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Leptin and Androgens in Male Obesity: Evidence for Leptin Contribution to Reduced Androgen Levels*

ANDREA M. ISIDORI, MASSIMILIANO CAPRIO, FELICE STROLLO, COSTANZO MORETTI, GAETANO FRAJESE, ALDO ISIDORI, AND ANDREA FABBRI

Cattedra di Andrologia, Dipartimento di Fisiopatologia Medica, Università La Sapienza (A.M.I., M.C., A.I., A.F.), Italian National Research Centers on Aging (F.S.), Cattedra di Endocrinologia, Universita' Tor Vergata (C.M., G.F.), 00100 Rome, Italy; and the Department of Endocrinology (A.F.), St. Bartholomew's Hospital, ECIA 7BE London, United Kingdom

ABSTRACT

Leptin circulates in plasma at concentrations that parallel the amount of fat reserves. In obese males, androgen levels decline in proportion to the degree of obesity. Recently, we have shown that in rodent Leydig cells leptin inhibits hCG-stimulated testosterone (T) production via a functional leptin receptor isoform; others have found that leptin inhibits basal and hCG-induced T secretion by testis from adult rats. In this study, we further investigated the relationship linking leptin and androgens in men. Basal and hCG-stimulated leptin and sex hormone levels were studied in a large group of men ranging from normal weight to very obese (body mass index, 21.8–55.7). Initial cross-sectional studies showed that circulating leptin and fat mass (FM) were inversely related with total and free T ($r = -0.51$ and $r = -0.38$, $P < 0.01$ and $P < 0.05$, respectively). Multiple regression analysis indicated that the correlation between leptin or FM and T was not lost after controlling for SHBG and/or LH and/or estradiol (E_2) levels and that leptin was the best hormonal predictor of the lower androgen levels in obesity. Dynamic studies showed that in obese men the area under the curve of T and free T to LH/hCG stimulation (5000 IU im) was 30–40% lower than in controls and inversely correlated with leptin levels ($r = -0.45$ and $r = -0.40$, $P < 0.01$ and $P < 0.05$, respectively). Also, LH/hCG-stimulation caused higher increases in 17-OH-progesterone to T ratio in obese men than in controls, whereas no differences were observed between groups either in stimulated E_2 levels or in the E_2/T ratio. In all subjects, the percentage increases from baseline in the 17-OH-progesterone to T ratio were directly correlated with leptin levels or FM ($r = 0.40$ and $r = 0.45$, $P < 0.01$), but not with E_2 or other hormonal variables. In conclusion, our studies, together with previous *in vitro* findings, indicate that excess of circulating leptin may be an important contributor to the development of reduced androgens in male obesity. (*J Clin Endocrinol Metab* **84:** 3673–3680, 1999)

OBESITY in western countries has become widespread and its prevalence continues to increase (1). The identification of obesity genes and transcription factors that regulate adipocyte differentiation, proliferation, and metabolism has produced significant advances in the understanding of the pathophysiology of adipose tissue (2). However, the recent and disparate insights into adipocyte biology has to be integrated with knowledge of classical metabolism, endocrinology, and nutrition to have a more complete picture of the clinical aspects of obesity.

Obesity is associated with a number of metabolic abnormalities, including a high prevalence and incidence of non-insulin-dependent diabetes mellitus, increased triglyceride levels, and decreased high-density lipoprotein cholesterol. Furthermore, obesity in men is associated with a decline in total plasma testosterone (T) and free testosterone (FT) (for review see Refs. 3–8), which parallels body fat mass (FM) (9). Of these alterations, some are most certainly secondary to the development of obesity, whereas others may be putative causative factors. In particular, the endocrine abnormalities observed in obese men may both derive from global impairment in metabolism and contribute to the increase and worsening of obesity. Adipose tissue and androgens in obese men are associated by a reciprocal link supported by two lines of evidence: first, T and FT are decreased in proportion to the degree of obesity (9); second, T regulates insulin sensitivity (10–12), increases lipolysis (13), and affects body composition (14–16). Thus, in men the effects of obesity on metabolic variables could involve the presence of lower androgens, which may carry an independent risk of cardiovascular disease (17) and diabetes (18).

The major pathogenic factors suggested as being responsible for T reduction in obesity are the decrease in the binding capacity of sex hormone-binding globulin (SHBG), the reduction of LH pulse amplitude, and hyperestrogenemia (19). Alternatively, an altered metabolism or an excess of fat-derived hormonal products may cause an impairment of testicular interstitial function.

Leptin, the obese (*ob*) gene product secreted from adipocytes, circulates in plasma at concentrations that parallel the amount of fat reserves (20, 21) and controls adiposity by modulating food intake and energy metabolism in the rodent (22–24). Recent research has shown that leptin also plays an important role in rodent and human reproduction (25–28). It has been demonstrated that leptin receptors are present in ovarian granulosa cells and that leptin treatment of rat granulosa cell cultures inhibits hormonal-stimulated estradiol (E_2) production (29, 30). Leptin receptors are also present in testicular tissue (27). Recently, we demonstrated that leptin

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Address correspondence and requests for reprints to: Andrea Fabbri, M.D., Ph.D., Cattedra di Andrologia, Dipartimento di Fisiopatologia Medica, Università di Roma «La Sapienza», 00161-Rome, Italy. E-mail: a.fabbri@caspur.it.
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directly inhibits human chorionic gonadotropin (hCG)-stimulated T secretion from rat Leydig cells in culture via a functional leptin receptor isoform and at concentrations within the range of obese men (32). Others have also shown that leptin inhibits basal and hCG-stimulated T secretion from incubations of rat testicular samples (33). Finally, several studies demonstrated that leptin levels are inversely correlated with T (34–36) and it has been recently proposed that T may regulate *ob* gene expression (36). All the above raise the possibility that leptin may directly regulate testicular steroidogenesis in humans.

In the present study, we examined the *in vivo* relationship between leptin concentrations and basal and hCG-stimulated sex hormone levels in a group of men ranging from normal weight to very obese. Our results demonstrate that excess of leptin may exert a direct negative action on LH/hCG-stimulated androgen production in *in vivo* models and that hyperleptinemia may have a role in the pathogenesis of the reduction of androgens in male obesity.

Materials and Methods

Subjects

A total of 38 healthy nonsmoking men were studied: 28 obese men [body mass index (BMI) >30] and 10 age-matched nonobese men (controls). Physical examination and blood and urine biochemistry were performed to exclude significant diseases. Some of the obese subjects had an impaired glucose tolerance test (according to the World Health Organization criteria), but none of them was overtly diabetic. Testicular size and secondary sexual characters were normal. Men older than 60 yr of age were not included in the study. All the men had a stable weight, and none of them was currently dieting nor was taking any medication. All subjects provided informed consent before taking part in the study, and the research protocol was approved by the Ethical Committee of the University of Rome La Sapienza.

Study Protocol

The study consisted of a 2-week protocol: during the first week physical examination, routine laboratory tests, an oral glucose tolerance test, hormonal assays, and body fat composition examinations were performed. Blood samples were obtained in the morning (0700–0800 h) after an overnight fast. Sera were frozen at −80 C until analysis. An oral glucose (75 g) tolerance test was performed, and samples were taken at 0, 30, 60, 90, 120, and 180 min for glucose and insulin determination. During the second week, a LH/hCG test was performed. A single dose of 5000 IU hCG (Serono, Rome, Italy) was injected im early in the morning; thereafter, blood samples were collected at 0, 24, 48, 72, and 96 h for the determination of hormone levels [T, FT, 17-α-hydroxyprogesterone (17-OH-P), SHBG, E_2, LH, FSH, and leptin]).

Measurements of total body fat and fat distribution

Body fat content and distribution were determined using the BMI and the dual energy x-ray absorptiometry (DEXA). BMI was calculated by dividing weight (kg) by the square of length (mt). DEXA measurements of lean body mass, FM and percentage of body fat were performed by using a total body scanner (Hologic QDR-2000; Hologic, Inc., Waltham, MA), as described elsewhere (37).

Determination of plasma hormones

T, FT, 17-OH-P, E_2, LH, and FSH were measured with solid phase commercial radioimmunoassay (Radim, Pomezia, Italy) (38); SHBG levels were measured by immunoradiometric assay (Radim). Leptin concentrations were measured by a commercial RIA kit (Linco Research, St. Louis, MO). For all hormonal parameters the intra- and interassay coefficients of variation ranged within 2–5% and 3–8%, respectively; all determinations were performed in duplicate.

Statistical analysis

The testicular T production stimulated by LH/hCG was evaluated measuring the area under the curve (AUC) obtained from the series of blood sample at 0, 24, 48, 72, and 96 h. Fat mass (kg), measured by DEXA analysis, was used as the best indicator of the amount of body fat. The differences between controls and obese men were tested for significance by Student's *t* test and analysis of variance. Leptin levels were sufficiently normally distributed and did not need logarithmic transformation. Relations between all variables under investigation were determined as univariate analysis and/or linear regression, computed by the method of least squares; Pearson's r, partial correlation, and multiple linear regression analysis were calculated. Data are presented as the mean ± SE, unless otherwise specified; *P* values less than 0.05 were considered to be statistically significant.

Results

The study population covered a wide range of body fatness as estimated by DEXA analysis (FM ranging from 8.9–72.3 kg). To identify a FM-related impairment in hormonal activity of the testis, the population was divided into three groups of subjects: not obese or controls (BMI, <30), moderately obese (BMI, 30–40), and massively obese (BMI, >40) men, according to the World Health Organization criteria (39, 40). Table 1 reports clinical and basal hormonal data of the study groups. In the moderate and massive obese, leptin levels were higher (two and six times, respectively), whereas T and FT levels were lower (33% and 45% for T and 22% and 30% for FT, respectively) than in controls. In both groups of obese, LH and FSH values were not different from controls, even if a tendency to LH reduction was observed in subjects with massive obesity. This group of subjects also showed a slight increase in plasma levels of E_2 and insulin, as well as a moderate increment of fast glucose and a marked reduction in 17-OH-P plasma concentrations compared with controls (Table 1).

Univariate correlations between all hormonal parameters investigated in basal conditions are reported in Table 2. As expected, leptin was highly correlated with BMI and FM (r = 0.85 and r = 0.87, respectively; $P < 0.001$). FM and leptin were negatively correlated with T, FT, and 17-OH-P, but only FM

TABLE 1. Clinical characteristics and hormonal levels of control and obese subjects

	Controls (BMI <30)	Moderate obese (BMI 30–40)	Massive obese (BMI >40)
No.	10	14	14
Age (range)	37.8 (23–58)	42.0 (27–58)	40.7 (18–58)
BMI (kg/m^2)	24.5 ± 0.47	32.7 ± 0.65[a]	45.7 ± 1.25[a,b]
FM (kg)	17.2 ± 1.63	27.3 ± 1.42[a]	51.4 ± 2.17[a,b]
Leptin (ng/mL)	6.1 ± 1.25	11.4 ± 2.94[a]	38.1 ± 3.41[a,b]
T (ng/mL)	5.5 ± 0.43	3.9 ± 0.38[c]	3.0 ± 0.30[d]
FT (pg/mL)	14.3 ± 1.10	11.1 ± 0.90[c]	9.7 ± 0.86[c]
17-OH-P (ng/mL)	2.8 ± 0.49	2.1 ± 0.38	1.3 ± 0.11[a,b]
SHBG (nM/L)	26.3 ± 2.19	20.5 ± 3.29	18.1 ± 2.69[c]
E_2 (pg/mL)	35.1 ± 3.31	40.6 ± 4.15	48.3 ± 3.08[c]
FSH (mIU/mL)	2.8 ± 0.37	3.4 ± 0.63	3.0 ± 0.54
LH (mIU/mL)	3.4 ± 0.26	3.3 ± 0.48	2.8 ± 0.65
Glucose (g%)	90.5 ± 5.2	98.9 ± 5.35	107.3 ± 3.9[c]
Insulin (IU/L)	8.3 ± 0.8	14.8 ± 2.5	25.6 ± 2.8[b,c]

[a] $P < 0.001$: obese *vs.* controls.
[b] $P < 0.05$: moderate obese (BMI 30–40) *vs.* massive obese (BMI >40).
[c] $P < 0.05$: obese *vs.* controls.
[d] $P < 0.01$: obese *vs.* controls.

TABLE 2. Correlation matrix of all parameters studied

	BMI	FM	Leptin	T	FT	17-OH-P	SHBG	E_2	LH	Insulin
BMI	—	0.97[a]	0.85[a]	−0.67[a]	−0.32[b]	−0.64[b]	−0.38[c]	0.20[d]	−0.27[d]	0.71[a]
FM		—	0.87[a]	−0.62[a]	−0.46[b]	−0.62[a]	−0.40[c]	0.25[d]	−0.21[d]	0.67[b]
Leptin			—	−0.51[b]	−0.38[c]	−0.49[b]	−0.28[d]	0.15[d]	−0.19[d]	0.55[c]
T				—	0.28[d]	0.37[c]	0.40[c]	0.18[d]	0.38[c]	−0.55[c]
FT					—	0.44[b]	0.04[d]	0.11[d]	0.02[d]	−0.27[d]
17-OH-P						—	0.01[d]	0.17[d]	0.24[d]	−0.38[d]
SHBG							—	0.37[c]	−0.27[d]	−0.45[c]
E_2								—	−0.33[d]	0.32[d]
LH									—	0.24[d]
Insulin										—

[a] $P < 0.001$.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] Not significant.

TABLE 3. Multiple regression analysis of the correlation between leptin and basal testosterone as dependent variables

	b[a]	SE(b)	P	$R^2 \times 100$[b]
Constant	5.6763	1.4878	0.0009	
Leptin	−0.0384	0.0153	0.0196	26%
E_2	−0.0510	0.0251	0.0535	35%
LH	0.2404	0.1381	0.0941	39%
SHBG	0.0438	0.0261	0.1065	41%
Insulin	−0.0538	0.0327	0.1200	42%
17-OH-P	0.1784	0.2176	0.4201	—

[a] Regression coefficient.
[b] $R^2 \times 100$ is expressed as increased explained variance, for the added variables.

(not leptin) was negatively correlated with SHBG. Insulin was positively correlated with FM and leptin, and negatively correlated with SHBG, T, but not FT. E_2 was directly correlated with SHBG, but not with any of the other parameters studied. LH was weakly correlated only with basal T levels, but not with AUC of T (data not shown); FSH was not correlated with any of the parameters investigated (data not shown).

We also explored the possibility of other variables that could explain the relationship between leptin concentration and basal T levels by multiple regression analysis, in which T levels were dependent and leptin, E_2, LH, insulin, SHBG and 17-OH-P levels were independent variables (Table 3). Multivariate analysis showed that leptin was the best hormonal predictor of reduced androgens in basal condition explaining up to 26% of the variance and that the relationship was slightly modified by including all other variables (from 26% to 42% in explained variance). Only E_2 added an independent minor contribution to the model, with a P value that was close to statistical significance. This is consistent with a contribution of low magnitude, independent of FM or leptin, on T reduction likely due to central and/or peripheral effects of E_2 (see *Discussion*). All correlations between leptin and basal sex hormones were lost when adjusting for the effect of FM.

Fig. 1, A–F, illustrates the hormonal response to hCG administration. The peak value of plasma T and FT (Fig. 1, A and B) were significantly lower in the moderate and massive obese than controls; in all groups, the highest T and FT values were reached between 48 and 72 h when 80–100% and 30–40% differences were observed between plasma T and FT values of controls and massive obese ($P < 0.01$). Peak values of 17-OH-P (Fig. 1C) were achieved 24 h earlier than those of T and FT and were not different between groups; however, and more interestingly, the incremental change from basal was much higher in obese (two times) than in controls ($P < 0.01$). E_2 levels (Fig. 1D) were highly variable during the test, with no mean differences between groups, and it was not possible to identify a FM-related tendency in the hormonal response; in addition, there was no relationship between the AUC of E_2 response and leptin or FM (data not shown). Either leptin or SHBG levels remained unmodified during hCG test, in all groups (Fig. 1, E and F).

In the moderate and massive obese, the decreased T response to hCG and the high net increase in 17-OH-P levels led to elevated 17-OH-P/T molar ratios and increased percentage changes from baseline of 17-OH-P/T, which were two to five times higher than in controls ($P < 0.05$) (Fig. 2A). The increases in 17-OH-P/T ratios, calculated at peak T values, were related to the amount of body fat and leptin ($r = 0.45$ at 48 h and $r = 0.40$ at 72 h, in all subjects; $P < 0.01$), but not with basal E_2, LH, or SHBG; the latter finding indicates the presence of an enzymatic defect in the conversion of 17-OH-P to T, revealed by LH stimulation. Reduced androgen response to hCG in obese individuals was not due to increased aromatase activity and consequent conversion of T to E_2, as shown by the lack of significant changes in E_2/T ratio in obese men when compared with controls (Fig. 2B).

The relationship between leptin and hCG-induced T production was evaluated by linear regression of the AUC of both T and FT on leptin levels (Fig. 3, A and B). The T AUC represents the total stimulated testicular production of T during the 5-day test and can be considered an accurate index of Leydig cell steroidogenic capacity. T AUC were lower in the massive (607.8 ± 37.5 ng/ml·h) and moderate obese (658.5 ± 36.4) compared with controls (930.2 ± 68.4; $P < 0.01$); also, considering the whole population, testicular steroidogenic capacity was inversely related to leptin serum levels ($r = -0.45$; $P = 0.018$; Fig. 3A). Differences in FT AUC were minor and reached significance in the group of massive obese in which values were 20% lower than in controls (2004.6 ± 155.7 *vs.* 2416.2 ± 209.9 pg/ml·h, $P < 0.05$). Interestingly, the regression of AUC of FT on leptin levels achieved the same statistical significance of T AUC only when adjusted for SHBG levels ($r = -0.40$, $P = 0.037$; Fig. 3B);

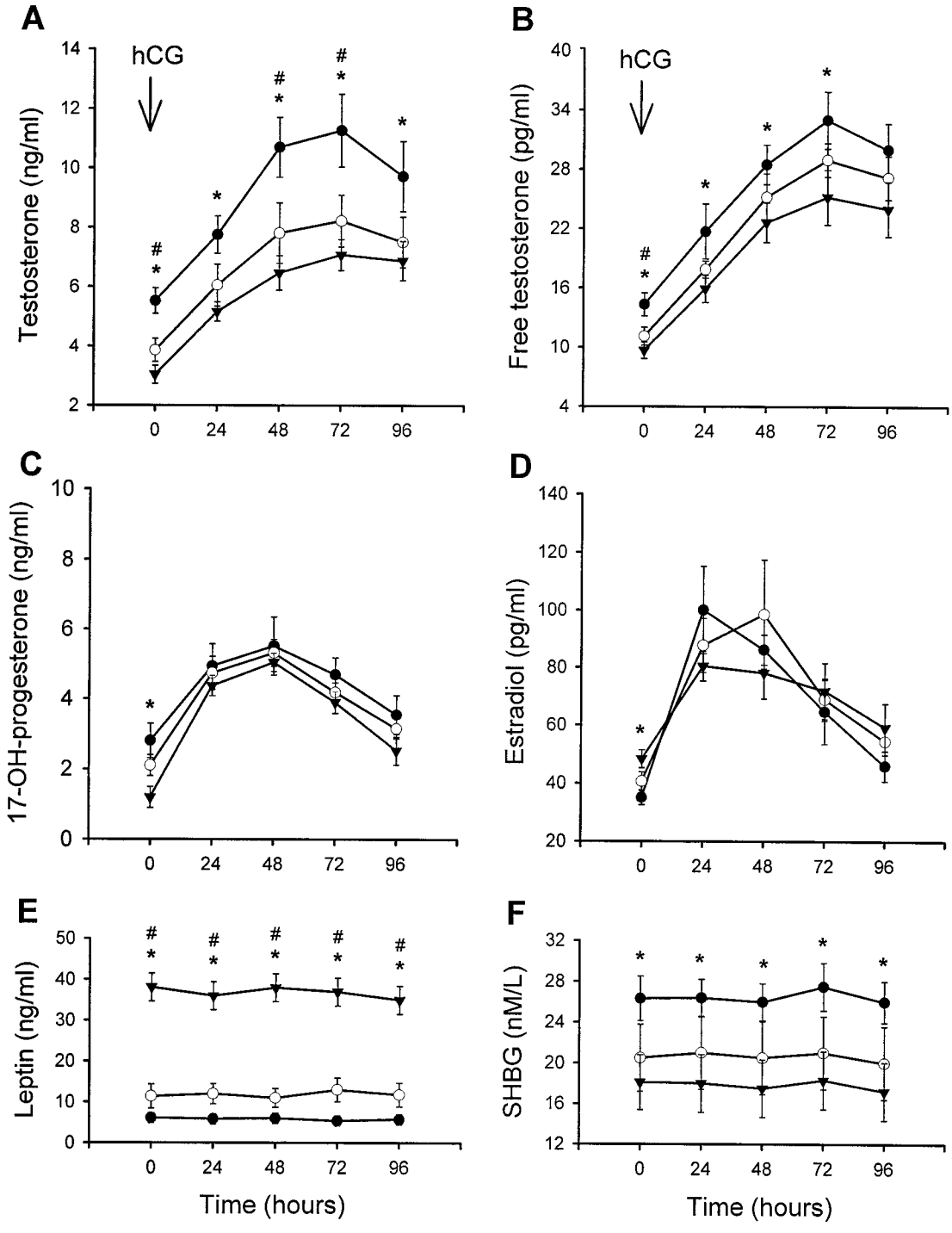

FIG. 1. Hormonal parameters after hCG-stimulation (5000 IU im) of all subjects treated as groups of BMI: ●, BMI <30; ○, BMI 30–40; ▼, BMI >40. The *arrows* indicate hCG injection. * indicates BMI <30 *vs.* BMI >40, $P < 0.05$; # indicates BMI <30 *vs.* BMI 30–40, $P < 0.05$.

since in obese patients SHBG levels were constantly lower than in controls throughout the study period (Fig. 1F), the latter finding further indicates the presence of a fat-related impairment of testicular steroidogenic capacity.

Discussion

These studies have shown that in male obesity basal and LH-stimulated androgen levels are reduced and inversely correlated with circulating leptin. The impaired androgen response to LH stimulus was due to a defect in the enzymatic conversion of 17-OH-progesterone to T, which was disclosed by a leptin-related increase in 17-OH-progesterone to T ratio. These results suggest that leptin may be an important contributor to the pathogenesis of reduced androgens in obese men. Because we have previously shown that functional leptin receptors are present in rodent Ley-

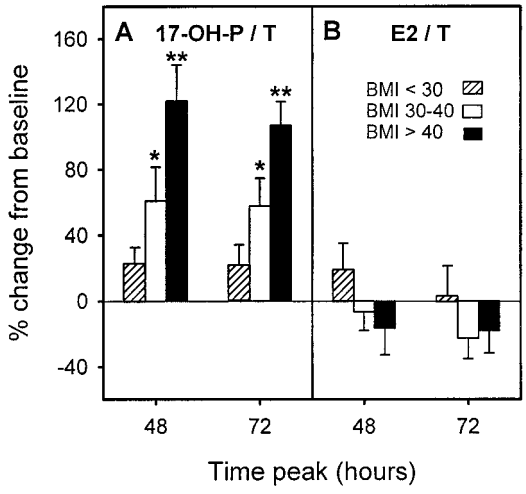

FIG. 2. Percentage changes in serum molar ratio of 17-OH-progesterone to T (A) and E_2 to T (B) after hCG-stimulation. ▨, BMI <30; □, BMI 30–40; ■, BMI 40>. ** indicates BMI <30 *vs.* BMI >40, $P < 0.01$; * indicates BMI <30 *vs.* BMI 30–40, $P < 0.05$ BMI 30–40 *vs.* BMI >40, $P < 0.05$.

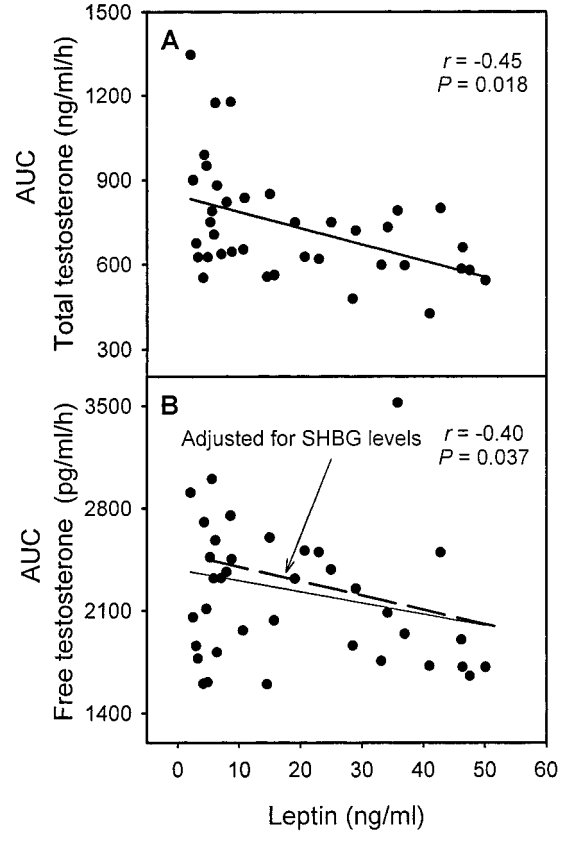

FIG. 3. Linear regression of the AUC of total T (A) and the AUC of free T (B) after hCG stimulation on basal leptin values in all subjects. B, r and *P* are the values of the correlation when adjusted for basal SHBG levels.

dig cells (31, 32), it is conceivable that in males high leptin concentrations may have a direct inhibitory effect(s) on Leydig cell function.

Evaluation of sex hormone profile in obese subjects showed significantly decreased mean serum levels of T, FT, and SHBG binding capacity, which were consistent with previous findings (41). In particular, we observed that plasma-free T levels were subnormal either in moderate or massively obese men, despite of reduced SHBG concentrations. These findings strongly suggest the presence of a defective production of testicular androgens directly related to the amount of body fat and/or leptin levels. Along with these results, it was found that the decline of androgens in obese men represents a continuum observable at any degree of obesity (9).

Is the androgen reduction in obesity due to a central or peripheral component? According to several studies, we found normal gonadotropin levels in obese men (41, 42), with a tendency to LH reduction in severe obesity. Vermeulen *et al.* (19) demonstrated that a reduced amplitude of LH pulses occurs in very obese men, leading to a mild hypogonadotrophic hypogonadism. In the present study, we did not evaluate the LH pulsatility, and we can not exclude that an alteration in LH pulses was present in patients with a high amount of FM. Estrogens, which are inhibitory modulators of LH pulsatility and bioactivity (43), were increased in massively obese patients, but not different from controls in moderately obese subjects, indicating that E_2 was not solely responsible for the observed reduction in androgen production. This is consistent with the observation that in massively obese men large weight loss is associated with a significant increase in plasma androgen levels that occurs before the decline of hyperestrogenemia (44).

It has been proposed that the reduction in total plasma T levels is mainly due to a decrease in SHBG binding capacity (45). In our study, SHBG levels had a trend toward reduction in moderately obese men and were significantly lower in the massively obese compared with control subjects. *In vitro* and *in vivo* studies demonstrated that insulin is an important inhibitor of the synthesis of SHBG (46–48). As expected, in our subjects, a strong negative correlation was found between insulin levels and SHBG, whereas there was no correlation between leptin and SHBG levels. It is, therefore, reasonable to suppose that the hyperinsulinemia of obese subjects is the major determinant of their lower SHBG levels. Most importantly, by controlling the correlation between leptin or FM and T for SHBG variability, there was still a strong significant relationship linking indices of adipose tissue and androgens ($r = -0.55$, $P < 0.05$). This latter result indicates that SHBG reduction in obesity is a minor determinant of lowered androgen levels. In particular, multiple regression analysis showed that SHBG can explain only up to 3% of the correlation (Table 3). These observations led us to investigate the steroidogenic function of the testis in a dynamic approach.

In obese men, T response to hCG stimulation was much lower than in control subjects. Testicular responsiveness to a maximal hCG dose (49) seemed reduced considering the absolute peak value, single day increase, or the AUC obtained during the 5 days after the stimulus. After hCG administration, lean and obese subjects showed a comparable trend in serum T increase, suggesting that the rate of hCG absorption and testicular androgen response did not differ

between groups, even if the T values of the obese men were constantly lower. These results confirm previous data observed in obese children (50) who exhibited a significantly lower increase in hCG-stimulated T than lean controls, even in the presence of a normal pituitary reserve of LH and FSH after the LHRH test. Amatruda *et al.* (51) and Glass *et al.* (41) reported that the increases in serum T after hCG were subnormal on an absolute basis, but when expressed as a percentage increase these changes were low-normal to normal. In our study, the AUC of T response, which is a more precise index of T production, was 30–40% lower in the obese than in controls; more important, it was negatively related to leptin, but not to E_2 or other hormonal variables. Also, the AUC of FT response was subnormal in respect to controls even in the presence of low SHBG, and, after correcting for SHBG levels, it was negatively correlated to leptin or FM (Fig. 3B). These combined results strongly indicate that testicular T *de novo* production is impaired in obese men and that leptin seems to be the best hormonal predictor of this blunted response to LH stimulation.

Interestingly, the pattern of response and absolute values of 17-OH-P (Fig. 1C) were similar between groups, although the basal levels were lower in obese men compared with control subjects. The low basal 17-OH-P levels found in massively obese men are consistent with a global impairment of Leydig cell steroidogenic function in this group of subjects. Other studies have investigated the adrenal function in male obesity and have shown that basal cortisol and 17-OH-progesterone levels tend to decrease with the increase in the degree of obesity (5, 52) and that there is a defect in 21-hydroxylase activity revealed by ACTH testing (52). In this study we investigated the efficiency of 17-OH-progesterone to T conversion, which is an index of 17,20-lyase activity, during hCG-stimulation test. The net increment of 17-OH-P at peak value was 40–50% higher in the massively obese than in controls, and the percentage changes in 17-OH-P/T ratio from basal were related to the amount of body fat and leptin levels. These findings indicate that obese men have a FM-related defect in the enzymatic conversion of 17-OH-P to T, which is revealed by hCG stimulation. High E_2 can inhibit the expression and activity of the 17,20-lyase and may be responsible for this steroidogenic lesion (53–55). However, stimulated E_2 levels were not higher in the obese than in controls, excluding the fact that the lower androgen response was due to an increased aromatization of T to E_2 and that estrogens have a major role in the observed defect of 17,20-lyase activity in obese men. More important, the percentage increase in the 17-OH-progesterone to T molar ratio paralleled the increase in leptin levels of obese men, but it was not correlated to E_2, SHBG, and LH concentrations of these subjects.

Multiple regression analysis indicated that the best hormonal predictor of the obesity-related reduction in T and FT basal levels and androgen changes after hCG stimulation was serum leptin concentration. Insulin added no significant prediction, and, even if it was inversely correlated to T, it did not correlate with FT. These results are consistent with the knowledge that insulin has no negative influences on androgen production in obese men (56). On the contrary, insulin is known to have stimulatory actions on T production that have been demonstrated in obese and normal weight men (57) and in Leydig cells in culture (58, 59). Eventually, the negative correlation between insulin and basal T can be partly explained by the inhibitory action of insulin on SHBG production (48). In obese men, the positive insulin-leptin correlations have been reported in several other studies and explained by the simple association of both hormones with obesity (60) and/or by long-term insulin stimulation of *ob* gene expression and release (61).

In our study, all correlations between leptin and basal androgens disappeared after adjustment for body FM. The loss of correlation has already been reported for basal androgens (62), indicating that *in vivo* correlation studies are mostly unable to discriminate between adipose tissue and leptin in the pathogenesis of FM-related androgen changes and that *in vitro* studies are needed to address causality in the relationship between leptin and steroidogenesis. Indeed, it is established that leptin levels are higher in females compared with males (63) and that this occurs even after correction for the degree of body fat mass (64–67). Also, it has been shown that hypogonadal men have higher circulating leptin levels compared with hypogonadal patients under effective androgen substitution therapy (35). Finally, *in vitro* studies have shown that a 6-day exposure of human fat cells to T or dihydrotestosterone inhibits leptin expression (36). These results lead to the accepted knowledge that T is an important contributor to the gender difference in serum leptin levels (36, 67). The lack of a direct effect of T on leptin secretion by the adipocytes has been also reported (68), and we showed in this study that elevated T up to two to three times above the baseline after hCG stimulation for 3–5 days did not modify plasma leptin concentrations in normal weight and obese men. Because T increases muscle size and modifies body composition parameters in favor of fat-free mass (16), it can not be excluded that a relevant part of the claimed androgen effects on leptin are indirect and exerted through changes in body composition, fat content, and adipose tissue distribution (69, 70).

In recent *in vitro* studies, we have demonstrated that the long and short leptin receptor isoforms are expressed in rodent Leydig cells (31, 32). We have shown that the testicular leptin receptor is functional and that leptin has a direct negative action on LH/hCG-stimulated T and androstenedione production from Leydig cells in culture at concentrations within the range of male obesity. The leptin inhibition of hCG-stimulated androgens was accompanied by a rise in precursor metabolites (*i.e.*, 17-OH-progesterone, progesterone, and pregnelone), indicating an enzymatic lesion at the level of 17,20-lyase (31, 32). Tena-Sempere *et al.* (33), by using incubation of rat testicular samples, have also reported that leptin inhibits basal and hCG-stimulated T secretion from adult but not prepubertal rat testis. Besides testis, ovary and adrenals are other possible steroidogenic targets for leptin action. It has been demonstrated that leptin suppresses insulin-induced progesterone and 17β-E_2 production by isolated bovine granulosa cells (29), impairs hormonally stimulated E_2 production in rat granulosa cells (71), and prevents insulin-induced progesterone and androstenedione secretion in bovine ovarian thecal cells (30). In other studies, leptin has been shown to inhibit cortisol release directly from cul-

tured bovine adrenocortical cells by reducing the accumulation of ACTH-stimulated cytochrome P450 17α messenger RNA (72). All these data indicate that leptin has negative actions on steroidogenesis that are mediated by specific receptors in the target cells and are likely to be exerted at different enzymatic steps of the steroidogenic pathway.

In conclusion, the clinical data of our study are consistent with an impairment of basal and LH/hCG-stimulated androgen production in obese men related to FM or leptin levels and, together with experimental results, indicate that leptin is a major and direct signal linking excess of adipose tissue to altered steroidogenic function of the testis. These studies complement and add significant information to the knowledge of the interaction between leptin and male reproductive function (26, 73). A dualistic function of the hormone emerges in which physiological leptin concentrations are necessary for proper reproductive function during puberty and in the postpubertal period (74, 75), whereas leptin excess and/or modifications of secretory rhythms as a result of obesity seem to have deleterious effects on the target steroidogenic cell.

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