Relationship between serum ferritin concentration and established risk factors among men in a population with a high mortality from cardiovascular disease

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Abstract

Objectives: To determine whether reported associations between serum ferritin and cardiovascular risk factors are due to confounding by diet.

Design: Cross-sectional survey in which exercise and smoking habits were collected by a questionnaire; BMI, waist to hips ratio and blood pressure were measured; blood was taken for measurement of lipids, glucose and ferritin; and nutrient and beverage intake were assessed using a validated food and beverage frequency questionnaire.

Subjects: Men randomly selected from the electoral rolls who had agreed to participate, had fasted and did not have diabetes, ischaemic heart disease or haemochromatosis. 165 men were selected of whom 154 participated and 131 had fasted and were free of disease.

Setting: A regional Australian city.

Main outcome measures: Blood pressure and plasma cholesterol, HDL cholesterol, triglycerides and glucose concentrations.

Statistical analyses: Correlation and multiple linear regression coefficients were calculated between serum ferritin and cardiovascular risk factors, correcting for: age; intake of specific nutrients and alcohol; anthropometry; smoking and exercise.

Results: There were significant correlations between serum ferritin and both BMI and waist to hips ratio ($r = 0.28$, $P = 0.001$ and $r = 0.26$, $P = 0.003$ respectively). When regressed against ferritin with confounders, only waist to hips ratio was associated with ferritin ($B = 1.61$, $P = 0.046$). Serum ferritin was also correlated with plasma cholesterol ($r = 0.28$, $P = 0.00$), HDL cholesterol ($r = -0.22$, $P = 0.01$), triglycerides ($r = 0.25$, $P = 0.00$) and glucose ($r = 0.18$, $P = 0.04$). When ferritin was regressed against each variable with confounders, only the association with triglycerides remained just significant ($B = 0.12$, $P = 0.04$).

Conclusion: Confounding by diet explained most of the associations between serum ferritin and cardiovascular risk factors.

Key words: ferritin, cardiovascular disease, risk factors, body mass index, cholesterol, HDL cholesterol, confounding

Introduction

The possibility that body iron stores are a risk factor for coronary heart disease was first postulated in 1981 (1). Early prospective epidemiological studies of serum ferritin concentration, a reliable indicator of stored iron, and coronary heart disease were supportive of the hypothesis (2). Moreover, a biological basis for an association between iron and coronary heart disease began to emerge (1). However, subsequent prospective studies failed to find an association between serum ferritin concentration and coronary heart disease and a recent meta-analysis of the prospective studies could not support the existence of a strongly positive association between iron status and coronary heart disease (3).

Although the meta-analysis was unable to rule out the possibility of a weak association between iron status and coronary heart disease because of potential limitations, the weight of evidence is against iron status being a strong independent risk factor for coronary heart disease (3). However, during the course of some of the prospective studies of serum ferritin and coronary heart disease (2,4), as well as during the course of other studies (5–11), various associations have been sought and found between serum ferritin concentration and cardiovascular disease risk factors. Men have been included in more studies than have women and, in men, serum ferritin concentration has been found to be associated with higher plasma triglycerides (2,4–7), glucose (2,6), total cholesterol (4) and fibrinogen concentrations (5), systolic (2,4,5,7) or diastolic (4,5,7) blood pressure, body mass index (BMI) (4,5,7–9) and waist to hips ratio (5). An inverse association has been reported with high density lipoprotein (HDL) cholesterol concentration (2,5,6), smoking (7) and exercise (10). Although included in fewer studies, similar associations have been found among women (4,7,8,10–13).

The association between serum ferritin and higher concentrations of plasma glucose and triglycerides, lower concentrations of HDL cholesterol, higher blood pressure and lack of exercise has attracted particular attention because of the association between such a profile and atherogenesis (6). The lower incidence of coronary heart disease in pre-menopausal women could also be explained...
in these terms. However, to date it is uncertain why ferritin is associated with these features of the insulin resistance syndrome and whether there is a causal relationship (6).

Rather than a causal connection, it has been suggested that the associations between serum ferritin concentration and both plasma lipids and blood pressure might be able to be explained by other associations that serum ferritin, plasma lipids and blood pressure have in common such as diet or alcohol consumption (2, 5–7). To date there has not been a study to determine whether the association between serum ferritin concentration and cardiovascular risk factors can be explained by confounding by diet or other factors. Studies which have collected data on serum ferritin concentration and cardiovascular risk factors have collected only limited or no dietary data.

We had conducted a prevalence study of cardiovascular disease risk factors in a population with a high mortality from cardiovascular disease (14). During the course of the study data on nutrient intakes had also been collected and stored frozen sera were available for iron studies. We therefore had the opportunity to determine whether the associations that have been found between serum ferritin concentration and plasma concentrations of glucose, triglycerides and HDL cholesterol as well as blood pressure, among men in previous studies, could be explained by confounding. If the associations are due to confounding by diet, alcohol consumption or other factors, then there is no reason to believe that iron status can influence the risk of coronary heart disease through conventional risk factors for the disease.

Methods

The study was conducted in Ballarat, a regional Australian city and approved by Ballarat Base Hospital ethics committee.

A simple random sample of 165 men from the electoral rolls of Greater Ballarat were sent a letter inviting them to attend a cardiovascular disease risk factor screening clinic at the base hospital. After two postal reminders and calling on their homes, 154 (95%) agreed to participate. Of those, 23 were excluded because they had not fasted (nine cases) and/or had ischaemic heart disease (eight cases), diabetes (five cases), or haemochromatosis (three cases) leaving 131 (80%) subjects for this study.

Participants had completed the same self-administered questionnaire as was used in the National Heart Foundation’s cardiovascular risk factor prevalence surveys in Australia’s capital cities (14). The data collected by the questionnaire included age, number of cigarettes smoked per day and hours of vigorous exercise in the week preceding interview and serum ferritin concentration (in µg/L) were calculated first (20). Serum ferritin concentration, number of cigarettes smoked and hours of vigorous exercise were not normally distributed and had to be transformed by a logarithmic or square root transformation (20).

Correlation coefficients between BMI, waist to hips ratio, number of cigarettes smoked per day and hours of vigorous exercise in the week preceding interview and serum ferritin concentration (in µg/L) were calculated first (20). Serum ferritin concentration, number of cigarettes smoked and hours of vigorous exercise were not normally distributed and had to be transformed by a logarithmic or square root transformation (20).

These anthropometric and lifestyle variables were then regressed on serum ferritin concentration with potential confounding variables using direct multiple linear regression. This enabled the regression coefficient between each of them and serum ferritin concentration to be determined whilst adjusting for all variables (20).

Potential confounding variables considered were age; dietary iron (in mg per day) or fat (in g per day) of which the commonest source of both nutrients among Australian men is meat (21); consumption of alcohol (in g per day); coffee and tea (in cups per month); and dietary vitamin C (in mg per day) and fibre (in g per day), both of which could be influenced by healthy food choices. These variables may be associated (positively or negatively) in men with both serum ferritin concentration (10,22,23) and obesity (24,25), smoking (24,26) or the taking of regular exercise (24,27). Dietary iron and fat were included among the potential confounders rather than meat consumption so as to avoid entering a large number of additional varia-
variables, each representing the frequency with which a different meat or meat product was consumed, in the multiple linear regression analyses.

Total energy intake (in kJ per day) was included in the multiple linear regression analysis to adjust for possible systematic over or under reporting of dietary intake (28). None of the men had an unphysiological energy intake (29). With the exception of age, all potential confounding variables and energy had to be transformed by a logarithmic or square root transformation. Use or non-use of aspirin or non-steroidal anti-inflammatory drugs was also included in the multiple linear regression analysis because they are known to increase the possibility of gastrointestinal bleeding which might reduce body iron stores and serum ferritin concentration (22). The potential confounders which most reduced the correlation between serum ferritin and the anthropometric and lifestyle variables were identified through backward elimination of variables in a stepwise process (20).

Because two potential confounders which are highly correlated with one another (collinear variables) will not only provide very similar information but may also lead to results which are un reproducible or difficult to interpret (28), the independent variables were screened for collinearity. Collinearity was assessed through a series of multiple linear regression analyses, using each independent variable in turn as a dependent variable and keeping all others as predictor variables. Dependent variables with a R^2 greater than 0.95 were excluded.

Correlation coefficients between serum ferritin concentration and fasting plasma concentrations of lipids (in mmol/L), glucose (in mmol/L) and fibrinogen (in g/L) and average systolic and diastolic blood pressures (in mmHg) were also calculated. With the exception of plasma cholesterol concentration, all variables were transformed using a logarithmic or square root transformation.

Serum ferritin concentration was next regressed against each variable with which it was found to be significantly correlated with potential confounding variables using multiple linear regression analyses. Potential confounding variables considered at this stage were age, BMI, waist to hips ratio, dietary iron or fat, dietary fibre, alcohol consumption, cigarettes smoked, hours of vigorous exercise, coffee consumption and dietary calcium (in mg per day). As well as being associated with serum ferritin concentration (10,22,23), these variables may be associated with plasma cholesterol (24,30), HDL cholesterol (24,31,30), triglycerides (24,30,31) or glucose (24,32,33) concentrations or blood pressure (24,34). Dietary total energy was again included in each multiple linear regression analysis. Dietary cholesterol, saturated, polyunsaturated and monounsaturated fatty acids, total fat and calcium were also not normally distributed and had to be transformed using a square root transformation. Collinearity and the potential confounders which most reduced the correlation between serum ferritin and the biochemical risk factors and blood pressure were assessed as before.

**Results**

Table 1 shows the medians and ranges of the subjects’ ages, serum ferritin concentrations and cardiovascular risk factors of interest. Table 2 shows the medians and ranges of the nutrient and beverage intakes of interest.

The correlation coefficients between BMI, waist to hips ratio, number of cigarettes smoked and hours of vigorous exercise and fasting serum ferritin concentration are shown in Table 3. There was a statistically significant correlation between BMI and waist to hips ratio and serum ferritin concentration.

Table 4 shows the regression coefficients between BMI and waist to hips ratio and serum ferritin concentration from the multiple linear regression analysis adjusting for one another, number of cigarettes smoked, hours of vigorous exercise and the other potential confounding variables. Only waist to hips ratio was significantly associated with serum ferritin concentration but only just. There were no redundant variables from the check for collinearity. Dietary energy was the variable that most reduced the correlation between BMI and serum ferritin.

The correlation coefficients between serum ferritin concentration and fasting plasma concentrations of cho-
lesterol, HDL cholesterol, triglycerides, glucose and fibrinogen and blood pressure are also shown in Table 3. There were statistically significant correlations between serum ferritin concentration and plasma cholesterol, HDL cholesterol, triglycerides and glucose concentrations, while the correlation between serum ferritin and systolic blood pressure \((P = 0.09)\) was not statistically significant.

Table 5 shows the regression coefficients between serum ferritin concentration and each of plasma cholesterol, plasma HDL cholesterol, plasma triglycerides, plasma glucose and systolic blood pressure from the multiple linear regression analyses adjusting for potential confounding variables. The only risk factor for cardiovascular disease with which serum ferritin was significantly associated, and only narrowly, was plasma triglycerides. There were no redundant variables from the collinearity checks, except for dietary starch and sugar in relation to plasma triglycerides.

Discussion

Ballarat was selected for the original cardiovascular disease risk factor prevalence survey because like other non-metropolitan areas of Australia it has a higher mortality from cardiovascular disease than the capital cities. After excluding people over the age of 69 years, for whom death certification may be unreliable, Ballarat Statistical Sub-Division has a mortality rate from cardiovascular disease among men which is 1.40 times that of the capital cities (95% confidence interval 1.22–1.61) (14). The serum ferritin concentration was similar to that reported for other parts of Australia (10).

Initially we found significant correlations between serum ferritin concentration and higher plasma concentrations of glucose, triglycerides and cholesterol, and lower concentrations of HDL cholesterol, triglycerides and glucose concentrations, while the correlation between serum ferritin and systolic blood pressure \((P = 0.09)\) was not statistically significant.

Table 4. Regression coefficients between lifestyle and anthropometric cardiovascular disease risk factors and serum ferritin concentration from multiple linear regression analysis(a)

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Predictor variable</th>
<th>Regression coefficients for predictor variables</th>
<th>Standard errors of regression coefficients</th>
<th>t ratios</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ferritin</td>
<td>Body mass index</td>
<td>0.01</td>
<td>0.01</td>
<td>1.28</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Waist to hips ratio</td>
<td>1.61</td>
<td>0.79</td>
<td>2.03</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>Cigarettes smoked</td>
<td>0.01</td>
<td>0.02</td>
<td>0.66</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>Hours of vigorous exercise</td>
<td>-0.04</td>
<td>0.10</td>
<td>-0.38</td>
<td>0.70</td>
</tr>
</tbody>
</table>

n = 131

(a) Adjusted for age; dietary iron, fibre, vitamin C and energy; alcohol, coffee and tea consumption; and use of aspirin or non-steroidal anti-inflammatory drugs.

Table 3. Correlation coefficients between serum ferritin concentration and cardiovascular disease risk factors from univariate analysis

<table>
<thead>
<tr>
<th>Outcome variable</th>
<th>Correlation coefficient ((r))</th>
<th>Significance ((P))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ferritin</td>
<td>0.28</td>
<td>0.001</td>
</tr>
<tr>
<td>ferritin</td>
<td>0.26</td>
<td>0.003</td>
</tr>
<tr>
<td>ferritin</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td>ferritin</td>
<td>-0.12</td>
<td>0.19</td>
</tr>
<tr>
<td>Plasma:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cholesterol</td>
<td>0.28</td>
<td>0.00</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>-0.22</td>
<td>0.01</td>
</tr>
<tr>
<td>triglycerides</td>
<td>0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>glucose</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>fibrinogen</td>
<td>0.04</td>
<td>0.65</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.15</td>
<td>0.09</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.13</td>
<td>0.15</td>
</tr>
</tbody>
</table>

n = 131
The correlation coefficients we found initially between serum ferritin concentration and fasting plasma cholesterol, HDL cholesterol, triglycerides and glucose concentrations were of the same order of magnitude as the unadjusted (or only age adjusted) correlation coefficients which previous workers have found between these variables (2,4,5). Previous workers did not proceed to regress serum ferritin against fasting plasma lipids and glucose concentrations with potential confounding factors as we have done. However, Salonen et al. found that meat intake was associated with both serum ferritin and serum low density lipoprotein cholesterol in their data (2). It is therefore possible that the association between concentrations of serum ferritin and triglycerides in their data would also have been much reduced had they regressed the two variables against one another with dietary lipids as we did.

That the correlation coefficients we and others have found between serum ferritin and plasma lipids and glucose concentrations are due to confounding is supported by the weakness of the correlations. It is supported, too, by the inconsistency between studies over the existence of an association between serum ferritin concentration and plasma concentrations of cholesterol (2,4,5), HDL cholesterol (2,5,6), triglycerides (2,4–7) and glucose (2,6). We are not aware of any biological mechanism being documented whereby a change in serum ferritin concentration can lead to a change in the actual concentrations of plasma cholesterol, HDL cholesterol, triglycerides and glucose. However, the biological basis of many associations found during epidemiological studies have only become apparent later.

Although we found no reason to believe that iron status influences men's risk of coronary heart disease through conventional risk factors for the disease, knowledge of the determinants of serum ferritin concentration is still important (for example, to assess the significance of high values in patients). Initially we found significant correlations between serum ferritin concentration and both BMI and waist to hips ratio, as previous workers have done (4,5,7–9). However, when they were regressed against serum ferritin with potential confounding variables, the associations were considerably reduced, as with waist to hips ratio, or lost altogether, as with BMI. It was dietary energy which most reduced the correlation between serum ferritin concentration and both BMI and waist to hips ratio. This is not unexpected as meat and meat products are both the second most common source of energy and the most common source of iron for Australian men (21). Diet is therefore the main non-constitutional determinant of men’s serum ferritin concentration in this population.

The correlation coefficients we found initially between BMI and waist to hips ratio and serum ferritin concentration were both of the same order of magnitude as the age adjusted correlation coefficients which previous workers have found between these variables (4,5,7). Rodger et al. also found no association between BMI and serum ferritin concentration in men after adjusting for potential confounding factors (age, smoking, alcohol consumption and exercise) using multiple linear regression analysis (11). Although Oshaug et al. found no difference in dietary iron and percentage of energy from fat between the different BMI and serum ferritin sub-groups in their study, they did not actually regress BMI against serum ferritin with these and other potential confounding factors (5). An association between BMI and serum ferritin found among adolescent males in Sweden has been linked to an effect of overweight on liver transaminases, which might give falsely high serum ferritin concentrations (9). The associations between BMI and serum ferritin that have been found in other populations might also be spurious.

The association we found between waist to hips ratio and serum ferritin concentration after adjusting for confounding factors was weak and only narrowly statistically significant. It is possible that, as with plasma triglycerides, the association was due to confounding by some unknown factor or our being unable to adjust fully for the confounding factors we had identified. If there is an association between waist to hips ratio and serum ferritin concentration, our findings would suggest that it is the distribution of body fat, particularly excess central fat, as measured by the waist to hips ratio, which affects serum ferritin concentration, rather than the body’s energy store corrected for height, as measured by the BMI (35).

Previous workers have failed to find an association between smoking and exercise and serum ferritin concentration as we did (2,7). Findings over an association between serum ferritin concentration and blood pressure have been inconsistent with studies reporting no
association (6), an association with only systolic blood pressure (2) and a weak association with both systolic and diastolic blood pressure (4,5,7).

**Conclusion**

We conclude that the correlation coefficients we found initially between anthropometric risk factors and serum ferritin concentration and serum plasma ferritin and biochemical risk factors were due to confounding by dietary and other factors. There is therefore no reason to believe that iron status influences men’s risk of coronary heart disease through conventional risk factors for the disease. If, in the future, studies of the effects of iron depletion were to show that it failed to change plasma lipid or glucose concentrations, this would help to confirm that the initial associations we found between serum ferritin concentration and these variables were non-causal.

**References**