Insulin Regulates Testosterone and Sex Hormone-Binding Globulin Concentrations in Adult Normal Weight and Obese Men

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ABSTRACT

There are no studies in vivo on the effects of insulin on androgens and sex hormone-binding globulin (SHBG) in men. We, therefore, investigated the effects of insulin suppression on testosterone and SHBG in two groups of eight non-diabetic adult obese men and six healthy normal weight men who underwent diazoxide treatment (100 mg, three times daily) for 7 days. Blood samples for hormone determination were obtained before the subjects had been selected for the study, immediately before diazoxide administration, and on the last day of treatment. A 24-h oral glucose tolerance test was also performed for glucose, insulin, and C-peptide determinations before and on the last day of treatment. Only one subject experienced significant side-effects, and no significant changes in mean body weight were found during the treatment. Diazoxide administration worsened glucose tolerance in several subjects and reduced fasting and glucose-stimulated insulin levels by approximately 50% in both control and obese subjects. No significant difference was present between historical and pretreatment hormone values in either group. Moreover, there were no differences in pretreatment gonadotropin and SHBG concentrations between the two groups, whereas testosterone (free and total) levels were lower in the obese than in the control subjects. After diazoxide administration, testosterone (free and total) decreased slightly, but significantly, whereas LH and SHBG significantly increased in both groups. Diazoxide treatment increased estradiol levels in controls, but not in obese men. In conclusion, these results indicate that in vivo, insulin is capable of stimulating testosterone production and, simultaneously, of inhibiting SHBG concentrations in both normal weight and obese men. (J Clin Endocrinol Metab 80: 654–658, 1995)

SEX HORMONE-binding globulin (SHBG) and testosterone levels are frequently reduced in obese males (1) and are inversely correlated with indices of body weight (2–4). Although the mechanisms responsible for these abnormalities are unknown, there is evidence that weight loss can reverse them (5), which suggests that they are secondary to the development of obesity. Moreover, it has been demonstrated that serum SHBG and testosterone concentrations are inversely correlated with fasting and glucose-stimulated insulin levels, regardless of the degree of overweight or pattern of body fat distribution (2, 3). This obviously implies that insulin may be involved in the regulation of both testosterone and SHBG synthesis and/or metabolism in men, as seems to occur in women. In fact, there is evidence from in vitro and in vivo studies that insulin may regulate ovarian androgen production (6, 7). Moreover, in hyperandrogenic women with polycystic ovaries, a positive correlation has been demonstrated between testosterone and insulin levels (6), and the reduction of insulin has been shown to decrease the degree of hyperandrogenism (8) and improve clinical features (9). Finally, studies in vitro have shown that insulin may inhibit SHBG synthesis (10), and suppression of insulin levels has been found to increase SHBG concentrations in obese hyperandrogenic women (8), but not in normal weight healthy women (11). There are no data in vivo on the action of insulin on sex hormones and SHBG in men. Therefore, in this study we investigated the effects of insulin suppression after long term diazoxide administration on testosterone and SHBG blood concentrations in two groups of obese and healthy normal weight men.

Subjects and Methods

Subjects

Obese subjects were selected from those attending the out-patient Endocrine Unit of the Institute of Clinical Medicine and Gastroenterology of the University of Bologna for the treatment of obesity. The presence of relevant metabolic or endocrine abnormalities other than obesity itself was excluded on the basis of clinical history, physical examination, and blood tests for routine biochemical and basal hormone determinations. Physical examination included measurement of body height without shoes to the nearest 0.5 cm and of body weight without clothes. Body mass index was calculated by dividing body weight (in kilograms) by height (in square meters). The waist (minimum values between the iliac crest and the lateral costal margin) and hip (maximum value over the buttocks) circumferences were measured to obtain information on the pattern of body fat distribution (12). Blood samples were obtained in the morning (0800–0830 h) after an overnight fast. A single sample was drawn for routine biochemical tests and steroid and thyroid hormone measurements, and three consecutive blood samples were taken at 10-min intervals for gonadotropin measurements.
None of these subjects had diabetes or other endocrine diseases, or relevant cardiovascular, hepatic, or renal abnormalities. Arterial blood pressure was normal in all subjects. A group of six healthy normal weight subjects was also included in the study, some of whom had followed the same selection protocol; two of them had come to our Unit for an overall evaluation of their clinical conditions, and the others were part of our medical team or students. All obese and control subjects gave their informed and written consent to the study. General data and historical blood hormone concentrations of these subjects are reported in Table 1.

Diazoxide study

After they had been selected, all subjects had pretreatment baseline blood samples taken for sex steroid (single sample) and gonadotropin (three samples at 10-min intervals) determinations. An oral glucose (75 g, Glucosol, Schil, Italy) tolerance test was then performed, and venous blood samples were drawn at 0, 60, and 120 min for glucose, insulin, and C-peptide determinations. To obtain an integrated response to the glucose challenge, insulin and C-peptide areas under the curve were calculated by the trapezoidal rule using absolute values. After baseline tests had been performed, all subjects underwent treatment with 100 mg diazoxide (Propillicm, Shering-Plough, Milan, Italy) orally, three times daily (300 mg/day) before each meal for 7 days. During this period, they were instructed to follow their usual dietary and lifestyle habits. The last dose of diazoxide was taken on the morning of the seventh day of treatment. Then, all subjects underwent the same protocol testing previously described.

Hormonal and biochemical assays

Blood glucose was determined immediately after each oral glucose tolerance test by the glucose oxidase method, whereas hormone assays were performed in duplicate on serum stored at -20°C until analysis. Insulin and C-peptide levels were determined using reagents purchased from Eiken Chemical Co. (Tokyo, Japan) and Technogenetics (Trezzano sul Naviglio, Milan, Italy). Hormonal, baseline, and postdiazoxide sera for gonadotropin determinations were pooled into three single samples to be assayed. Gonadotropin LH (First International Reference Preparation 68/40) and FSH (Second International Reference Preparation 78/549) were measured with reagents obtained from Tosoh (Tokyo, Japan), SHBG with a noncompetitive liquid phase immunoradiometric assay with reagents obtained from Pharmos Diagnostic (Oulunsalo, Finland), estradiol with reagents obtained from Diagnostic Products Corp. (Los Angeles, CA), and free testosterone by a solid phase assay with reagents obtained from Diagnostic Products. Finally, total testosterone was measured by a high pressure liquid chromatography/RIA method. Briefly, serum was extracted using Bond-Elut C18 disposable columns (Varian, Arbor City, CA); the reconstituted residue was injected into the high pressure liquid chromatograph and eluted isocratically using a ternary mobile phase [37% A/B, where A = methanol-tetrakis(tetramethylethyl)ether (70% / 30%) and B = water]. The fractions corresponding to the retention time of testosterone were collected, dried, reconstituted with RIA buffer, and measured by RIA. Polyclonal antiserum for testosterone was raised in rabbits according to a method previously described (13). This antiserum showed a cross-reactivity of 20.7% and 0.8% for 5α-dihydrotestosterone and androstenedione, respectively. The coefficient of variation of the retention times of 10 injections of testosterone was 0.3%. The recovery of the overall procedure was 82.5 ± 2.8%.

Intra- and interassay coefficients of variation in our laboratory are, respectively, 4.6% and 5.7% for insulin, 6.7% and 15.8% for C-peptide, 6.0% and 9.5% for LH, 6.0% and 8.2% for FSH, and 8.0% and 11.1% for estradiol, 8.0% and 14% for SHBG, 5.6% and 6.2% for free testosterone, and 9.0% and 9.3% for testosterone.

Statistics

All results are reported as the mean ± sd. Comparisons between obese and control subjects were performed using the nonparametric Mann-Whitney U test, whereas comparisons among historical, pretreatment, and postdiazoxide hormone concentrations were performed by the Wilcoxon rank sum test. The comparison between baseline and historical hormone data was made to correct for the lack of a placebo control test in both obese and normal weight individuals. P < 0.05 was used to define statistical significance.

Results

General

Treatment with diazoxide was well tolerated by all subjects except one in the control group, who complained of fluid retention and substantial weight increase (+2.5 kg), which, however, returned to baseline 1 week after the cessation of treatment. As a group, there was no significant change in body mass index in either obese or control subjects.

Glucose, insulin, and C-peptide (Table 2)

Four obese subjects had impaired glucose tolerance before diazoxide administration. Treatment suppressed fasting and glucose-stimulated insulin and C-peptide levels in both obese and control subjects. Glucose tolerance worsened in several of them, particularly in the obese group, and one obese subject developed postdiazoxide glucose values during the oral glucose tolerance test compatible with type II diabetes.

Gonadotropins and sex steroids (Table 3)

No difference was found between historical and pretreatment values of sex steroid, SHBG, and gonadotropin in either control or obese subjects. Under basal conditions, free (71.8 ± 17.0 vs. 43.3 ± 11.4 pmol/L·10⁻¹⁷, respectively) and total (14.5 ± 2.0 vs. 9.7 ± 2.5 nmol/L, respectively) testosterone values were significantly lower in obese subjects than in controls; the free fraction decreased slightly, but significantly (P < 0.05), after diazoxide treatment in both groups (56.2 ± 13.2 vs. 36.0 ± 9.7 pmol/L·10⁻¹⁷, respectively), whereas total testosterone fell significantly (P < 0.05) only in the obese group (8.4 ± 2.8 nmol/L). There was no difference in baseline LH levels between the groups (controls, 3.65 ± 2.60 IU/L; obese, 2.53 ± 0.92 IU/L), and a similar significant (P < 0.05) increase was found after diazoxide treatment in both obese men (3.35 ± 0.61 IU/L) and controls (4.63 ± 2.58 IU/L). Conversely, FSH concentrations did not change significantly.
TABLE 2. Fasting and glucose-stimulated glucose and insulin blood concentrations in normal weight and obese male subjects in basal conditions and after diazoxide treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose</th>
<th>Insulin</th>
<th>C-peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting (mmol/L)</td>
<td>OGTT AUC (mmol/L/min)</td>
<td>Fasting (pmol/L)</td>
</tr>
<tr>
<td>Obese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>5.37 ± 0.97</td>
<td>1,040 ± 267</td>
<td>345 ± 400</td>
</tr>
<tr>
<td>After</td>
<td>6.69 ± 1.56b</td>
<td>1,149 ± 350b</td>
<td>182 ± 144b</td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>4.23 ± 0.65</td>
<td>579 ± 113</td>
<td>97 ± 47</td>
</tr>
<tr>
<td>After</td>
<td>5.04 ± 0.43b</td>
<td>692 ± 80b</td>
<td>65 ± 13</td>
</tr>
</tbody>
</table>

a P < 0.05 for basal values between the groups.
b P < 0.05, after vs. before diazoxide treatment.
c P < 0.01, after vs. before diazoxide treatment.

TABLE 3. Sex steroids, gonadotropins, and sex hormone-binding globulin in normal weight and obese male subjects in basal conditions and after diazoxide treatment

<table>
<thead>
<tr>
<th>Hormones</th>
<th>Basal</th>
<th>Diazoxide</th>
<th>Basal</th>
<th>Diazoxide</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (IU/L)</td>
<td>5.37 ± 0.92</td>
<td>3.35 ± 0.54a</td>
<td>3.65 ± 2.60</td>
<td>4.63 ± 2.58a</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>4.16 ± 1.91</td>
<td>4.49 ± 1.61</td>
<td>3.48 ± 2.48</td>
<td>4.14 ± 2.85</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>0.65 ± 0.20</td>
<td>0.81 ± 0.27</td>
<td>0.92 ± 0.20b</td>
<td>1.18 ± 0.43</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>3.48 ± 2.8b</td>
<td>14.5 ± 3.3</td>
<td>14.1 ± 3.3</td>
<td>14.1 ± 3.3</td>
</tr>
<tr>
<td>Free testosterone (pmol/L·10-18)</td>
<td>433 ± 11.4</td>
<td>36.0 ± 9.7c</td>
<td>71.8 ± 17.0b</td>
<td>56.2 ± 13.2c</td>
</tr>
<tr>
<td>Estradiol (pmol/L)</td>
<td>11.7 ± 0.05</td>
<td>0.12 ± 0.02c</td>
<td>0.26 ± 0.05b</td>
<td>0.20 ± 0.03c</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>17.6 ± 5.8</td>
<td>19.8 ± 6.9c</td>
<td>16.3 ± 2.5</td>
<td>20.4 ± 5.6c</td>
</tr>
<tr>
<td>Testosterone/SHBG ratio</td>
<td>1.17 ± 0.05</td>
<td>1.02 ± 0.17</td>
<td>1.41 ± 0.81</td>
<td>2.52 ± 1.02</td>
</tr>
<tr>
<td>Estradiol/SHBG ratio</td>
<td>1.15 ± 0.84</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a P < 0.05, diazoxide vs. baseline.
b P < 0.05 for baseline values between the groups.

No significant variation occurred in the LH/FSH ratio; the baseline values were, however, lower in obese subjects than in controls. The two groups had similar pretreatment estradiol values (controls, 84.4 ± 9.2 pmol/L; obese, 59.8 ± 24.9 pmol/L), but diazoxide administration did not modify its levels in the obese (60.9 ± 27.9 pmol/L), whereas it significantly (P < 0.05) increased its values in controls (186.1 ± 92.9 pmol/L). Baseline SHBG concentrations in obese subjects (17.6 ± 5.8 nmol/L) were similar to control values (16.3 ± 2.5 nmol/L), and they increased slightly, but significantly (P < 0.05), in both groups after diazoxide administration (obese, 19.8 ± 6.9 nmol/L; controls, 20.4 ± 5.6 nmol/L). Pretreatment testosterone/SHBG ratios were significantly lower in obese subjects than in controls; however, they significantly decreased in both groups after diazoxide treatment. On the contrary, diazoxide increased the estradiol/SHBG ratio in controls, whereas no changes were observed in the obese groups.

Individual values of testosterone (total and free), SHBG, and estradiol in obese and control subjects before and after diazoxide treatment are depicted in Fig. 1.

Discussion

The results of the present study demonstrate that insulin is capable of regulating testosterone and SHBG in adult normal weight healthy controls and obese individuals. Until now there were no data available on the effects of insulin on sex steroids in males. However, several recent studies suggested this possibility. Seidell et al. (2) and ourselves (3), in fact, demonstrated that in adult males, ranging from normal to obese, insulin was inversely correlated with testosterone and SHBG, and this relationship was statistically independent of body weight or the pattern of body fat distribution. These findings suggested that insulin may inhibit both testosterone and SHBG concentrations. On the contrary, there are studies demonstrating that streptozotocin-induced diabetic rats had decreased testosterone levels and a reduced capacity of the Leydig cells to synthesize androgens (14, 15), probably as a consequence of impaired gonadotropin secretion at the pituitary level (14) or reduced LH receptor function in the testes (16). Moreover, in the same rats it was demonstrated that insulin administration restored the receptor-binding capacity to normal values (16) and increased testosterone synthesis by stimulating testicular β-hydroxysteroid dehydrogenase activity (14). In addition, there are clinical studies showing that poorly controlled diabetic men had significantly lower free testosterone levels compared to those with good metabolic status and healthy age-matched controls (17). Taken together, these data seem, conversely, to suggest that insulin may have stimulatory effects on testosterone production.

Our findings indicate that insulin suppression by diazoxide reduced testosterone while it increased SHBG concentrations in both normal weight and obese male subjects. There are no available data on a possible direct effect of diazoxide on steroid metabolism (8), although it has been demonstrated that it can stimulate the release of catecholamines (18), which could theoretically interfere with testosterone secretion and metabolism in vivo. At the
preliminary level, our findings, therefore, support the concept that in vivo, insulin stimulates testosterone production and simultaneously inhibits SHBG synthesis. With regard to SHBG, they are in accordance with previously cited studies performed in vitro (10) and in vivo in hyperandrogenic women with polycystic ovaries (8, 19) and confirm that insulin may have inhibiting effects on SHBG synthesis. The rise in SHBG would be expected to cause free testosterone levels to fall, which, in turn, should favor a rise in LH as a compensatory response at the neuroendocrine level. This is exactly what we observed in our study. Moreover, insulin may directly operate, synergistically with LH, to increase testosterone production, possibly by stimulating hydroxysteroid dehydrogenase activity in the target tissues. Changes in total testosterone would obviously be expected to parallel those in its free fraction. The fact that its levels fell significantly only in the obese and not in the normal weight individuals seems, however, likely to be due to the small number of subjects enrolled in this latter group. Another way to regulate testosterone metabolism by insulin may be at the level of the aromatase enzyme system (20), as the rise in estradiol concentrations in the normal weight group seems to indicate, which suggests that under basal conditions, insulin probably has a suppressive effect on aromatase activity in males. Accordingly, the relative increase in aromatase after insulin suppression can obviously contribute to the fall in testosterone, because it is actively converted to estradiol. However, we did not find any increase in estradiol levels after diazoxide treatment in the obese group. It can, therefore, be suspected that in obese males, who have baseline hyperinsulinemia, the effects of insulin on aromatase activity are somewhat greater than those in nonobese individuals. The slight fall in insulin concentrations induced by diazoxide in the obese group, which was insufficient to achieve insulin levels within the physiological range, could have favored only a partial reduction of the aromatase activity, which, in turn, can explain the lack of an estradiol increase in this group.

Neither an increase in SHBG concentrations nor a direct effect of insulin on aromatase can adequately explain lowered baseline testosterone concentrations in the obese. It is, therefore, probable that other mechanisms may be involved to adequately explain the lowered testosterone concentrations in obesity. For example, recent studies of Vermeulen et al. (21) reported that a group of obese men had decreased LH pulse amplitude and decreased free testosterone concentrations, which, together with the finding that Leydig cells normally respond to hCG stimulation (22), makes it probable that mechanisms responsible for the mild hypogonadotropic state related to obesity in males (1) may be at least in part of neuroendocrine origin.

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Fig. 1. Individual SHBG (A), free (B), and total (C) testosterone and estradiol (D) concentrations in obese and normal weight male subjects before and after diazoxide (300 mg/day) treatment for 7 days.
References