Serum Ferritin, Sex Hormones, and Cardiovascular Risk Factors in Healthy Women

Lillian Nordbø Berge, Kaare H. Benaa, Arne Nordøy

Abstract  The protective effect of endogenous sex hormones is commonly believed to explain the gender gap in the risk of coronary heart disease and the diminished protection in women when menopause occurs. Recent reports indicate that iron overload, due to cessation of menstrual bleeding, may be an important factor. We therefore investigated iron stores by serum ferritin measurements in healthy premenopausal (n=113) and postmenopausal (n=46) women. Ferritin levels were higher in postmenopausal than in premenopausal women, both in blood donors (43.4 versus 23.1 μg/L, P<.001) and in nondonors (71.7 versus 32.8 μg/L, P<.001). Serum ferritin was positively correlated with age (r=.36, P<.001). After age adjustment, serum ferritin was positively correlated with hemoglobin, hematocrit, serum total cholesterol, and low-density lipoprotein (LDL) cholesterol. Total cholesterol was correlated with age (r=.66, P<.001), as were LDL cholesterol (r=.60, P<.01) and high-density lipoprotein cholesterol (r=.32, P<.01). Neither ferritin nor serum lipids were directly associated with female sex hormone levels. The mutual relation between ferritin, hemoglobin, and hematocrit probably only indicates their usefulness as measures of body iron. The parallel rise in serum ferritin, total cholesterol, and LDL cholesterol might contribute to the increased risk of coronary heart disease among postmenopausal women.

Key Words  • cholesterol • ferritin • menopause • women

Coronary heart disease (CHD) is an uncommon disease in premenopausal women. Most often, this has been attributed to the endogenous sex hormones, although the effects of age and menopause are difficult to separate.

The benefits of estrogens have hitherto been related to the effects on serum lipids, as postmenopausal women experience elevation of serum low-density lipoprotein (LDL) cholesterol and triglyceride concentrations, factors associated with an increased risk of CHD.

It has been speculated that factors other than serum lipids are influenced by the menopause. Data from the Framingham Study suggested that removal of the uterus was as important as oophorectomy or a natural menopause in regard to the risk of CHD. This led Sullivan to propose regular menstrual blood loss as the protective factor and related the postmenopausal risk of CHD to an accumulating iron overload, reflected in raised levels of the iron storage protein, ferritin. The mechanisms, he suggested, might involve oxidant activity of iron-dependent enzymes. Recently, Lauffer reported a positive correlation between CHD mortality and the amount of iron in the liver. Similarly, Salonen et al found an increased risk of myocardial infarction in men with elevated serum ferritin, and the association was even stronger in subjects with high serum LDL cholesterol levels.

The aim of the present study was to investigate the cross-sectional relation between serum ferritin, sex hormones, and cardiovascular risk factors in healthy women.

Methods

The present investigation was part of another study described earlier. In the original study 50 women in late pregnancy were also included for comparison with premenopausal women aged younger than 40 years (n=55), and postmenopausal women were compared with premenopausal women aged older than 40 years (n=58); the age limits were arbitrarily chosen to reduce the age differences between the groups compared. In the present study all premenopausal women (n=113) were included to achieve a larger sample.

Volunteers were recruited among staff members of the Institute of Clinical Medicine and University Hospital of Tromsø by distribution of a questionnaire containing inclusion and exclusion criteria. The postmenopausal women were older (55.3±6.1 years) than the premenopausal women (38.9±7.2 years). Menopausal state was confirmed by the serum concentration of estradiol and luteinizing hormone. A natural menopause had occurred at least 1 year ago (mean, 7.1 years; range, 1 to 37 years). In premenopausal women cycles were regular (28±4 days), and the phase of the cycle was determined by the last menstrual period and estradiol and progesterone analysis.

None of the subjects were using any medication, including oral contraceptives or other hormones. Blood donation, lifestyle habits, and family history of premature cardiovascular disease were recorded. The subjects were examined in the sitting position after an overnight fast. After blood pressure recording, blood was drawn for analysis of serum ferritin, sex hormones, lipids, fibrinogen, and hematologic variables. Written informed consent was obtained from all participants.

Hematology

Blood was drawn into Vacutainers containing EDTA, and hematologic analysis including platelet count and mean platelet volume was performed on a Coulter Counter model S-plus III (Coulter Electronics Inc).
Ferritin
Blood was allowed to clot for 1 hour at 37°C, centrifuged at 2000g for 10 minutes, and stored at -20°C until testing. Ferritin was analyzed by an immunometric technique based on enhanced luminescence, using monoclonal mouse anti-ferritin (Amerlite Diagnostics Ltd, Amersham).

Fibrinogen
Blood was drawn into Vacutainers containing citrate, giving a 9:1 blood/anticoagulant ratio, and immediately placed on ice. Analysis was performed by turbidimetric rate measurement of fibrin polymer formation in an Automatic Clinical Analyzer (DuPont).

Hormone Analysis
Serum estradiol and progesterone were measured by a competitive assay based on enhanced luminescence, with a kit from Amersham and readings in an Amerlite Analyzer (Amersham). In postmenopausal women, serum luteinizing hormone was measured by fluorometric enzyme immunoassay with a commercial kit (Baxter Healthcare Corp). Serum testosterone was analyzed by a no-extraction, solid-phase radioimmunoassay with a commercial kit (Diagnostic Products Corp).

Lipid and Lipoprotein Analysis
Total cholesterol and triglycerides in serum were measured with a Hitachi 737 Automatic Analyzer and reagents from Boehringer Mannheim. High-density lipoprotein (HDL) cholesterol was measured in the supernatant after precipitation of very-low-density lipoproteins and LDL according to Burstein et al. LDL cholesterol was calculated by the formula of Friedewald et al. Statistical analyses were performed on a personal computer with STATGRAPHICS (STSC, Inc) and SAS (SAS Institute Inc) statistical software programs. We regarded \( P<0.05 \) as significant. The values are reported as mean±SD unless otherwise specified.

Results
In women of reproductive age, the phase of the menstrual cycle at the time of examination was as follows: menstruation (\( n=27 \)), follicular (\( n=47 \)), and luteal (\( n=39 \)), with estradiol and progesterone levels within the expected range (0.31 to 1.55 and 12.3 to 78 nmol/L, respectively) (mean; range). Serum testosterone was 1.23±0.54 mmol/L. Postmenopausal women had significantly lower serum estradiol (<0.07 nmol/L), progesterone (<1 nmol/L), and testosterone (0.78±0.49 mmol/L) than premenopausal subjects \( (P<0.01 \) for all hormones).

Among premenopausal women, 37.2% reported daily smoking, compared with 21.8% in the postmenopausal group. Regular physical exercise (>2 h/wk) was reported by 46.0% of premenopausal women and by 34.8% of postmenopausal women. The differences in smoking and exercise habits were not significant. The percentages of blood donors were similar in the two groups (18.6% of premenopausal and 17.4% of postmenopausal women). The two groups differed with respect to clinical and laboratory data (Table 1). After age adjustment (by ANCOVA), only serum ferritin, total cholesterol, and LDL cholesterol remained significantly different. In these analyses, age and menopausal status were independently related to total cholesterol and LDL cholesterol, whereas age was not related to ferritin when controlling for menopausal status. Controlling for smoking did not change the results (data not shown). For the groups combined, blood donors had lower serum ferritin levels than nondonors (Table 2).

Serum ferritin was positively correlated with age, systolic and diastolic blood pressure, hemoglobin, he-

### Table 1. Characteristics and Laboratory Values of Healthy Premenopausal and Postmenopausal Women

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal (n=113)</th>
<th>Postmenopausal (n=46)</th>
<th>Unadjusted</th>
<th>Age Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.1±3.2</td>
<td>23.3±2.8</td>
<td>.78</td>
<td>.50</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>114.0±14.4</td>
<td>120.2±14.9</td>
<td>.02</td>
<td>.71</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>75.3±7.9</td>
<td>78.3±6.2</td>
<td>.02</td>
<td>.73</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>13.2±1.2</td>
<td>13.6±0.8</td>
<td>.05</td>
<td>.31</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>38.3±3.1</td>
<td>39.5±2.5</td>
<td>.02</td>
<td>.30</td>
</tr>
<tr>
<td>Platelets, 10⁹/L</td>
<td>274±61</td>
<td>258±57</td>
<td>.14</td>
<td>.45</td>
</tr>
<tr>
<td>Mean platelet volume, fl</td>
<td>8.6±0.9</td>
<td>8.4±0.7</td>
<td>.19</td>
<td>.21</td>
</tr>
<tr>
<td>Fibrinogen, g/L</td>
<td>2.5±0.5</td>
<td>2.8±0.5</td>
<td>.01</td>
<td>.31</td>
</tr>
<tr>
<td>Ferritin, µg/L</td>
<td>31.2±20.5</td>
<td>66.7±43.9</td>
<td>.0001</td>
<td>.0003</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.58±0.87</td>
<td>7.24±1.33</td>
<td>.0001</td>
<td>.001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.71±0.37</td>
<td>1.87±0.42</td>
<td>.02</td>
<td>.48</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.48±0.75</td>
<td>4.93±1.35</td>
<td>.0001</td>
<td>.0003</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.87±0.43</td>
<td>1.03±0.43</td>
<td>.01</td>
<td>.18</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol. Values are mean±SD. Differences between groups were tested by ANCOVA.
TABLE 2. Serum Ferritin Concentration in Healthy Premenopausal and Postmenopausal Women

<table>
<thead>
<tr>
<th></th>
<th>Blood Donors (n=19)</th>
<th>Nondonors (n=94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal</td>
<td>23.1 ± 8.7</td>
<td>32.8 ± 21.8</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>43.4 ± 31.6</td>
<td>71.7 ± 44.9</td>
</tr>
<tr>
<td>All women</td>
<td>29.1 ± 20.3</td>
<td>43.9 ± 34.8*</td>
</tr>
</tbody>
</table>

Values are mean ± SD, expressed in micrograms per liter. *P < .05 compared with blood donors.

matocrit, total cholesterol, HDL cholesterol, and LDL cholesterol for the total sample (Table 3, Fig 1, and Fig 2). Inverse relations were seen between log ferritin and platelet count. Adjusting for blood donation did not change the correlations (data not shown). After age adjustment, the associations with hemoglobin, hematocrit, platelet count, total cholesterol, and LDL cholesterol remained significant (Table 3). Serum ferritin was not correlated with any of the female sex hormones (alone or combined), serum testosterone, the phase of the menstrual cycle, or the duration of the postmenopausal period.

Total cholesterol and LDL cholesterol were both correlated with age (r = .66, P < .001 and r = .60, P < .01, respectively), as was HDL cholesterol (r = .32, P < .01). To further investigate the effects of age and menopause, we calculated the correlation coefficients between risk factors and the female sex hormones. The estradiol and progesterone levels of regularly menstruating women fluctuate within the same range regardless of age. Similarly, all postmenopausal women have the same measurable serum levels of these hormones. Correlation between risk factors and female sex hormones is thus only meaningful in premenopausal women. Except for an inverse relation between diastolic blood pressure and progesterone (r = −.21, P < .05), none of the risk factors under investigation were associated with any of the female sex hormones alone or in combination. Identical results were obtained when controlling for age, confirming the lack of association between age and hormone level within this group.

The associations between ferritin and hemoglobin and between ferritin and hematocrit also remained significant when controlling for sex hormones. No significant correlations were seen between serum ferritin and family history of heart disease, smoking, physical exercise, frequency of meat dinners, body mass index, fibrinogen, or serum triglycerides (data not shown).

Discussion

The positive correlation between serum ferritin and menopause fits well into the theory of increasing iron stores when regular bleeding ceases. The influence of age on serum ferritin is well documented. The age-independent correlation between ferritin and hematocrit in healthy subjects is a common observation, indicating that they are all useful measures of body iron.

The close relation between age and menopause makes the determination of their relative influence on risk factors difficult. However, in the present study the

![Fig 1. Scatterplot showing serum ferritin concentration in healthy premenopausal and postmenopausal women, blood donors excluded.](http://atvb.ahajournals.org/)

TABLE 3. Crude and Partial Pearson Correlations Between Serum Ferritin and Cardiovascular Risk Factors in Healthy Women

<table>
<thead>
<tr>
<th></th>
<th>Premenopausal (n=113)</th>
<th>Postmenopausal (n=46)</th>
<th>Total Sample (n=159)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>.10</td>
<td>.18</td>
<td>.36†</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>.08</td>
<td>.17</td>
<td>.21†</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>.07</td>
<td>.16</td>
<td>.16*</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>.19*</td>
<td>.21</td>
<td>.36†</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>.20*</td>
<td>.20</td>
<td>.37†</td>
</tr>
<tr>
<td>Platelet count</td>
<td>-.18</td>
<td>-.12</td>
<td>-.20*</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>.16</td>
<td>.16</td>
<td>.40†</td>
</tr>
<tr>
<td>HDL-C</td>
<td>.16</td>
<td>.30*</td>
<td>.24†</td>
</tr>
<tr>
<td>LDL-C</td>
<td>.10</td>
<td>.07</td>
<td>.34*</td>
</tr>
</tbody>
</table>

BP indicates blood pressure; HDL-C, high-density lipoprotein cholesterol; and LDL-C, low-density lipoprotein cholesterol.

*P < .05, †P < .01, ‡P < .001.
best-fit curve for the relation between serum ferritin and age was nonlinear, and menopause but not age was significantly related to ferritin in the multivariate analyses. The lack of measurable associations between serum ferritin and the female sex hormones may indicate that the effect of menopause on iron stores is an indirect one, mediated via the cessation of bleeding.

In accordance with our findings, total cholesterol and LDL cholesterol have previously been reported to increase as a function of biological age, but are probably also associated with menopause. Increasing serum levels of total cholesterol and LDL cholesterol premenopausally have been reported, interpreted as a consequence of declining ovarian function. Although the present study showed no correlation between serum lipids and sex hormone levels in premenopausal women, the permanent lowering of these hormones in the postmenopausal period obviously has a different effect. The covariations of total cholesterol and LDL cholesterol with age and menopause probably explain their relation to serum ferritin.

Both serum ferritin and cholesterol are influenced by dietary habits. Intake of meat, a common source of iron and saturated fat, was reported to be positively correlated with serum ferritin in Australian women and with the risk of acute myocardial infarction in Italian women. With the crude measure of meat dinners per week, we found no associations with ferritin or cholesterol. A recent publication from the Framingham Study reported limited associations between dietary fat and serum cholesterol in women.

Salonen et al. reported a synergistic effect of serum ferritin and LDL cholesterol on the risk of developing ischemic heart disease, although ferritin and LDL cholesterol were not significantly associated. In addition, they found an inverse age-adjusted correlation between ferritin and HDL. In our data, ferritin and HDL cholesterol were not significantly related when controlling for age. In a meta-analysis of data from several large studies, Lauffer found no correlation between nonheme hepatic iron and cholesterol. Iron and HDL cholesterol values were inversely correlated, although the relation was significant only when both sexes were included in the analysis. Salonen et al. investigated men, who exhibited markedly higher serum ferritin levels (mean, 166 μg/L) than the women of the present study. This might have contributed to the more pronounced effects on risk factors and end points. Another important difference from the present study was a decrease of serum ferritin levels with age, whereas we found the opposite.

The relative influence of age and menopause on serum HDL cholesterol is controversial. Like the present study, some previous cross-sectional studies indicate an age-related increase, whereas unchanged or decreasing HDL cholesterol levels have also been reported. Cross-sectional studies of HDL cholesterol and menopause have demonstrated no change. While in two longitudinal investigations, HDL cholesterol was found to decrease slightly postmenopausally. A longitudinal design is probably more reliable to detect the small changes in HDL cholesterol that occur perimenopausally.

One theory about serum ferritin as a risk factor is that iron has a potentially oxidizing effect on LDL cholesterol. Because oxidatively modified LDL cholesterol may be particularly atherogenic, the coexistence of high serum ferritin and LDL cholesterol levels may have an effect that is at least additive.

In men, serum ferritin and LDL cholesterol levels increase at adolescence. In women, both variables are lower than in men from puberty to menopause, when they both increase. According to these considerations, the interrelation between ferritin and LDL cholesterol may be similar in the two sexes, the main difference being the time at which important alterations in the serum levels occur. Because atherosclerosis is a slowly developing process and menopause does not occur at the same age in all women, the influence of menopause on the development of CHD is not precluded by epidemiological studies showing no distinct bend of the incidence curve at some specific age.

The inverse relation between serum ferritin and platelets across groups remained significant after age adjustment. Although the platelet count was not significantly different in the two groups, the finding may indicate that both ferritin and platelet count are associated with the menopause. This is consistent with previous observations of a lower platelet number in postmenopausal women. At present it is not known whether serum ferritin and platelets have any mutual relation that may influence the risk of cardiovascular disease.

In conclusion, menopause is a major determinant of the serum ferritin level in women. The lack of significant associations with female sex hormones in our study may indicate that the cessation of regular bleeding contributes to higher ferritin levels postmenopausally. Independent of age, serum ferritin is associated with known cardiovascular risk factors. The parallel increase in serum LDL cholesterol and ferritin concentration after the menopause may contribute to the increased risk of CHD in elderly women.

References
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