# Effects of Acute Hyperinsulinemia on Testosterone Serum Concentrations in Adult Obese and Normal-Weight Men

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**In a previous study performed in adult obese and normal-weight male subjects, we found that suppression of insulin levels by diazoxide reduced testosterone and increased sex hormone–binding globulin (SHBG) blood concentrations. These and other data suggested that insulin may have a regulatory capacity in testosterone secretion and/or metabolism in men, similar to what has already been demonstrated in women. In this study, we investigated the effects of acute hyperinsulinemia on major androgen levels, including testosterone, in two groups of normal-weight (n = 11) and obese (n = 9) men. Acute hyperinsulinemia was obtained by the euglycemic-hyperinsulinemic clamp technique. Relationships between the degree of insulin resistance (ie, total glucose disposal [M value]) and testosterone levels were also evaluated. Basal testosterone levels in obese subjects (10.40 ± 3.02 nmol/L) were significantly lower than in normal-weight controls (15.50 ± 4.65 nmol/L, $P < .01$), whereas no difference was present in androstenedione and dehydroepiandrosterone sulfate (DHEA-S) concentrations. During the clamp study, testosterone was significantly increased in the obese group (11.79 ± 3.64 nmol/L, $P < .05$) but not in the control group (15.81 ± 4.54 nmol/L, $P$ = NS). The other two androgens did not significantly change in either the obese or control group. There was a highly significant correlation between baseline testosterone concentrations, with M values suggesting a relationship between impaired peripheral insulin sensitivity and reduced plasma testosterone concentrations. It should be pointed out that there was a certain discrepancy in the testosterone variations, particularly in the control group, in which two thirds of the subjects had no change or some decrease in testosterone levels, whereas in the remainder testosterone increased over the values of the assay variation coefficient. These findings are consistent with the hypothesis that insulin may regulate testosterone blood levels also in male subjects. Whether these effects are primarily due to increased hormone secretion or reduced clearance needs to be investigated.**


IN MALE SUBJECTS, testosterone blood concentrations are inversely correlated with body weight.[1-3] Therefore, obese subjects have lower mean testosterone concentrations than normal-weight healthy controls.[4] Several factors may be responsible for the decreased plasma testosterone levels in obese people, including decreased sex hormone–binding globulin (SHBG) synthesis,[5] decreased pituitary gonadotropin secretion,[6] and altered aromatase system activity in peripheral tissue.[7] Insulin represents an additional factor capable of regulating both testosterone secretion and metabolism. Therefore, hyperinsulinemia or an impairment in insulin's peripheral action may influence testosterone levels in blood, but at present their relative contribution is still unclear. Available data indicate that this may occur differently in male and female subjects. In women, insulin appears to stimulate ovarian androgen synthesis by interaction with its own receptors and/or the insulinlike growth factor-I receptor.[8,9] In addition, there is in vitro[10] and in vivo[11] experimental evidence that insulin inhibits SHBG synthesis, and this appears to occur in both sexes, which is consistent with the fact that, at least in part, reduced testosterone blood levels in obese males may be dependent on decreased SHBG concentrations, which in turn may reflect prevailing hyperinsulinemia in both the bloodstream and peripheral target tissues.[12]

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In a previous study, we demonstrated that inhibition of insulin by long-term diazoxide treatment decreased total and free testosterone and increased SHBG blood concentrations in healthy normal-weight and obese individuals.[13] These findings suggested that insulin may have a regulatory role in testosterone secretion and/or metabolism also in men, by mechanisms that may be different from those documented in women.

The aim of this study was to investigate the effects of acute hyperinsulinemia on major androgen blood concentrations, including testosterone, in two groups of normal-weight healthy controls and obese insulin-resistant men.

## MATERIALS AND METHODS

### *Subjects*

Nine obese subjects (body mass index [BMI] >28 kg/m$^2$) were selected from patients attending the Semeiotica Medica Institute of the University of Padua for the treatment of obesity. The presence of other relevant metabolic or endocrine abnormalities was excluded on the basis of clinical history, physical examination, and blood tests for routine biochemistry and basal hormone determination. Arterial blood pressure was normal in all subjects. Anthropometry included measurement of height without shoes to the nearest 0.5 cm and of weight without clothes. BMI was calculated by dividing body weight in kilograms by height in square meters. A group of 11 healthy normal-weight subjects (BMI <27 kg/m$^2$) were also included in the study as controls. No subjects were dieting or taking medications in the month before the study. All subjects were instructed to consume a weight-maintaining diet with at least 250 to 300 g/d carbohydrates during 3 days before the study. All obese and control subjects volunteered for the study and provided informed written consent. Their general characteristics are reported in Table 1.

### *Clamp Study*

All tests were performed in the morning (8:30 AM) after a 12-hour overnight fast, while subjects had been quietly recumbent for 10 to 15

**Table 1. General Characteristics (mean ± SD) of the Normal-Weight and Obese Subjects**

| Characteristic | Subjects | | Significance (*P*) |
|---|---|---|---|
| | Obese | Control | |
| No. of subjects | 9 | 11 | |
| Age (yr) | 39.5 ± 12.4 | 30.9 ± 5.3 | NS |
| Body weight (kg) | 112.0 ± 19.0 | 70.0 ± 8.6 | <.0001 |
| Height (cm) | 1.74 ± 0.09 | 1.73 ± 0.07 | NS |
| BMI (kg/m$^2$) | 37.2 ± 7.0 | 23.2 ± 2.2 | <.0001 |

minutes. A teflon catheter was inserted into an antecubital vein for glucose and insulin infusion. A second catheter was inserted retrogradely into a vein of the contralateral hand, which was maintained in a hot box (70°C) to arterialize venous blood samples. Plasma glucose was determined in basal conditions and every 5 minutes during the test. After establishing the baseline glucose concentration, a 5-minute priming insulin infusion (Actrapid HM; Novo, Copenhagen, Denmark) was followed by a constant infusion of 40 mU/m$^2$ body surface area per minute throughout the test. To maintain each subject at his basal arterial plasma glucose concentration, a 20% glucose solution was infused, with modification of the infusion rate on the basis of arterialized 5-minute glycemia determinations.[14] The rate of insulin infusion was chosen because it was sufficient to suppress hepatic glucose production (HGP) in both control and obese subjects without impaired glucose tolerance.[15] To confirm this assumption, we measured HGP in a reduced number of subjects during the clamp study, using [3-$^3$H]-D-glucose as tracer. Starting 180 minutes before the insulin infusion, subjects received a primed (15 μCi) infusion (0.15 μCi/min) of [3-$^3$H]-D-glucose, which was maintained unchanged until the end of the study. Blood samples for determination of [3-$^3$H]-D-glucose specific activity were drawn at −30, −20, 10, and 0 minutes, and during the last 60 minutes of the clamp, every 10 minutes. Overall glucose disposal was estimated from [3-$^3$H]-D-glucose specific activity using Steele's non–steady-state equations.[16] In our experimental conditions, HGP was completely suppressed both in normal and in obese men; therefore, we can indicate the glucose infusion rate (milligrams per kilogram per minute) as an index of total body glucose disposal (M value).

The duration of the clamp was approximately 120 minutes in each individual. Plasma insulin concentrations at the end of the clamp increased to approximately 100 μU/mL. Samples for androgen concentrations were obtained before (−10 and 0 minutes) and at the end of the clamp study during the last 30 minutes.

### *Hormones and Biochemistry*

Plasma glucose concentrations were measured immediately by the glucose oxidase method (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Plasma insulin was determined by radioimmunoassay (RIA) using commercial kits (INSIK-5; Sorin, Saluggia, Italy). Testosterone, androstenedione, and dehydroepiandrosterone sulfate (DHEA-S) levels were measured by a high-performance liquid chromatography RIA method, as previously described.[17] Interassay coefficients of variation in our laboratory are 5% for insulin, 9.3% for testosterone, 7.7% for androstenedione, and 9.7% for DHEA-S.

### *Statistics*

All results are reported as the mean ± SD. All comparisons were made using the Student *t* test, when appropriate. Spearman correlation coefficients between several variables and chi-square analysis, when adequate, were also applied. $P < .05$ was used to define statistical significance.

## RESULTS

Basal testosterone concentrations in the obese group (10.40 ± 3.02 nmol/L) were significantly lower than in the control group (15.50 ± 4.65 nmol/L, $P < .01$), whereas no significant differences between the two groups were present for androstenedione and DHEA-S concentrations. Basal testosterone levels were significantly correlated with BMI ($r = -.61$, $P < .01$).

During the clamp study, testosterone significantly increased in the obese group (11.79 ± 3.64 nmol/L, $P < .05$; +13.8% ± 13.6%), but not in the control group (15.81 ± 4.54 nmol/L, $P$ = NS; +3.8% ± 17.0%) (Fig 1). No significant change occurred in androstenedione (obese, 3.46 ± 1.29 *v* 2.65 ± 0.91 nmol/L, $P$ = NS; controls, 3.49 ± 1.50 *v* 3.14 ± 0.40 nmol/L, $P$ = NS) or DHEA-S (obese, 5.62 ± 4.90 *v* 5.50 ± 3.85 μmol/L, $P$ = NS; controls, 6.73 ± 5.07 *v* 7.28 ± 2.96 μmol/L, $P$ = NS).

Fasting insulin levels were significantly higher in obese subjects (116.2 ± 48.1 pmol/L) than in controls (81.1 ± 66.0 pmol/L, $P < .05$). After the 2-hour insulin infusion, HGP was suppressed in both groups (control *v* obese, −0.2 ± 0.4 *v* −0.7 ± 0.2 mg/kg · min). At the end of the clamp study, the M value in obese subjects was significantly lower than in the control group (3.9 ± 0.5 *v* 7.0 ± 0.5 mg/kg · min, $P < .0005$). As expected, there was also a highly significant negative correlation between the M value and BMI ($r = -.86$, $P < .001$). Moreover both basal ($r = .67$, $P < .01$) and clamp ($r = .73$, $P < .01$) testosterone concentrations were significantly correlated with the M value.

It should be pointed out that particularly in the control group there was a certain discrepancy in testosterone variations during the clamp study. In fact, seven controls (64%) had no change or some decrease in testosterone levels, whereas in four controls testosterone increased over 9%, which is a value similar to the coefficient of variation of the assay in our laboratory. On the contrary, only two obese subjects failed to show increased testosterone levels over that value during the test. No significant

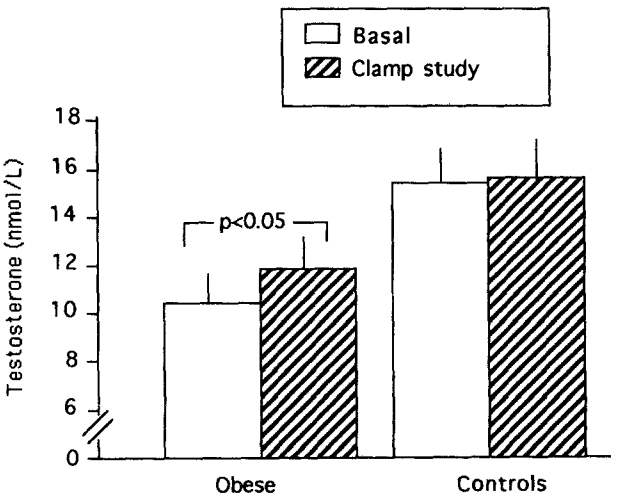


**Fig 1. Serum testosterone concentrations (mean ± SD) in basal conditions and during the clamp study in obese and control male subjects.**

difference was evident in BMI, basal androgen and insulin levels, or M value in either group between responders and nonresponders. Moreover, there was no significant difference in percent testosterone increase between responder in the two groups.

## DISCUSSION

The results of this study confirm that, as in women,[8] insulin is capable of regulating serum testosterone concentrations in vivo in men also. In fact, acute hyperinsulinemia was capable of significantly increasing testosterone blood concentrations, at least in obese men. It is unlikely that the data are subject to methodological problems, since the hormone increase exceeded the coefficients of variation of the assay. Moreover, they also do not appear to be related to circadian variations, since a reduction,[18] rather than an increase, in testosterone levels would have been expected. Mechanisms by which insulin acts obviously need to be clarified. On the other hand, it has been demonstrated that insulin can stimulate gonadal testosterone synthesis and secretion in obese hyperandrogenic women.[9] It is therefore not surprising that this can also occur in men. The results of a previous study,[13] in which we demonstrated that chronic suppression of insulin levels reduced blood testosterone in normal-weight and obese subjects, agree with this suggestion. Specific insulin receptors have been demonstrated in rat Leydig cells, in which insulin has been proved to stimulate testosterone synthesis.[19-21] Moreover, in vitro studies have shown that testosterone synthesis can also be stimulated by insulin-like growth factor-I.[19] Since insulin and insulin-like growth factor-I receptors have similar biochemical and functional structure, it could be argued that insulin action may occur through its own receptors or, alternatively, through the insulin-like growth factor-I receptors themselves, particularly in conditions of moderate to severe hyperinsulinemia as usually occurs in obese individuals.

Insulin can also affect testosterone transport into the bloodstream, since it is capable of suppressing hepatic SHBG synthesis.[10] Clinical and epidemiological studies have clearly demonstrated that SHBG and testosterone concentrations are invariably low in obese male subjects and inversely correlated with insulin concentrations and body weight.[1-5] Unfortunately, we could not determine SHBG concentrations for technical reasons. On the other hand, it was to be expected that a decrease in SHBG would have been observed during the test, at least in controls. In fact, by reducing insulin concentrations, an increase of SHBG levels has been demonstrated both in women with obesity and polycystic ovary syndrome[11] and in obese and normal-weight males.[13] Insulin might also regulate testosterone blood levels by acting on the aromatase system. In fact, although studies performed in rats have shown that chronic hyperinsulinemia may increase aromatase activity,[22] human studies performed in both hyperandrogenic women and obese males suggest exactly the contrary.

Interestingly, a certain discrepancy was observed for testosterone variations in the subjects we studied, especially in the control group, similar to what emerged from the data of similar studies on the effects of acute hyperinsulinemia on androgen concentrations in women with the polycystic ovary syndrome or in normal control women.[23,24] We suggest that these discrepancies may reflect the fact that insulin may affect testosterone blood levels in many ways, and that the sum of its effects may not be evident in experimental conditions similar to those of our study.

In conclusion, we report that in obese and in some normal-weight men, acute hyperinsulinemia significantly increased testosterone blood levels, without affecting other major androgen concentrations. These findings are therefore consistent with the hypothesis that in vivo insulin is capable of regulating testosterone secretion and/or metabolism even in males. They obviously cannot help to explain why obese men have reduced testosterone concentrations in the blood. On the other hand, several other factors seem to be involved in determining this abnormality, which especially include reduced pulsatile luteinizing hormone release from the pituitary.[25] Whether hyperinsulinemia and impaired luteinizing hormone secretion may be totally responsible for hypotestosteronemia in obese males still remains to be proven.

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