribution of true ferritin between individual subjects. Here we combined descriptive evidence from both the serial measurements and case-control subjects to confirm that log ferritin was approximately normally distributed in men.

A final stage in our modeling process was to derive prior distributions for all unknown parameters in all stages of our model. We used “low-information” prior distributions, where the entire ranges of plausible parameter values are covered by a roughly “flat” prior distribution. Therefore, all likely values are approximately equally likely a priori. This allowed the data to dominate the prior distributions, which then have little influence. The only exception to this was the prior parameters for the distribution of ferritin values in the population, for which we plotted previous estimates that have appeared in the literature [17–29] and used prior parameter values that covered the range suggested by these studies. To check the robustness of the estimated OR for log ferritin, we varied the prior parameters in a sensitivity anal-

ysis. Measurement error adjustment was performed for males only, since repeated measurements of ferritin were not available for females.

An exact analytic solution for this complex model is impossible, so that we followed the methods of Richardson and Gilks [10] in using the Gibbs sampler, a Markov chain Monte Carlo approach to numerical integration [30] wherein random samples from the marginal distribution of each parameter of interest are generated by intensive computer calculations. After ensuring convergence, empirical summary statistics can be formed and used to make inferences about the true values of the quantities of interest. This computational work was performed using BUGS software [31]. We ran 10,500 iterations of the Gibbs sampler, the first 500 to ensure convergence of the algorithm, and the last 10,000 for inference. This ensured a high degree of accuracy in the final estimates, as we verified using the methods of Raftery and Lewis [32].

3. Results

Over the study period, 244 cases and 140 controls had ferritin levels drawn before the index angiograms. Of these, 201 subjects were males and 183 subjects were females. About 85% of females had reached menopause. Cases exhibited slightly higher cardiac risk profiles than controls (Table 1). Median ages were 63 and 58 years among the cases and controls, respectively. By design, the sex distributions were similar. During the second half of the study, women and controls only were recruited to increase their proportion. A higher proportion of cases had diabetes, hypertension, and hypercholesterolemia. The smoking prevalences were similar in the two groups, as were the prevalences of a family history of heart disease. The levels of serological markers for conditions that could potentially affect the ferritin measurement which include liver disease, an ongoing infection or renal dysfunction were similar in cases and controls. Unadjusted median plasma ferritin levels of the cases and controls and that of different subgroups of interest are presented in Table 2. Overall, plasma ferritin levels did not substantially differ between the cases and controls. Females, especially menopausal cases, exhibited a higher median ferritin levels, but this trend was not statistically significant.

The multivariate analyses among females, males and females and males combined did not support an association between plasma ferritin levels and CAD even after adjustment for menopausal status in females (OR for one unit change in log ferritin 1.01, 95% CI 0.71–1.44; OR 0.95, 95% CI 0.66–1.37; and OR 0.95, 95% CI 0.75–1.21, respectively) (Table 3). Calculations of the OR between low and high ferritin levels did not reveal any important associations between ferritin and CAD. For example, among females, a change from a ferritin level of 19 (25% quartile) to 63 (75% quartile) mg/L yielded an OR of 1.01, 95% CI 0.66–1.54. Among males, a change in ferritin level of 32 (25% quartile) to 104 (75% quartile) mg/L yielded an OR of 0.95, 95% CI 0.62–1.45. Age, HDL cholesterol, total cholesterol, and diabetes were the other risk factors that were selected in the final models. The strength of those factors was less than expected likely because of the process of selection of cases and controls. Patients who undergo angiography are selected for this test based on symptoms and risk factors. Consequently, by design, the risk factors profile of cases and controls might be very similar. We included these known risk factors for CAD in the multivariate analysis to assess the independent effect of ferritin. Additionally, in the model that combines males and females, age had a weak relationship with CAD. This is because by design, there was a high proportion of older females with CAD. However, because ferritin level was unknown at the time of selection of cases and controls and not used as a criterion for selection, its potential effect on CAD can be assessed.

Table 1
Demographic and clinical characteristics of the case-control study population

	Cases $n = 244$	Controls $n = 140$
Median age (years)[a]	63 (54, 70)	58 (49, 68)
Females (%)	46	50
Menopausal females (%)	88	84
Females on hormonal therapy (%)	20	36
Family history (%)	25	26
Diabetes (%)	34	19
Smoking		
Current (%)	14	13
Never (%)	38	43
Hypertension (%)	59	47
Cholesterol lowering drug (%)	18	6
Total cholesterol (mg/dl)	218 (190, 248)	211 (183, 234)
HDL cholesterol (mg/dl)	36 (30, 45)	41 (36, 52)
LDL cholesterol (mg/dl)	141 (114, 165)	134 (114, 157)

[a]Continuous variables are presented as median and interquartile range.

Table 2
Median plasma ferritin levels (mg/L) in various subgroups

	Cases	Controls
Overall group (*n*)	244	140
Mean (SD)	64 ± 56	62 ± 55
Median (interquartile range)	48 (28, 86)	45 (24, 85)
Males (*n*)	131	70
Mean (SD)	76 ± 64	80 ± 58
Median (interquartile range)	56 (30, 101)	65 (37, 108)
Females (*n*)	113	70
Mean (SD)	51 ± 42	44 ± 46
Median (interquartile range)	41 (22, 66)	31 (16, 53)
Menopausal females (*n*)[a]	99	59
Mean (SD)	48 ± 37	47 ± 46
Median (interquartile range)	40 (22, 64)	32 (17, 59)
Nonmenopausal females (*n*)	8	10
Mean (SD)	61 ± 73	17 ± 11
Median (interquartile range)	41 (9, 77)	16 (6, 24)

[a]Missing information on menopausal status in 7 females.

Table 3
Multivariate models for the relationship between plasma ferritin and coronary artery disease

Patient subgroup	Odds ratio	95% Confidence interval
Females only		
HDL cholesterol	0.96	0.93–0.98
Diabetes	3.73	1.72–8.10
Log ferritin	1.01	0.71–1.44
Males only		
HDL cholesterol	0.96	0.93–0.99
Age	1.05	1.02–1.08
Log ferritin	0.95	0.66–1.37
Males and females		
HDL cholesterol	0.95	0.93–0.97
Age	1.04	1.02–1.06
Total cholesterol	2.25	1.37–3.68
Log ferritin	0.95	0.75–1.21
Males (incorporating measurement error)		
HDL cholesterol	0.97	0.94–0.99
Age	1.04	1.01–1.06
Log ferritin	0.94	0.69–1.30

*HDL = high density lipoprotein.

There was wide variability in within-subject standard errors about the mean ferritin measurements among the male subjects who were followed over time. The maximum standard deviation among the repeated measurements was 380 mg/L, while the minimum was 1.2 mg/L. The average standard deviation was 41 mg/L, and the median was 27 mg/L, so that there was a total range of approximately 100 to 160 mg/L (four times the standard deviation) in typical subjects. About 25% of subjects had standard deviations below 15 mg/L, while another 25% of subjects had standard deviations above 47 mg/L. Despite evidence of large variations in within-subject ferritin measurements over time, adjustment for measurement error in ferritin values did not substantially affect the point estimate of the OR, but did slightly improve the precision of the estimate (OR 0.94, 95% CI 0.69–1.30). Therefore, we did not uncover any evidence that measurement error is responsible for masking the effects of ferritin on CAD.

To verify if our methodology would uncover such masking effects, if indeed they were present, we reran our measurement error model on the identical data set (both case-control and serial measurement components), changing only the values for presence or absence of CAD, according to the value of ferritin, i.e., induction of an artificial effect of true ferritin on CAD, which is masked by the measurement error. We then ran the same two models as above, one that ignores measurement error, and one that adjusts for measurement error. While a non-measurement error adjusted logistic regression model did not capture the "true" induced OR in its 95% CI, our measurement error model did capture the correct OR value. Therefore, it is likely that our model would have adequately adjusted for measurement error if necessary, but that this was not indicated by our (non-simulated) data. Similarly, the use of different prior distributions that described the distribution of ferritin in the population did not substantially alter any of our estimates.

4. Discussion

This study suggests that ferritin does not seem to play a large role, if any, in the development of CAD. While the ferritin level of females with CAD was higher than females without CAD (both in menopausal and non-menopausal females), ferritin was not a predictor of CAD in adjusted analyses. Adjustment for measurement error with a Bayesian conditional independence model [10,11,16] did not reveal large changes in estimated ORs in our study. Our analysis allowed for the utilization of all available information, not only from the two different study populations for which we collected data, but also the information about ferritin levels from the literature for individuals with and without CAD. Second, our methods allowed for the simultaneous estimation of all model parameters. For example, the measurement error variance parameter was estimated using information from both of our data sets.

Several recent epidemiological studies have investigated the association between iron metabolism and CAD. The results of these studies are inconsistent. The first large epidemiological study was reported by Salonen and coworkers [23]. In a cohort of Finnish men, a positive association was found between serum ferritin and myocardial infarction. However, since this publication, other epidemiological studies have failed to replicate findings of similar magnitude. Only one other study reported on ferritin, which is thought to correlate best with body iron stores [14]. In a cohort of Icelandic men and women, Magnusson and coworkers did not find any association between serum ferritin and the risk of myocardial infarction [24]. However, plasma total iron binding capacity was inversely related to the risk of myocardial infarction in both genders.

The remaining studies looked at various other indicators of iron metabolism. Two of these studies used the first National Health and Nutrition Examination Survey to investigate the potential association between iron and CAD. In the first study, Sempos and coworkers found that transferrin saturation was not related to the risk of coronary heart disease and non-fatal myocardial infarction but that transferrin saturation was inversely related to CAD mortality and all cause mortality in both genders [25]. In the second study, Liao and coworkers also failed to show an association between transferrin saturation and myocardial infarction [26]. However, unlike Sempos and coworkers, they reported an inverse association between transferrin saturation and coronary heart disease. In addition, they studied plasma and dietary iron, total iron binding capacity, hemoglobin, and hematocrit. Plasma iron was inversely related to myocardial infarction only in women while the other indicators were not related to either outcome in either gender.

A study of a different American cohort assessed the association between dietary iron and CAD. Ascherio and coworkers found that, among a cohort of U.S. male physicians, there was no association between total dietary iron intake and the risk of myocardial infarction [27]. However, when a separation was made between heme iron found in red meat and non-heme iron from other food sources, heme iron was weakly associated with myocardial infarction whereas non-heme iron showed no significant association. In contrast, in a case-control study of Greek patients, Tzonou et al. found a relationship between total dietary iron and CAD. Finally, Morrison and coworkers also investigated the association between dietary iron and CAD in the Nutrition Canada Survey cohort [29]. In this study, no association was found between the risk of fatal acute myocardial infarction and neither total dietary iron (heme and non-heme) nor iron supplement use.

The results of these epidemiological studies are inconsistent, and they do not definitively answer the question as to whether high iron level is a cardiac risk factor. Several factors may explain the lack of consistency between these recently published epidemiological studies. First, despite the large total sample sizes of the previous cohort studies, very few cardiac events were available for analysis [3]. Second, these studies measured different indicators of iron metabolism. Thus, comparison of the study results is difficult. Third, some indicators of iron metabolism such as ferritin are a better measure of iron stores than others [14]. Ferritin has not been consistently used as a measure of iron stores. Fourth, in all studies, measurements of the various indicators of iron metabolism were done at one point in time and assumed to be representative of long term exposure. This could lead to decreased power to find clinically important differences due to measurement error. Finally, most studies did not include females and did not investigate the association between iron metabolism and the gender differences in CAD risk.

Our study was a case-control study that allowed the identification of a large number of individuals with angiographically documented CAD. Cases and controls were referred for angiography based on the physician's evaluation of the patient's pre-test probability of CAD but without the knowledge of ferritin levels. As a result, the prevalence of cardiac risk factors was by design quite similar between the two groups. This method of selection allowed the identification of cases and controls highly matched on cardiac risk factors. Plasma ferritin, which is thought to best represent iron stores, was the exposure. Data were collected on both men and women to allow the investigation of the association between iron metabolism and CAD risk in both sexes. Finally, repeated measurements of ferritin over time in the same individuals were obtained to estimate measurement error and adjust for it in the analysis of the case-control study. A conditional independence model based on Richardson and Gilks [10,11,16] was developed to incorporate measurement error in the assessment of a logistic regression analysis. Adjustment for any potential bias that measurement error might have induced in our estimates did not reveal any substantial effects, so that it does not seem likely that measurement error played a role in masking any true effects of ferritin on CHD, even though within-subject ferritin measurements in males were not as stable as previously assumed. However, our analysis failed to find an effect for ferritin suggesting that factors other than measurement error explain the inconsistencies between previous studies.

Several limitations could be, at least in part, responsible for our inability to find an association between iron metabolism and CAD. First, the role of iron in the atherosclerosis process could occur at a different step in the CAD pathway than the one that was studied here. By using angiography to distinguish cases and controls, a true effect of iron might have been missed if the effect occurred at another step of the pathway. Second, the use of a current rather than a past measure of ferritin might have been inadequate. Among the few studies that have used a measure of iron metabolism obtained over 20–25 years before a cardiac event, most have failed to show an association between iron metabolism and CAD (25–27). Third, the recruitment of controls was a limiting factor for the sample size of this study. On average, 15 to 25% of coronary angiograms are normal (as defined by stenosis 50% or less) [13]. Definition of controls as those without any visible coronary lesion decreased this average to about 2–5%. However, given the large volume of angiographies at the Cleveland Clinic Foundation, we were able to identify 140 individuals without CAD for whom we have complete data. Also, premenopausal women with CAD are rare, leading to a low event rate in females.

While the serial measurements allowed for the adjustment for measurement error in plasma ferritin, there are also several limitations to this component of our study. First, we did not have access to serial data of ferritin measurement in females. Second, the serial measurements were taken on subjects different from those in whom we estimated the logistic regression model. Therefore, we could not more accurately pinpoint each subject's true value by averaging over within-subject repeated measurements. Instead, the serial data provided only a range of plausible values for each subject, from which an "optimal" value could be chosen which maximized the likelihood function of our logistic regression model. A more informative measurement error model (which we did not have the data to create) might have better corrected for any potential measurement error. We tried to provide better true ferritin estimates via the fitting of a linear regression model that would predict true ferritin values using other variables measured in our case-control subjects, but no important predictors could be found among those we tried (presence or absence of ongoing infection, liver disease or renal dysfunction). Nevertheless, our simulation showed that if there were real effects that were masked by measurement error, our model did have the ability to adjust for it. Therefore, we do not feel that we have missed any strong effects of ferritin due to measurement error.

In conclusion, in females, high ferritin level before or after menopause does not appear to substantially contribute to CAD. In males, adjustment for measurement error slightly improved the precision of the estimate without changing the OR. Our results do not provide any evidence that ferritin is a risk factor for CAD. Measurement error might be accounted for in the evaluation of risk factors, especially in situations where a one time measurement is assumed to be representative of long-term exposure.

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Appendix

Our measurement error model includes four stages. At the first stage, the unobserved (latent) true log ferritin level, along with our covariates are related to the presence or absence of CAD through a logistic regression model, so that

$$\text{logit}\ (\text{CAD}_j) = \alpha + \beta_1 \times \text{TLF}_i + \beta_2 \times \text{AGE}_i + \beta_3 \times \text{HDL}_i$$

where CAD_i indicates the presence or absence of CAD in patient i, α is the intercept, and β_1, β_2, and β_3 represent the logistic regression coefficients for true log ferritin, age, and HDL cholesterol, respectively, for patient i.

Observed log ferritin levels were then related to true log ferritin levels via a normal distribution such that

$$\text{OLF}_{ij} \sim \text{N}\ (\text{TLF}_i, \sigma^2_i),$$

where OLF_{ij} represents the j^{th} repeated log ferritin measurement in patient i, and σ^2_i is the variance of log ferritin measurements for patient i. The collection of $\log\ (1/\sigma^2_i) = \tau_i$ values were then assumed to follow a normal distribution $\tau_i \sim \text{N}\ (\mu_\tau, \sigma^2_\tau)$. This hierarchial distribution formed the link between the two data sets, allowing control over the spread of variances in the ferritin data set without repeated measures. Diffuse prior distributions were used for μ_τ and σ^2_τ. This formed the second stage of the model.

While observed log ferritin measurements were assumed to vary around the true values within each patient, variability of true log ferritin between patients also followed a normal distribution. Thus, in the third stage of our model

$$\text{TLF}_i \sim \text{N}\ (\mu_{\text{TLF},} \sigma^2_{\text{TLF}}),$$

where μ_{TLF} and σ^2_{TLF} denote the overall mean and variance of true log ferritin values in the population of patients represented by those in our study.

At the fourth stage of our model, prior distributions were specified as described in the text. In particular, based on an extensive literature review, we used $\mu_{\text{TLF}} = 4.83$ and $\sigma^2_{\text{TLF}} = 0.65$, and non-informative (flat) prior distributions for all other parameters. A robustness analysis however, showed that our conclusions did not substantially change across a range of other plausible values for μ_{TLF} and σ^2_{TLF}.

Since closed form formulae are not available for estimation of parameters in this model, we used the Gibbs sampler [16,30], an iterative Markov chain Monte Carlo technique for inferences. Briefly, the model works as follows: Each of the four model stages described above contributes terms to the likelihood function and/or the prior distribution. Multiplying all of the terms together provides a joint posterior distribution over all parameters, upon which all inferences are based. The Gibbs sampler provides a sample from the marginal posterior distribution of each parameter.

In each iteration of the Gibbs sampler, a tentative "true ferritin value" is selected for each patient. This selection is based on the marginal posterior distribution of true ferritin for each subject, which is calculated from the information in four different sources; the observed ferritin value for that subject, the within-subject variability of ferritin, the current estimate of the relationship between ferritin and CAD, and the distribution of ferritin in the population. Once a tentative "true" value is selected, the other parameters are updated, and the next iteration begins. By allowing a range of values for "true ferritin" that changes from iteration to iteration, within each subject, the uncertainty in "true ferritin" values is fully incorporated into the model. By selecting these values using all available information, the model is able to adjust for measurement error, as appropriate.

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