Body iron stores and glucose intolerance in premenopausal women: role of hyperandrogenism, insulin resistance and genomic variants related to inflammation, oxidative stress and iron metabolism

Mª Ángeles Martínez-García, Ph.D., Manuel Luque-Ramírez, M.D., Ph.D., * José L. San-Millán, Ph.D. † and Héctor F. Escobar-Morreale, M.D., Ph.D.

Departments of Endocrinology and †Molecular Genetics, Hospital Universitario Ramón y Cajal & Universidad de Alcalá, Madrid, and Centro de Investigaciones Biomédicas en Red de Diabetes y Enfermedades Metabólicas Asociadas CIBERDEM, Spain.
*Present address: Department of Endocrinology Hospital Universitario de La Princesa, Madrid, Spain.

Corresponding author:
Héctor F. Escobar-Morreale, M.D., Ph.D
E-mail: hescobarm.hrc@salud.madrid.org

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Objective: Increased serum ferritin levels and iron stores might be involved in the development of abnormal glucose tolerance in women presenting with obesity and or polycystic ovary syndrome (PCOS). We aimed to study the determinants of serum ferritin levels in premenopausal women among indexes of insulin resistance, adiposity, hyperandrogenism and genotypes pertaining to inflammation, oxidative stress and iron metabolism.

Research Design and Methods: Two hundred fifty-seven premenopausal women, classified depending on the presence or absence of PCOS, obesity and/or abnormal glucose tolerance, were submitted to a complete metabolic evaluation, serum ferritin, haptoglobin and C-reactive protein (CRP) measurements, and genotyping for proinflammatory and prooxidant variants and mutations in the HFE gene.

Results: Serum ferritin concentrations were increased in women presenting with PCOS and/or abnormal glucose tolerance, independently of obesity. A stepwise multivariate linear regression analysis ($R^2 = 0.18$, $P < 0.0001$) retained menstrual regularity ($\beta = 0.14$, $P = 0.035$), free testosterone ($\beta = 0.14$, $P = 0.052$), insulin sensitivity index ($\beta = 0.12$, $P = 0.012$), His63Asp variant in HFE ($\beta = 0.16$, $P = 0.008$) and abnormal glucose tolerance ($\beta = 0.15$, $P = 0.015$) as significant predictors of the logarithm of ferritin levels, whereas neither CRP, haptoglobin, waist to hip ratio nor variants in the TNFα, TNFRSF1B, IL6, IL6ST, IL6Ra, PON-1 and HFE Cys282Tyr mutation exerted any influence.

Conclusions: Androgen excess (partly because of hyperandrogenemia and partly because of menstrual dysfunction), insulin resistance, abnormal glucose tolerance and HFE His63Asp variant correlate with ferritin levels in premenopausal women.
Mounting evidence suggests that increased body iron stores are involved in the pathogenesis of insulin resistant disorders such as the metabolic syndrome and type 2 diabetes in the general population (1). This involvement appears to be bidirectional, because iron accumulation does not only favor insulin resistance and may contribute to pancreatic β-cell dysfunction and diabetes, but also insulin resistance may in turn facilitate iron accumulation within the body (2, 3). Interestingly, oxidative stress and inflammation are involved in the interplay between iron overload and insulin resistance (2, 4).

Although most of the evidence linking iron metabolism and disorders of glucose metabolism has been provided from the study of middle-aged or older men and of postmenopausal women (2, 5), body iron stores also influence insulin resistance and glucose metabolism in premenopausal women (6, 7).

Ferritin is the cellular storage protein for iron. We have reported that serum ferritin levels are increased in women presenting either with obesity or the polycystic ovary syndrome (PCOS), and very especially when both conditions are present in the same patient (6). Therefore, both androgen excess and insulin resistance may underlie this finding. And because these changes occurred independently of changes in serum inflammatory markers, the increased ferritin levels indicates that body iron stores are actually increased in these women and do not result from the secondary role of ferritin as an acute-phase marker (6).

Of note, serum ferritin levels were clearly increased in the small subset of these women presenting with abnormal glucose tolerance, both in the PCOS and non-hyperandrogenic subgroups (6). This finding suggests that increased body iron stores could be related to the development of abnormalities in glucose metabolism in these patients, because progressive iron accumulation in the pancreas is a recognized pathogenic mechanism of disorders of glucose tolerance in patients with iron overload (8).

We hypothesized that the reduced menstrual losses secondary to the oligomenorrhea present in most women with PCOS could contribute to their increased iron stores, yet recent data from our group suggest that insulin resistance is actually one of the major players explaining their increased serum ferritin levels: whereas serum ferritin levels did not change after restoring regular menses by using an oral contraceptive for 6 months, these levels decreased markedly after insulin-sensitization using metformin (3).

The present study was undertaken with the aim of determining, in a large series of premenopausal women, which are the determinants of increased serum ferritin levels (an index of body iron stores and a risk factor for the development of abnormalities of glucose tolerance) among markers of hyperandrogenism, adiposity, insulin resistance and genomic variants related to chronic inflammation, oxidative stress and iron metabolism.

**RESEARCH DESIGN AND METHODS**

**Subjects:** Two hundred fifty-seven premenopausal women were included. The group was composed by consecutive patients reporting to the Department of Endocrinology because PCOS and/or weight excess and by healthy non-hyperandrogenic non-obese volunteers recruited from the staff of Hospital Universitario Ramón y Cajal. Women were classified according to their BMI into obese (BMI $\geq 30$ kg/m$^2$, $n = 128$) and non-obese (BMI < 30 kg/m$^2$, $n = 129$) subgroups. PCOS was diagnosed in 148 women presenting with clinical and/or biochemical hyperandrogenism in addition to oligo-ovulation as previously
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reported (9) - thereby fulfilling all the current definitions of the syndrome (10-12) - whereas 109 women showed no sign of hyperandrogenism, had normal androgen levels and had regular ovulatory menstrual cycles.

Menstrual and ovulatory dysfunction were defined by the presence of oligomenorrhea (cycles longer than 35 days), or amenorrhea (absence of menstrual bleeding for at least three usual cycle lengths) or, in women presenting with regular menstrual cycles (cycles between 26 and 35 days), by lack the of ovulation according to body temperature charts and/or by serum progesterone levels less than 12.7 nmol/l during the luteal phase of the menstrual cycle (9).

None of the women had a personal history of hypertension, diabetes mellitus or cardiovascular events. Women who took oral contraceptives, antiandrogens, insulin sensitizers, iron supplements or drugs that might interfere with blood pressure regulation, lipid profile or carbohydrate metabolism within the previous 6 months, or who were referred for any medical reason aside from androgen and/or weight excess, were automatically excluded. Written informed consent was obtained from all the participants, and the study was approved by the Ethics Committee of Hospital Universitario Ramón y Cajal.

**Study protocol:** Clinical and anthropometrical variables, including the hirsutism score, BMI, waist circumference and WHR were determined. WHR was calculated by dividing the minimal waist circumference by the hip circumference at the level of greater trochanters, using a non-stretchable measuring tape.

Whole blood, serum and plasma samples were obtained between days five and 10 of the menstrual cycle, or during amenorrhea after excluding pregnancy. After a 3-day 300-g carbohydrate diet and 12-h overnight fasting, samples were obtained early in the morning for the measurement of total testosterone, sex hormone-binding globulin, 17-hydroxyprogesterone, androstenedione, dehydroepiandrosterone-sulfate, luteinizing hormone, follicle-stimulating hormone, estradiol, thyrotropin and prolactin. A complete hemogram, serum biochemistry and lipid profiles were also obtained. Then, a 75-g oral glucose tolerance test was performed, and samples were obtained for measurement of serum insulin and plasma glucose at 0, 30, 60, 90 and 120 min. Samples were immediately centrifuged, and serum and plasma were separated and frozen at -20°C until assayed.

The assays used for these measurements have been described in detail elsewhere (9). Free testosterone concentrations were calculated from total testosterone and sex hormone-binding globulin levels (9). Serum ferritin and C-reactive protein (CRP) concentrations were measured by automated immunochemiluminescence (Immulite 2000 Ferritin and High Sensitivity CRP, Diagnostic Products Corporation, Los Angeles, CA) with lower limit of detection of 0.88 pmol/L and 0.1 mg/L respectively, and intra- and inter-assay coefficients of variation below 10%. The circulating concentrations of haptoglobin were assayed by a commercial immunonephelometry method (Dade Behring, Marburg, Germany) calibrated against the international CRM 470 reference material. The composite insulin sensitivity index was calculated from circulating glucose and insulin concentrations during the oral glucose tolerance test as described by Matsuda & DeFronzo (13). Disorders of glucose tolerance were diagnosed following the recommendations of the American Diabetes Association (14).

**DNA analysis:** Genomic DNA was obtained from whole blood samples using a Nucleon BACC3 DNA isolation kit
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(Amersham, Buckingham, U.K.) and was used to genotype several polymorphisms related to inflammation or oxidative stress that had been previously found to be associated with metabolic disorders such as PCOS, obesity, diabetes and insulin resistance, and variants involved in iron metabolism. Genotyping of the dinucleotide CA-repeat in intron 8 of the IL6R-α locus (rs57636717) was performed by polymerase chain reaction using a fluorescently labeled forward primer (15). Amplified fragments were resolved by capillary electrophoresis on an ABI Prism 3100 automated genetic analyzer (Applied Biosystems, Foster City, CA) and their sizes were determined using the GeneMapper 4.0 software. We detected 13 different alleles with sizes ranging from 143 to 169 bp of which the 149 bp allele was the more frequent. For statistical analyses subjects were genotyped as homozygous for 149 bp alleles, heterozygous for 149 bp alleles, and non-carriers of any 149 bp allele (15). Several genomic variants were analyzed by PCR-RFLP as previously described: variants His63Asp (c.187C>G) and Cys282Tyr (c.845G>A) in exons 2 and 4, rs1799945 and rs1800562 respectively, of the HFE gene (7); polymorphism -308G>A (rs1800629) in the promoter of the TNF-α gene (16); variant Met196Arg (c.587T>G; rs1061622) in exon 6 of TNF receptor 2 (TNFRSF1B) gene (17); polymorphism -174G>C (rs1800795) in the IL-6 gene promoter (18); variant Gly148Arg (c.442G>C; rs2228044) in exon 5 of the IL-6 signal transducer (IL6ST) gene (15) and polymorphism -108C>T (rs705379) in the paraoxonase (PON1) gene (19).

Statistical analysis: Data are shown as means ± SD unless otherwise stated. The Kolmogorov-Smirnov statistic was applied to continuous variables. Logarithmic or square root transformations were applied as needed to ensure normal distribution of the variables. Univariate general linear models were used to evaluate the influence of obesity, PCOS and glucose tolerance on clinical and biochemical variables, introducing age as a covariate to control for possible age differences among the groups. A multivariate linear regression analysis was used to determine the influence of clinical, biochemical, metabolic and genetic variants on the logarithm of serum ferritin concentrations as described below. Depending on the median of the whole population of premenopausal women, serum ferritin levels were categorized into values below, or equal or above, the median value. The associations of these two groups with categorical and continuous variables were analyzed by the \( \chi^2 \) and Fisher’s exact tests as appropriate. \( P < 0.05 \) was considered statistically significant. Analyses were performed using SPSS Statistics 17 (SPSS Inc, Chicago, Illinois).

RESULTS

The influence of PCOS and of obesity on clinical and biochemical variables is summarized in Table 1. Because PCOS patients were younger compared with non-hyperandrogenic women, and obese women were older compared with non-obese women, the influence of age was controlled by introducing this variable as a covariate in the comparisons described below.

PCOS patients had increased serum androgen and luteinizing hormone levels and decreased estradiol concentrations, and were insulin resistant and had increased WHR, compared with their non-hyperandrogenic counterparts irrespective of obesity. Obesity was characterized by increased indexes of insulin resistance, increased frequency of abnormalities in glucose tolerance, and increased serum CRP, haptoglobin and free testosterone levels compared with non-obese women, both in PCOS patients and in non-hyperandrogenic women.

Serum ferritin levels were influenced independently by PCOS and glucose
intolerance (Figure 1). PCOS patients presented with increased serum ferritin levels when compared with non-hyperandrogenic women. When considered as a whole, women presenting with abnormal glucose tolerance had higher serum ferritin concentrations compared with those showing normal glucose values during fasting and after an oral glucose tolerance test. Of note, these results occurred both in the non-obese and in the obese subgroups, and obesity did not influence serum ferritin concentrations after controlling for both PCOS and glucose tolerance (Figure 1).

Because serum ferritin levels were not distributed normally, their logarithm was introduced as dependent variable in multiple linear regression models using age, BMI, glucose tolerance (codified as normal or abnormal), menstrual cycles (codified as regular menstruation, oligomenorrhea or amenorrhea), serum free testosterone, C-reactive protein and haptoglobin levels, the insulin sensitivity index, and the genomic variants related to iron metabolism, inflammation and oxidative stress as independent variables. The model that considered all independent variables explained 22% of the variability in the logarithm of serum ferritin concentrations ($R^2 = 0.22, P < 0.0001$, Figure 2).

On the contrary, when the independent variables were introduced using a stepwise method (probability to enter $\leq 0.05$, probability to remove $\geq 0.10$), the model ($R^2 = 0.17, P < 0.0001$) adjusted for age and BMI (these variables were manually entered into the model) retained only menstrual regularity ($\beta = 0.18, P = 0.003$), abnormal glucose tolerance ($\beta = 0.17, P = 0.013$), H63A variant in $HFE$ ($\beta = 0.17, P = 0.004$) and insulin sensitivity index ($\beta = -0.145, P = 0.031$) as predictors of the logarithm of serum ferritin levels (Figure 2).

When considering the median of the serum ferritin concentrations of the whole population (83 pmol/l) as a cut-off value, premenopausal women presenting with a serum ferritin level above the median had an odds ratio for abnormal glucose tolerance of 2.4 (95% confidence interval: 1.3 - 4.4, $\chi^2 = 7.420, P = 0.009$) and an odds ratio for PCOS of 2.2 (95% confidence interval: 1.3 - 3.7, $\chi^2 = 9.524, P = 0.002$).

**DISCUSSION**

The metabolic consequences of iron overload are exemplified by the development of abnormalities of glucose tolerance in primary or secondary hemochromatosis. But less severe iron overload also play an important role in the development of abnormalities in glucose tolerance (2), as demonstrated by the improvement in insulin resistance and glucose tolerance in type 2 diabetes patients after the iron depletion achieved by repeated blood letting (20) or the higher insulin sensitivity associated with reduced iron stores in frequent blood donors from the general population (21).

Our present results demonstrate that serum ferritin levels, an accurate marker of body iron stores in the absence of acute inflammatory syndromes (2), are also related to abnormal glucose tolerance in premenopausal women. Serum ferritin levels above the median associated a 2.4-fold odds ratio for presenting with abnormal glucose tolerance, and ferritin concentrations were clearly higher in women in whom fasting or
postload glucose levels were above the normal range.

Furthermore, our present results suggest that the body iron stores of premenopausal women are associated with several factors including menstrual dysfunction, insulin resistance and the His63Asp variant in HFE even after controlling for the difference in age among PCOS patients and non-hyperandrogenic controls, and between obese and non-obese women.

We have reported previously, in a much smaller series, that body iron stores are increased in premenopausal women presenting with PCOS and obesity and that these associations were independent of mutations in HFE and were not influenced by changes in markers of chronic inflammation (6, 7). Our present findings confirm that serum ferritin levels are increased in PCOS patients and that, accordingly, women presenting with serum ferritin levels above the median associate a 2.2 odds ratio of having PCOS. Yet when controlling for the presence or absence of abnormal glucose tolerance, the previously reported association with obesity disappears, suggesting that it is the increased prevalence of disordered glucose tolerance in obese women, and not weight excess by itself, the actual responsible of increased body iron stores in these women.

According to the regression analyses presented here, menstrual dysfunction, increased androgen levels and insulin resistance, together with the HFE His63Asp variant and abnormal glucose tolerance, are among the strongest predictors of serum ferritin levels. On the contrary, these levels are not influenced by indexes of global or abdominal adiposity or by genomic variants related to chronic inflammation and oxidative stress.

Therefore, it appears that androgen excess and insulin resistance, which are present in many PCOS patients, collaborate in increasing body iron stores in premenopausal women. The effect of insulin resistance and hyperinsulinism on body iron stores might depend on a direct insulin stimulation of intestinal iron absorption by up-regulating activity of hypoxia-inducible factor-1 alpha and down-regulating hepcidin expression (22, 23), and may be counteracted in PCOS patients by administering insulin-sensitizers (3).

The effect of androgen excess on body iron stores might result from the well-known stimulatory effect of androgens on erythropoiesis, thereby increasing intestinal iron absorption (24), but may also result from the iron sparing effect of reduced menstrual losses secondary to the chronic menstrual dysfunction of PCOS. This iron sparing mechanism may take years to result in increased iron stores, explaining why regularization of menstrual bleeding in PCOS patients by administering antiandrogenic contraceptive pills for 6 months had no evident impact on serum ferritin levels in our previous report (3).

Our present results also suggest that the His63Asp variant in HFE influences body iron stores in premenopausal women, in conceptual agreement with the partial loss of HFE function induced by this mutation in animal models, leading to a variable degree of hepatic iron loading (25). But more importantly, the relatively strong association of increased serum ferritin levels with abnormal glucose tolerance raises the possibility that increased iron stores played some pathogenic role in the development of such metabolic derangements, given that progressive iron accumulation in the pancreas contributes to β-cell dysfunction and abnormal glucose tolerance in syndromes of iron overload (8). However, because association does not imply causality, this hypothesis is somehow speculative.

In summary, body iron stores, as reflected by serum ferritin concentrations, are interrelated with androgen excess, insulin resistance and glucose tolerance in women.
resistance and the His63Asp variant in \textit{HFE} in premenopausal women, and are associated with the development of abnormal glucose tolerance in this particular population.

\textbf{ACKNOWLEDGMENTS}

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Table 1. Influence of obesity and PCOS on clinical and biochemical variables of premenopausal women.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-obese women (n = 129)</th>
<th>Obese women (n = 128)</th>
<th>PCOS (n=149) vs controls (n=108)</th>
<th>Obese (n=128) vs non-obese (n=129)</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCOS patients (n = 82)</td>
<td>Non-hyperandrogenic women (n = 47)</td>
<td>PCOS patients (n = 67)</td>
<td>Non-hyperandrogenic women (n = 61)</td>
<td>F/χ²</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>24 ± 6</td>
<td>29 ± 7</td>
<td>27 ± 7</td>
<td>33 ± 8</td>
<td>43.97</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.4 ± 3.5</td>
<td>24.4 ± 4.1</td>
<td>36.9 ± 5.5</td>
<td>36.7 ± 5.4</td>
<td>1.73</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>73 ± 10</td>
<td>76 ± 11</td>
<td>97 ± 13</td>
<td>96 ± 13</td>
<td>0.94</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.75 ± 0.07</td>
<td>0.76 ± 0.08</td>
<td>0.82 ± 0.08</td>
<td>0.80 ± 0.08</td>
<td>8.37</td>
</tr>
<tr>
<td>Hirsutism score</td>
<td>11 ± 6</td>
<td>2 ± 2</td>
<td>10 ± 6</td>
<td>1 ± 2</td>
<td>217.83</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>37 ± 18</td>
<td>19 ± 8</td>
<td>54 ± 25</td>
<td>24 ± 10</td>
<td>116.10</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>134 ± 80</td>
<td>245 ± 233</td>
<td>180 ± 129</td>
<td>231 ± 198</td>
<td>10.80</td>
</tr>
<tr>
<td>Luteinizing hormone (U/l)</td>
<td>6.6 ± 3.9</td>
<td>5.3 ± 3.1</td>
<td>6.1 ± 3.8</td>
<td>4.9 ± 3.1</td>
<td>5.20</td>
</tr>
<tr>
<td>Follicle-stimulating hormone (U/l)</td>
<td>5.7 ± 4.2</td>
<td>6.4 ± 6.1</td>
<td>6.2 ± 4.5</td>
<td>5.8 ± 1.8</td>
<td>0.361</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>75 ± 58</td>
<td>58 ± 46</td>
<td>137 ± 75</td>
<td>84 ± 44</td>
<td>15.19</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>4.9 ± 0.4</td>
<td>5.0 ± 0.4</td>
<td>5.3 ± 0.5</td>
<td>5.2 ± 0.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Insulin sensitivity index</td>
<td>5.4 ± 3.0</td>
<td>7.0 ± 3.8</td>
<td>2.8 ± 2.1</td>
<td>4.8 ± 3.6</td>
<td>27.34</td>
</tr>
<tr>
<td>C-reactive protein (mg/l)</td>
<td>1.3 ± 1.7</td>
<td>1.3 ± 1.6</td>
<td>6.0 ± 5.6</td>
<td>5.6 ± 5.1</td>
<td>1.68</td>
</tr>
<tr>
<td>Haptoglobin (µmol/l)</td>
<td>12 ± 4</td>
<td>12 ± 4</td>
<td>17 ± 5</td>
<td>17 ± 5</td>
<td>0.01</td>
</tr>
<tr>
<td>Regular menstruation (n, %)</td>
<td>16, 20</td>
<td>47, 100</td>
<td>10, 15</td>
<td>61, 100</td>
<td>171.00</td>
</tr>
<tr>
<td>Oligomenorrhea (n, %)</td>
<td>47, 57</td>
<td>0, 0</td>
<td>35, 52</td>
<td>0, 0</td>
<td>191.00</td>
</tr>
<tr>
<td>Amenorrhea (n, %)</td>
<td>19, 23</td>
<td>0, 0</td>
<td>22, 33</td>
<td>0, 0</td>
<td>0.512</td>
</tr>
<tr>
<td>Normal glucose tolerance (n, %)</td>
<td>74, 90</td>
<td>40, 85</td>
<td>46, 70</td>
<td>43, 70</td>
<td>0.512</td>
</tr>
<tr>
<td>Abnormal glucose tolerance (n, %)</td>
<td>8, 10</td>
<td>7, 15</td>
<td>21, 31</td>
<td>18, 30</td>
<td>0.512</td>
</tr>
</tbody>
</table>

Data are means ± SD or raw numbers and percentages. Continuous data were submitted to univariate general linear models and, because age was different between PCOS patients and non-hyperandrogenic women, and between obese and non-obese women, age was introduced as a covariate in the analysis of all the other variables. Categorical data were analyzed by χ² tests.
**Figure 1.** Serum ferritin levels depending on the presence or absence of abnormalities of glucose tolerance and polycystic ovary syndrome (PCOS) in non-obese and obese women. The box-plot includes the median (horizontal line) and the inter-quartile range, and the whiskers indicate the minimum and maximum data values, unless outliers are present in which case the whiskers extend to a maximum of 1.5 times the inter-quartile range. The figures inside the boxes are the number of women in each subgroup. The logarithm of serum ferritin levels was submitted to a general linear model in which glucose tolerance, PCOS and obesity were introduced as independent variables and age was introduced as covariate to correct for the difference in age between patients and controls and between obese and non-obese women. No interaction was found among independent variables.

* $P = 0.001$ between women presenting with or without abnormalities of glucose tolerance.

† $P = 0.034$ between polycystic ovary syndrome patients and non-hyperandrogenic control women.

**Figure 2.** Multiple linear regression analysis of the logarithm of serum ferritin concentrations. The squares are the standardized regression coefficients ($\beta$, or the change in terms of standard deviations in the dependent variable that results from a change of one standard deviation in an independent variable) and the error bars indicate the 95% confidence interval of $\beta$. Menstrual history and genomic variants were coded as dummy variables: regular menstruation was coded 0, 1 was used for oligomenorrhea 1 and 2 was used for amenorrhea. Variants in $TNF-\alpha$, $TNFRSF1B$, $IL-6$, $IL6ST$, $HFE$ and $PON-1$ loci were coded as 0 for homozygosity for wild-type alleles, 1 for heterozygosity, and 2 for homozygosity for mutant alleles. The $IL6R-\alpha$ polymorphism was coded 0 for homozygosity for 149 bp alleles, 1 for subjects carrying only one 149 bp allele, and 2 for subjects carrying two non-149 bp alleles. Finally, $HFE$ His63Asp / Cys282Tyr double heterozygotes were coded 1 and subjects without double heterozygosity were coded 0.
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Predictors of Ferritin Levels (log)

All-Factors Regression ($R^2 = 0.22, P < 0.0001$)

- Age (yr)
- BMI (kg/m²)
- Waist to hip ratio
- Menstrual regularity
- Abnormal glucose tolerance
- Free testosterone (pmol/l)
- Insulin sensitivity index
- C-reactive protein (mg/l)
- Haptoglobin (μmol/l)
- TNF-α -308GA
- TNFRSF1B Met196Arg
- IL-6 -174GC
- IL6R-α intron 5 CA repeat
- IL1ST Gly148Arg
- HFE His63Asp
- HFE Cys282Tyr
- HFE Cys282Tyr / His63Asp double heterozygosity
- PON1 -108C/T

Stepwise Regression ($R^2 = 0.18, P < 0.0001$)

- Menstrual regularity
- Abnormal glucose tolerance
- Free testosterone (pmol/l)
- Insulin sensitivity index
- HFE His63Asp

Stepwise Regression (Age- and BMI-adjusted, $R^2 = 0.17, P < 0.0001$)

- Menstrual regularity
- Abnormal glucose tolerance
- Insulin sensitivity index
- HFE His63Asp

Standardized Coefficients of Regression ($β$, 95% CI)