INTRODUCTION

Changes in sex hormone-binding globulin (SHBG) plasma levels may be found in several physiological and pathological conditions (Selby, 1990). Increased SHBG concentrations are common in cirrhotic patients (Becker and Gluud, 1991) and seem to correlate with the degree of liver failure (Gluud et al., 1987). Plasma SHBG is also elevated in alcoholics, even in the absence of marked liver damage (Myking et al., 1987), showing a fast decrease after 10 days of abstinence (Iturriaga et al., 1995). This observation has recently been challenged (Ruusa et al., 1997). The significance of these changes is not known, but they could contribute to the hypo-androgenism that has been described in alcoholics (Van Thiel et al., 1974; Välimäki et al., 1982). The present work is aimed to study in male alcoholic patients: (1) the rate of changes in sex hormones and SHBG during both short-term and more prolonged abstinence; (2) the occurrence of clinical hypo-androgenism in these patients; (3) the presence of possible determinant factors for the SHBG increase; (4) the effect of ethanol intake on SHBG, observed in those patients who relapsed following abstinence.

PATIENTS AND METHODS

Male alcohol-misusing and -dependent patients admitted to the Alcoholism Ward of San Borja Hospital for detoxification and treatment of their addiction were studied. Inclusion criteria were: (a) age 20–50 years old; (b) history of excessive alcohol ingestion (150 g/day for 2 years or more, uninterrupted during the last month); (c) absence of clinical signs of liver failure, such as jaundice, ascites,
oedema, splenomegaly, encephalopathy or bleeding disorders; (d) absence of endocrine or urogenital pathology; (e) absence of concomitant systemic illness; (f) abstinence period shorter than 5 days; (g) informed written consent.

Clinical data

On admission, a complete history of alcoholism was obtained and physical examination was performed. Data on alcohol consumption given by the patients were validated by a trained psychologist by interviewing close relatives. The amount of alcohol ingested during the month before admission was recorded and calculated in g of pure ethanol per day, irrespective of the beverage type, which was usually wine.

Andrological evaluation included: (a) a structured interview to detect characteristics and changes in libido, sexual potency and intercourse frequency; (b) physical examination to register secondary sexual characteristics, testis volume and gynaecomastia.

Most patients at admission presented mild to moderate withdrawal symptoms, which were treated with diazepam initiated after the first blood sample was drawn.

Laboratory tests

Within 24 h of admission, blood samples were obtained for plasma or serum determinations of prothrombin time, total bilirubin, alkaline phosphatase, aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), albumin, creatinine, cholesterol (total, high-density lipoprotein and low-density lipoprotein), triglycerides and fasting and postprandial glucose. All determinations were performed using automated clinical laboratory methods. All tests were repeated at discharge (10 days later). Red and white blood cell counts and a urine sample for enzymatic determination of albumin were also obtained.

Hormone determinations

On admission and on the day of hospital discharge, fasting-blood samples were obtained for determination of total testosterone (T), free testosterone (Tf), sex hormone-binding globulin (SHBG), oestradiol (E2), oestrone (E1), dihydrotestosterone (DHT), follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), triiodothyronine (T3), thyroxine (T4), thyrotropin (TSH), insulin (I) and cortisol (C). The gonadotropins FSH and LH were determined in all collected samples by radioimmunometric assays (RIA) with commercially available kits (Diagnostic Products Corp., Los Angeles, CA, USA) as previously described (Ronco et al., 1992). The mean intra-assay and inter-assay coefficients of variation (CV) were 3.1% and 6.0% for LH and 4.1% and 7.8% for FSH respectively, which were within the relevant range of concentrations for this study. T, E1 and E2 were assayed using RIA kits supplied by the World Health Organization; the intra- and inter-assays coefficients of variation were below 6% and 8% respectively. Plasma SHBG was measured using a commercial RIA kit (Diagnostic Products Corp.), providing 3%, 6% and 2% intra-assay and 10%, 8% and 7% inter-assay CV at SHBG levels of 12, 36 and 126 nmol/l respectively, and a sensitivity equal to 5 nmol/l.

Two hours after a standard breakfast, blood samples were drawn for plasma glucose and insulin determinations.

On days 2, 4 and 7 after admission, additional blood samples were obtained for determination of albumin, SHBG, T, Tf and LH.

Histology

A liver biopsy was performed within 5 days of admission, using a Menghini needle. This is a routine diagnostic procedure for all patients admitted to the Ward, upon their informed consent.

Follow-up

Patients were given appointments for follow-up, at 2, 6 and 12 weeks after discharge. On each visit, at 08:00, dietary and alcohol intakes were recorded. Presence of withdrawal symptoms such as anxiety, tremor, perspiration, visual or auditory hallucinations and high blood pressure were recorded. A recently voided urine sample was obtained, for alcohol determination. Fasting blood samples were also drawn for determinations of albumin, GGT, SHBG and the following hormones: T, Tf, LH, FSH, E1 and E2.

Criteria for alcoholism relapse were: self-report of ethanol ingestion, presence of alcohol in the urine sample or presence of two or more withdrawal symptoms.

Controls

Twenty-one healthy volunteers, who admitted occasional alcohol consumption only, formed the control group.
Statistics

Student’s or paired t-tests and analysis of variance (ANOVA) were used to calculate differences between or within subjects. Pearson or Spearman correlations were also calculated.

This study was approved by the Ethics Committees on Medical Research of San Borja Hospital and the Institute of Nutrition, University of Chile.

RESULTS

The general features and some laboratory tests of alcoholic patients, at admission and discharge (Table 1) showed values slightly different to controls but within normal ranges for most parameters, except AST and GGT, which were moderately increased, decreasing after 10 days. Liver biopsy was obtained in 18 cases (three patients refused the procedure) and showed only mild or moderate steatosis.

Serum SHBG showed a 3-fold increase at admission (Table 2). It then went down slowly, but remained significantly higher than controls for the remainder of the study period. T was slightly lower than in controls on the second day only. TF, however, was much lower at admission and showed a gradual increase to normal values by the 10th day. There was a significant negative correlation between SHBG and TF during the first 10 days ($r = -0.53$, $P < 0.000$). LH was also increased, compared to controls, up to the 10th day of abstinence. FSH, PRL and oestradiol were not different from controls. In all cases, all hormonal values were within normal ranges, with the exception of SHBG and TF at admission (Table 2). No correlation was found between SHBG and age, years of excessive drinking, amount of ethanol and body mass index. In multiple regression analysis, the amount of ethanol ingested, as an independent variable, was the only parameter to reach significance ($t = -3.156$, $P < 0.007$).

Non-sex hormone analyses showed that mean cortisol was higher than controls, with seven cases (33%) above the upper normal level, at admission. Insulin and T3 were lower than in controls, but within their normal ranges. There was no correlation between SHBG and any of these hormones. At discharge, no significant differences were found, compared to admission (paired t-test).

Andrological evaluation showed decreased libido in 50% of patients, erectile dysfunction in 40% and gynaecomastia in 33%. Their occurrence did not correlate with SHBG or with sex hormone values.

During follow-up, SHBG continued to decrease, but with values still above those of the controls, even after 12 weeks. LH remained increased at 2 weeks. The other sex hormones did not show significant differences from control values.

When alcoholics were grouped according to their alcohol relapse, the normalization of SHBG occurred only in those who remained abstinent, whereas in those who relapsed, high SHBG levels continued and even increased in some cases (Fig. 1).

### Table 1. General features of patients and controls

<table>
<thead>
<tr>
<th>Feature</th>
<th>Controls ($n = 21$)</th>
<th>Alcohols ($n = 21$)</th>
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<tr>
<td></td>
<td>At admission</td>
<td>At discharge</td>
</tr>
<tr>
<td>Age (years)</td>
<td>33.1 ± 7.6</td>
<td>39.0 ± 5.0$^b$</td>
</tr>
<tr>
<td>Period of alcohol ingestion (years)</td>
<td>20.1 ± 7.3</td>
<td>20.1 ± 7.3</td>
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<tr>
<td>Amount of alcohol (g/day)</td>
<td>300 ± 116</td>
<td>250 ± 110</td>
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<tr>
<td>Total bilirubin (μmol/l)</td>
<td>13.7 ± 5.1</td>
<td>18.8 ± 6.8$^a$</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>15 ± 7</td>
<td>51 ± 44$^c$</td>
</tr>
<tr>
<td>GGT (IU/l)</td>
<td>16 ± 12</td>
<td>93 ± 80$^d$</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>47 ± 4</td>
<td>43 ± 4$^d$</td>
</tr>
<tr>
<td>Prothrombin time (INR)</td>
<td>0.94 ± 0.05</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.8 ± 1.9</td>
<td>22.7 ± 2.9$^b$</td>
</tr>
</tbody>
</table>

Values are means ± SD.

$^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ compared to controls.

Normal ranges: bilirubin 5–20 μmol/l; AST (aspartate aminotransferase) = 5–16 IU/l; GGT (gamma-glutamyltransferase) = 6–28 IU/l; albumin = 40–53 g/l; prothrombin time (INR) = 0.8–1.3.
DISCUSSION

The main findings of the present paper are: (1) the marked elevation of SHBG in alcoholics and its slow decrease during a 12-week follow-up period, when patients remained abstinent; (2) the biochemical hypo-androgenism exhibited by alcoholic patients and expressed as low Tf plasma levels and concomitant high LH plasma concentrations; (3) a relative dissociation between SHBG and Tf, as the latter reached normal values despite SHBG levels remaining elevated; (4) the SHBG sensitivity to ethanol, as shown by its increase, or its tendency to remain elevated in those patients who, after abstinence, resumed alcohol ingestion.

SHBG is a glycosylated protein synthesized by the liver, with a half-life of 7 days (Selby, 1990). High SHBG plasma levels are found in some conditions, such as advanced age (Gray et al., 1991), hyperthyroidism (Sarine et al., 1988) and especially in liver cirrhosis of different aetiologies (Kaymakoglu et al., 1995).

We have already found that SHBG is greatly increased in alcoholic patients, irrespective of the presence or absence of hepatic damage, as assessed by liver biopsies (Iturriaga et al., 1995). In this latter study we did not investigate further what other factors, besides ethanol, could have influenced SHBG.

The main mechanisms controlling SHBG levels are sex hormones, especially E2, which induces an increase. In the present study, as well as in our previous work, E2 plasma levels were similar to controls, without changes during the whole study. Therefore, a role for oestradiol in explaining the observed variations in SHBG is unlikely.

Many factors, besides sex hormones, are associated with changes in circulating levels of SHBG, e.g. insulin (Pugeat et al., 1991; Birkeland et al., 1993), cortisol (Tegelman et al., 1990), thyroid hormones (Sarine et al., 1988) and body weight (Field et al., 1994). Some of these have been reported to be altered in alcoholics, in whom hyperinsulinism and insulin resistance (Adner and Nygren, 1990), and increased cortisol levels (Heinz et al., 1995) have been described, although not always confirmed (Iturriaga et al., 1986; Bunout et al., 1989). In our patients, cortisol was increased compared to controls and 33% showed values above the normal range. On the other hand, mean fasting insulin and T3 levels were lower than controls. However, no correlation was found between any of these hormones and SHBG. Furthermore, their values at discharge, compared to admission, did not differ significantly. From these data, a role for non-sex hormones in explaining changes in SHBG is highly improbable.

Increased SHBG plasma levels could be the result of increased hepatic synthesis, increased hepatic release or decreased blood clearance. Mechanisms through which chronic ethanol ingestion could influence these processes are not currently known, as far as we are aware. We have already found that SHBG in our alcoholics is a mixture of several molecular forms with different

<p>| Table 2. Hormone values in controls and alcoholics during hospitalization |</p>
<table>
<thead>
<tr>
<th>Days</th>
<th>Controls</th>
<th>1</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHBG (nmol/l)</td>
<td>43.1 ± 10.0</td>
<td>119.4 ± 19.4(^c)</td>
<td>114.3 ± 18.5(^c)</td>
<td>110.8 ± 17.7(^c)</td>
<td>101.9 ± 15.9(^c)</td>
<td>90.5 ± 15.4(^c)</td>
</tr>
<tr>
<td>Total testosterone (nmol/l)</td>
<td>34.7 ± 9.4</td>
<td>30.2 ± 6.9</td>
<td>28.8 ± 4.8(^b)</td>
<td>31.5 ± 6.2</td>
<td>33.3 ± 6.9</td>
<td>35.0 ± 7.6</td>
</tr>
<tr>
<td>Free testosterone (pmol/l)</td>
<td>99.2 ± 30.9</td>
<td>62.5 ± 25.3(^c)</td>
<td>76.3 ± 26.0(^b)</td>
<td>72.9 ± 20.8(^b)</td>
<td>74.6 ± 21.9(^b)</td>
<td>84.7 ± 22.2</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>6.6 ± 3.9</td>
<td>12.8 ± 5.8(^c)</td>
<td>11.0 ± 4.6(^b)</td>
<td>10.6 ± 4.4(^b)</td>
<td>10.5 ± 4.2(^b)</td>
<td>9.6 ± 3.6(^c)</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>9.6 ± 3.9</td>
<td>14.2 ± 12.9</td>
<td>13.1 ± 13.4</td>
<td>13.1 ± 13.4</td>
<td>13.1 ± 13.4</td>
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<tr>
<td>Prolactin (pmol/l)</td>
<td>222 ± 124</td>
<td>302 ± 111</td>
<td>320 ± 98</td>
<td>320 ± 98</td>
<td>320 ± 98</td>
<td>320 ± 98</td>
</tr>
<tr>
<td>Oestradiol (pmol/l)</td>
<td>34.5 ± 10.3</td>
<td>34.5 ± 7.7</td>
<td>30.5 ± 8.1</td>
<td>30.5 ± 8.1</td>
<td>30.5 ± 8.1</td>
<td>30.5 ± 8.1</td>
</tr>
</tbody>
</table>

Values are means ± SD.
\(^aP < 0.05, \(^bP < 0.01, \(^cP < 0.001 versus controls.

Normal ranges: SHBG (sex hormone-binding hormone) 10–43 nmol/l; testosterone >10.4 nmol/l; free testosterone 45–139 pmol/l; LH (luteinizing hormone) 1–15 mIU/ml; FSH (follicle stimulating hormone) 1–15 mIU/l; oestradiol 36–180 pmol/l; prolactin 84–520 pmol/l.
degrees of glycosylation (L. Valladares et al., unpublished observations). It has been found that other liver proteins such as transferrin also show changes in glycosylation in alcoholic subjects (Stibler, 1991). These alterations could be the result of changes in glycosyltransferase and sialidase activities produced by ethanol (Xin et al., 1995). It has been recently shown that the degree of glycosylation influences the half-life of SHBG (Cousin et al., 1998) and would deserve further study in our alcoholic patients.

On the other hand, acetaldehyde adducts with different proteins are also a common finding in alcoholics (Lieber, 1988) and may result in functional changes of the protein (Lindros, 1995). To our knowledge, this mechanism has not been studied in the case of SHBG. Our finding that the amount of ethanol ingested was the only independent variable associated with SHBG levels could support some kind of direct effect of alcohol on the protein properties.

The existence of hypo-androgenism in alcoholics has been repeatedly documented (Van Thiele et al., 1974; Castilla-Garcia et al., 1987; Becker and Gluud, 1991). In the present study, we confirmed it with both clinical parameters and the low Tf and increased LH values in recently intoxicated patients. We previously postulated that high levels of SHBG could partially determine these changes (Iturriaga et al., 1995). The present results partially support this hypothesis, as there was a significantly negative correlation between SHBG and Tf during the first 10 days after admission. However, Tf values were normal after 10 days of abstinence, despite SHBG remaining high.

A study similar to the first part of ours has been recently published (Ruusa et al., 1997), except that it did not include a control group. The results of such a study also agree with our findings in regard to T, LH and FSH values, but are completely different regarding SHBG, which was found to be decreased during early withdrawal. We are not able to explain this discrepancy. The characteristics of patients were the same in both studies, but the assay technique used a different commercial kit; it has been described that both methods produce statistically identical but widely spread results (Brotherton, 1990). Another obvious difference is the ethnic origin of the two populations studied.

In our study, during follow-up, patients who remained abstinent exhibited normal SHBG values only after 12 weeks. This is a much slower change, when compared to GGT, which decreased to normal in 2 weeks. In the group of patients who relapsed to alcohol ingestion, which occurred mainly at 6 and 12 weeks of follow up, SHBG levels presented small fluctuations, in contrast to GGT, which was slightly increased at 2 and 6 weeks and only went up at 12 weeks. Therefore, the increase and
normalization of SHBG plasma levels seems to be a slow process.

The association between SHBG increase and ethanol consumption leads us to consider SHBG as a candidate marker for excessive alcohol ingestion. If so, this long-lasting response to ethanol could represent an advantage for screening. The validation of this hypothesis, however, requires the study of a large number of alcohol excessivedrinkers and comparison with other markers, such as GGT, mean corpuscular volume and especially with carbohydrate-deficient transferrin (Yerin et al., 1995).

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REFERENCES


