Liver iron overload is associated with elevated SHBG concentration and moderate hypogonadotrophic hypogonadism in dysmetabolic men without genetic haemochromatosis

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Abstract

Aims: To assess the relation between moderate iron overload on sex hormone binding globulin (SHBG) levels and gonadotroph function in men with dysmetabolic iron overload syndrome and the effects of phlebotomy.

Methods: The relationship between magnetic resonance imaging assessed liver iron concentration (LIC) and plasma ferritin levels with total testosterone, bioavailable testosterone (BT), SHBG and LH levels, were studied in 50 men with moderate dysmetabolic iron excess, in the absence of genetic haemochromatosis, who were randomised to phlebotomy therapy or to normal care.

Results: Four patients (8%) had low total testosterone (<10.4 nmol/l) and 13 patients (26%) had low BT (<2.5 nmol/l). In the entire population, those with LIC above the median (90 µmol/l) had a higher mean SHBG (P = 0.028), lower LH (P = 0.039) than those with LIC below the median. In multivariable analysis (adjusted for age, and fasting insulin) LIC was significantly associated with SHBG (positively) and LH (negatively). Patients in the highest quartile of SHBG had higher LIC (P = 0.010) and higher ferritinaemia (P = 0.012) than those in the three other quartiles. Iron depletion by venesection did not significantly improve any hormonal levels.

Conclusions: Hypogonadism is not infrequent in men with dysmetabolic iron overload syndrome. Liver iron excess is associated with increased plasma SHBG and moderate hypogonadotrophic hypogonadism. Phlebotomy therapy needs further investigation in symptomatic hypogonadal men with dysmetabolic iron excess.

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Introduction

Testosterone is lower in men with the metabolic syndrome or with type 2 diabetes, in comparison to metabolically healthy men (1, 2). Dysfunction of the gonadal axis is central (hypogonadotrophic hypogonadism) as gonadotropins are within the normal range. In addition, SHBG levels are usually low in men with insulin resistance (3).

In haemochromatosis, where iron overload is significant, excess iron is deposited in the pituitary gland with predilection for the gonadotrophs, with subsequent impairment of gonadotroph function (4, 5). Decreased expression of transferrin and transferrin receptor in iron loaded gonadotrophs probably reflects a down-regulation effect, and therefore suggests that these cells are a target of iron overload (6).

Currently, there is no published data on the effect of mild iron overload on the gonadal axis in the absence of genetic haemochromatosis. The dysmetabolic iron overload syndrome has been defined as a condition with hyperferritinaemia, mild liver iron overload, and various features of the metabolic syndrome (7). It is frequently associated with insulin resistance (8). However, the relationship between moderate dysmetabolic iron overload and both gonadotroph function and SHBG concentration has not been investigated. The aim of our study was to assess the effects of iron excess and removal under phlebotomy on the gonadal axis by investigating the relations between SHBG, sex hormone levels and liver iron stores, as assessed by a non-invasive magnetic resonance imaging (MRI) method. In addition, we analysed the prevalence of hypogonadism in men with dysmetabolic iron overload syndrome.
Patients and methods

We performed ancillary analysis from a randomised control trial on the effect of iron overload on CYP2E1 activity (9).

Study population

In this study, 50 men presenting with dysmetabolic iron overload syndrome were enrolled. Dysmetabolic iron overload syndrome was diagnosed using the following criteria: i) evidence of iron overload: liver iron concentration (LIC), measured by liver resonance MRI as described previously (10, 11), higher than 36 μmol/g; ii) presence of at least one of the following metabolic features: a) body mass index (BMI) ≥ 25 kg/m², b) systolic/diastolic blood pressure ≥ 130/85 mmHg or current antihypertensive drug therapy, c) fasting blood glucose ≥ 7 mmol/l or diabetes therapy, d) serum triglycerides levels ≥ 1.7 mmol/l and e) serum high-density lipoprotein cholesterol levels <0.9 mmol/l and iii) absence of the classical causes of hyperferritinaemia and iron overload, i.e. excessive alcohol consumption > 30 g pure alcohol/day, homozgosity for the C282Y mutation of the HFE gene, aceruloplasminemia, haematological disorder or a chronic increase in serum transaminase levels, except when related to non-alcoholic fatty liver disease. No patient had clinical evidence of cirrhosis.

Study design

The fifty patients were randomised into i) a treatment group in which iron depletion was performed by regular removal of 300–500 ml of blood every 14 days until serum ferritin levels dropped under 100 μg/l and ii) a control group without phlebotomy therapy. One patient who had been randomised to the treatment group, died during the study, before Test 2 was performed. His death was not related to the study but to acute renal insufficiency and septic shock. This patient and his matched control were excluded from the analysis. Therefore, analysis was for 48 patients. Each individual in the control group was matched with a patient in the treatment group, to ensure a similar observation time. The study protocol was approved by the ethics committee of the Hospital in Rennes and was registered on clinical trial.gov (2005-08-29). Patients and controls had blood tests at inclusion in this study, and returned 14 days later for additional blood tests.

Description of the intervention

In the treatment group, it was necessary to remove a mean quantity of 3.9 ± 1.3 l blood to obtain low serum ferritin levels (74 ± 34 μg/l at the end of treatment). These results confirm, in accordance with LIC measurement, that these patients had mild but real iron overload as previously discussed by Guillygomar’ch et al. (8). The time interval between Tests 1 and 2 was 161 ± 63 days. Five patients in the phlebotomy group and eight patients in the control group were on statin medication. No drug was withdrawn or introduced during the study. Body weight remained stable in both groups.

Clinical, biochemical and hormonal assessment

Examinations were at the Clinical Investigation Centre of the Hospital Pontchaillou, Rennes, France. Age, BMI, waist circumference, blood pressure and medications used were recorded. Hypertriglyceridaemia, hypercholesterolaemia, diabetes and abdominal obesity were defined according to the NCEP Adult Treatment Panel III criteria (12). Blood samples were collected, after an overnight fasting, at inclusion and 14 days later. Serum samples were obtained by centrifugation, immediately frozen at −20 °C and stored pending further analysis. SHBG was determined by IRMA (Immunotech, Beckman Coulter, Villepinte, France), LH and total testosterone by the automated chemiluminescent ADVIA-Centaur (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Bioavailable testosterone (BT) was determined using the Immunotech testosterone kit after ammonium sulphate precipitation and extraction with ethylic ether (within- and between-run coefficients of variation for concentrations varying from 2.2 nmol/l to 7 nmol/l ≤ 13.1% and ≤ 15.1% respectively). We defined clinical hypogonadism by total testosterone < 10.4 nmol/l (< 3000 pg/ml), BT < 2.5 nmol/l (<700 pg/ml) (13).

Statistical analysis

All analyses used R version 2.10.0 (Free Software Foundation, Boston, MA, USA), and a two-sided P < 0.05 was considered statistically significant. At baseline, comparisons between the phlebotomy group and the non-treated group were performed with the Wilcoxon test for continuous variable and χ² tests for categorical variables (or Fisher’s exact test as appropriate). Hormone levels according to LIC above and below the median (90 μmol/g) and mean ferritin and LIC across SHBG quartiles were compared by the Mann–Whitney rank sum tests. Linear regression was used to study the relation between LIC and SHBG or LH. All variables were log-transformed before entering the models. At the end of treatment, the variation of each variable between Tests 1 and 2 was calculated as Test 2 minus its value at Test 1. Then we compared the variation of the variables between the two groups with the Wilcoxon test.

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Results

Characteristics at baseline of the 48 participants randomised into our study are presented in Table 1. There were no significant differences between groups according to age and iron parameters, but the two groups differed significantly according to BMI and fasting insulinemia. After iron depletion by venesection, we did not observe significant improvements in the frequency of hypogonadism based on BT are scarce in overweight individuals in Europe (14). Data on the frequency of hypogonadism based on BT are scarce in men with the metabolic syndrome or type 2 diabetes. In a cohort of 355 men, aged 58.0 ± 0.5 years with type 2 diabetes, Kapoor et al. (2) found 16% of hypogonadism based on BT < 2.5 nmol/l. However, hypogonadism defined by total testosterone was more frequent in the same cohort: 20% had total testosterone < 8 nmol/l and 31% had total testosterone between 8 and 12 nmol/l. A confounding factor may be that SHBG is usually low in insulin resistant individuals, and thus total testosterone can be low while BT remains within the normal range. Therefore, it is important to measure non-SHBG bound testosterone in the setting of altered levels of SHBG (13). An original result of our study is the

Discussion

The main finding of this study is that liver iron overload is positively associated with SHBG and inversely with LH levels without significant changes in hormonal concentrations, after phlebotomy-induced iron depletion.

To our knowledge, this study is the first to investigate the prevalence and the nature of gonadal dysfunction in men with the dysmetabolic iron overload syndrome, a situation which is observed in about 10–20% of overweight individuals in Europe (14). Data on the frequency of hypogonadism based on BT are scarce in men with the metabolic syndrome or type 2 diabetes. Kapoor et al. (2) found 16% of hypogonadism based on BT < 2.5 nmol/l. However, hypogonadism defined by total testosterone was more frequent in the same cohort: 20% had total testosterone < 8 nmol/l and 31% had total testosterone between 8 and 12 nmol/l. A confounding factor may be that SHBG is usually low in insulin resistant individuals, and thus total testosterone can be low while BT remains within the normal range. Therefore, it is important to measure non-SHBG bound testosterone in the setting of altered levels of SHBG (13). An original result of our study is the

Table 1 Baseline characteristics of men studied. Data are presented as mean ± s.d. or n.

<table>
<thead>
<tr>
<th></th>
<th>Phlebotomy group (n=24)</th>
<th>Control group (n=24)</th>
<th>P value (T1)</th>
<th>P value (T2–T1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.4 ± 8.4</td>
<td>54.2 ± 10.4</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2 ± 3.9</td>
<td>30.4 ± 3.9</td>
<td>0.279</td>
<td>0.75</td>
</tr>
<tr>
<td>Insulinemia (µU/l)</td>
<td>13.3 ± 6.1</td>
<td>11.1 ± 5.0</td>
<td>0.029</td>
<td>0.77</td>
</tr>
<tr>
<td>Ferritinaemia (µg/l)</td>
<td>715 ± 397</td>
<td>74 ± 34</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Liver iron concentration (µmol/g)</td>
<td>100.5 ± 42.2</td>
<td>109.4 ± 51.5</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>16.9 ± 6.0</td>
<td>15.7 ± 5.8</td>
<td>0.75</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Deficiency in total testosterone n</td>
<td>3</td>
<td>1</td>
<td>0.61</td>
<td>1</td>
</tr>
<tr>
<td>Bioavailable testosterone (nmol/l)</td>
<td>3.38 ± 1.47</td>
<td>3.88 ± 1.86</td>
<td>0.76</td>
<td>0.28</td>
</tr>
<tr>
<td>Deficiency in bioavailable testosterone n</td>
<td>6</td>
<td>1</td>
<td>0.75</td>
<td>0.42</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>43.0 ± 19.8</td>
<td>38.5 ± 15.9</td>
<td>0.84</td>
<td>0.24</td>
</tr>
<tr>
<td>LH (UI)</td>
<td>3.9 ± 1.9</td>
<td>4.1 ± 2.4</td>
<td>0.97</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Defined by MRI or biopsy.

**Defined by total testosterone < 10.4 nmol/l (<3000 pg/ml).

†Defined by bioavailable testosterone < 2.5 nmol/l (<700 pg/ml).

‡Defined by calculated free testosterone < 0.255 nmol/l.

Figure 1 SHBG levels (A), LH levels (B) and bioavailable testosterone levels (C) according to the median liver iron concentration (<85 µmol/g, left boxplot; ≥80 µmol/g, right boxplot) in 50 men with dysmetabolic iron overload syndrome.

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A positive relationship between levels of SHBG and intrahepatic iron accumulation. Despite low insulin sensitivity and a high frequency of obesity in our cohort, men with the dysmetabolic iron overload syndrome did not have low SHBG levels. Age, a potential confounding factor associated with higher plasma SHBG, was taken into account in the analysis and participants had no liver disease, other than steatosis. In particular, no patient had clinical or biological signs of cirrhosis. Adjustment on fasting insulin, HOMA-IR, BMI or waist, as well as statin medication and alanine aminotransferase, did not modify the relationship (Table 2). These results suggest that liver iron accumulation may have a direct impact on the level of SHBG. To our knowledge, there are no previous reports of SHBG levels in cases of iron overload in the absence of cirrhosis. The molecular mechanisms involved in the increase in plasma SHBG associated with iron excess in the liver, are yet to be further characterised and may involve an increase in gene expression and/or reduced catabolism.

Our results provide indirect evidence that moderate iron overload is associated with gonadotroph suppression, as iron load was significantly related with LH concentration in a multivariable model. The negative relation between LIC and LH levels was independent of fasting insulin or the HOMA-IR, a validated marker of insulin resistance. The relationship was also independent of BMI or waist, a proxy of adipose tissue and aromatase activity.

We did not observe any significant improvement in either LH, SHBG or sex hormones levels after phlebotomy in our study. Increased iron deposition in tissues is cytotoxic and leads to cell death. Heavy iron deposition in the pituitary in β-thalassemia major, causes severe damage and shrinkage of the pituitary gland (15). However, there have been reports in men with genetic haemochromatosis, of reversal of hypogonadotropic hypogonadism, following aggressive venesection therapy (16). To our knowledge, there has been no previous description of the impact of bloodletting on gonadal function in the dysmetabolic iron overload syndrome, where iron overload is moderate and hypogonadism less severe. However, even though the ferritin was normalised at the final examination, the mean length of the phlebotomy intervention was limited to about 6 months. Therefore, one may speculate that reduction in excess iron deposits in the pituitary needs more time to fully reverse hypogonadotropic hypogonadism. A discrepancy between pituitary and hepatic iron depositions is also possible. LIC is considered as the gold standard for the evaluation of body iron load, but iron overload may have some organ specificity (17).

This study has some limitations including the size of the sample, the absence of measurements of aromatase activity and adipose tissue depots, the use of direct immunoassays and not mass spectrometry steroid assays. However, our work should be considered as a pilot study assessing, for the first time, the relationship between gonadal function and moderate liver iron overload assessed by MRI, in the absence of genetic haemochromatosis. In conclusion our study shows that the dysmetabolic iron overload syndrome is associated with elevated SHBG and with low LH and bio-T levels, yet without significant changes in hormonal concentrations after short term phlebotomy-induced iron depletion. These results may stimulate the initiation of further studies to better characterise the molecular mechanisms underlying altered SHBG and LH plasma concentrations in such patients. A prospective randomised study in symptomatic hypogonadal men with the dysmetabolic iron overload syndrome is warranted to assess whether phlebotomy therapy is a valuable therapeutic option to reverse hypogonadotropic hypogonadism in individuals with dysmetabolic iron excess.

### Table 2 Effect of MRI assessed LIC on SHBG or LH levels. Linear regression derived β coefficients and P values associated with LIC.

<table>
<thead>
<tr>
<th>Liver iron concentration</th>
<th>SHBG level</th>
<th>LH level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Univariable</td>
<td>β (0.047)</td>
<td>P (0.059)</td>
</tr>
<tr>
<td>Model 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31</td>
<td>0.047</td>
</tr>
<tr>
<td>Model 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.32</td>
<td>0.028</td>
</tr>
<tr>
<td>Model 3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.33</td>
<td>0.027</td>
</tr>
<tr>
<td>Model 4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.31</td>
<td>0.070</td>
</tr>
</tbody>
</table>

<sup>a</sup>Model 1 adjusted on age and LH or SHBG.
<sup>b</sup>Model 2 = model 1 + adjusted on baseline fasting insulin.
<sup>c</sup>Model 3 = model 2 + adjusted on BMI.
<sup>d</sup>Model 4 = model 3 + adjusted on statin medication and alanine aminotransferase all variables have been log-transformed before entering the models.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.
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Author contribution statement
A Gautier analysed the data and wrote parts of the manuscript, F Laine recruited patients and reviewed the manuscript, C Massart performed hormonal assessment and reviewed the manuscript, L Sandret analysed the data, P Brissot reviewed the manuscript, B Balkau reviewed and edited the manuscript, Y Deugnier reviewed the manuscript and F Bonnet wrote parts of the manuscript.

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