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Testosterone Substitution Normalizes Elevated Serum Leptin Levels in Hypogonadal Men

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ABSTRACT

The *ob* gene product leptin (OB) is a feedback signal from the adipocyte to the hypothalamus and is involved in regulation of food intake and energy expenditure in rodents. A major determinant of serum OB levels is fat mass. Several studies suggest that men have lower OB levels than women even after adjustment for percent body fat. We, therefore, investigated the influence of testosterone (T) substitution in hypogonadal men on serum OB levels.

Hypogonadal men with T levels of 3.6 nmol/L or less and off substitution therapy for at least 3 months were assigned to two treatment groups: testosterone enanthate (TE; 250 mg, im, every 21 days; n = 10) or a single sc implantation of 1200 mg crystalline T (TPEL; n = 12). Blood samples for determination of T, 5α-dihydrotestosterone (DHT), sex hormone-binding globulin, and 17β-estradiol were obtained before therapy and then every 21 days until day 189 and at follow-up visits on days 246 and 300. Serum OB levels were assessed on days 0, 42, 84, 126, 168, and 300. OB levels were referred to a normal range for men based on the analysis of OB levels in 393 adult men.

Substitution with T led to a large rise in T and DHT in both groups compared to baseline values (average T, days 21–189: TE, 14.33 ± 2.63 nmol/L; TPEL, 24.98 ± 1.64; average DHT, days 21–189: TE, 4.20 ± 0.57 nmol/L; TPEL, 5.11 ± 0.56; $P \leq 0.05$). Concomitantly, 17β-estradiol increased in both groups, and sex hormone-binding globulin levels were significantly decreased. At baseline, serum OB levels in hypogonadal men were 3-fold elevated compared to those in normal men (12.39 ± 2.93 μg/L *vs.* 4.28 ± 0.52; $P < 0.01$) and not different between groups (TE, 13.7 ± 5.6; TPEL, 11.3 ± 2.9 μg/L). This elevation was retained after adjustment for body mass index in the normal control group [TE, 1.45 ± 0.51 SD score ($P < 0.0001$); TPEL, 0.98 ± 0.35 SD score ($P < 0.0008$)]. During T substitution serum OB was completely normalized (trough levels: TE, 4.6 ± 1.0 μg/L; TPEL, 4.3 ± 0.9 μg/L). In multiple regression analysis, the androgen (T plus DHT)/estrogen ratio was the only significant determinant of OB levels ($r = -0.32$; $P < 0.01$). At baseline, OB levels did not correlate with body mass index, but during substitution, the correlation was considerably improved.

We conclude that hypogonadal men exhibit elevated OB levels that are normalized by substitution with T. The only determinant of OB levels was the androgen/estrogen ratio, indicating a major influence of sex steroids on OB production. The interaction of T and OB might be part of a hypothalamic-pituitary-gonadal-adipose tissue axis that is involved in body weight maintenance and reproductive function. (*J Clin Endocrinol Metab* **82:** 2510–2513, 1997)

LEPTIN (OB), the 167-amino acid peptide product of the *ob* gene (1), is highly specifically produced in white and brown adipose tissue (2). Recent studies in rodents suggest that OB is a feedback signal from adipose tissue to the hypothalamus, suppressing food intake and stimulating energy expenditure (3, 4). OB acts through a specific receptor (5) and inhibits synthesis of the appetite stimulant neuropeptide Y (NPY) (6). Mutations in the genes for OB or the OB receptor cause phenotypically identical forms of massive obesity by inhibiting the action of OB (5, 7).

Initial studies in humans show that serum OB levels are most closely correlated with the percentage of body fat (8, 9). Nevertheless, men have lower serum OB concentrations than women with similar body mass indexs (BMIs) (8–11). As the factors responsible for these differences are unknown, we undertook the present work to shed light on the effect of testosterone (T) on OB levels in men by substituting T in hypogonadal men.

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Subjects and Methods

Patients and study design

In this single center study 22 men with hypogonadism, as confirmed by 2 determinations of serum T yielding serum concentrations less than 3.6 nmol/L (normal, >10 nmol/L), were randomly assigned to T enanthate (TE; Testoviron depot 250, Schering, Berlin, Germany) or T pellets (TPEL), a single sc implantation of 6 testosterone pellets each containing 200 mg crystalline T (Organon, Oss, The Netherlands; Table 1) (12). Previous testosterone medication (TE or T undecanoate) had been suspended at least 3 months before the study began. On days 0, 21, 42, 63, 84, 105, 126, 147, 168, and 189, blood samples were drawn, and TE injections were administered. Study medication lasted until day 210, and follow-up visits were conducted on days 246 and 300. Serum samples for OB analysis were available for days 0, 42, 84, 126, 168, and 300. BMI was assessed on days 0, 105, and 300. All men gave written informed consent; the study was approved by the ethics committee of the University of Essen and followed the guidelines of the Declaration of Helsinki 1975.

Hormone assays

Hormones were measured by commercially available immunoassays; T, sex hormone-binding globulin (SHBG), and 17β-estradiol (E_2) were

TABLE 1. Patients characteristics at baseline (mean ± SEM)

	TE	T-Pellets
Number	10	12
Age (yr)	28.96 ± 2.30[a]	36.27 ± 3.29
BMI (kg/m^2)	24.76 ± 1.10	26.33 ± 0.86
Type of hypogonadism (primary/secondary)	4/6	5/7
T (nmol/L)	1.57 ± 0.40	1.90 ± 0.33
DHT (nmol/L)	1.70 ± 0.39	1.25 ± 0.18
E2 (pmol/L)	69.7 ± 7.2	71.4 ± 12.4
SHBG (nmol/L)	39.7 ± 7.0	45.6 ± 7.0
Cholesterol (mg/dL)	160.7 ± 6.2[a]	188.7 ± 10.8
LDL-Cholesterol (mg/dL)	73.9 ± 3.7[a]	97.2 ± 11.3
HDL-Cholesterol (mg/dL)	69.3 ± 6.9	74.1 ± 3.4
Triglycerides (mg/dL)	87.3 ± 7.8[a]	122.0 ± 11.6
Leptin (μg/L)	13.70 ± 5.62	11.30 ± 2.87

The type of hypogonadism refers to primary *vs.* secondary hypogonadism. Significant differences between groups were detected for age, estradiol (E2), cholesterol, LDL-cholesterol, and triglycerides.
[a] $P < 0.05$.

determined by RIA (Diagnostic Products Corp., Los Angeles, CA), and 5α-dihydrotestosterone (DHT) was determined by RIA after oxidative destruction of T (Amersham, Braunschweig, Germany). Inter- and intraassay variations were below 8% for all assays except DHT (17%) (12).

OB was measured by a specific RIA as previously described (13). Recombinant human OB (gift from Dr. Heiman, Eli Lilly Research Laboratories, Indianapolis, IN) was used for production of antiserum in rabbits and for preparation of tracer by the chloramine-T method (14) and of standards. The assay buffer was composed of 0.05 mol/L sodium phosphate (pH 7.4), 0.1 mol/L NaCl, 0.05% (wt/vol) NaN_3, 0.1% (vol/vol) gelatin from teleost fish (Sigma, Munich, Germany), and 0.1% (vol/vol) Triton X-100. The assay volume was 0.3 mL. After incubation at room temperature overnight, bound and unbound tracers were separated using the second antibody technique (14). Maximal binding of tracer was 37–45%, and half-maximal binding occurred at 0.9 μg/L unlabeled OB. Excellent parallelism was obtained with serial dilutions of human serum, and spiking experiments with 0.1 ng/tube yielded a recovery of 97 ± 2.1%. The sensitivity was 0.03 μg/L, and the intra- and interassay variations were 0.8% and 8.5%, respectively.

With this assay system a normal reference range of serum OB levels was established based on the analysis of sera from 393 healthy men, which proved to be BMI, but not age, dependent. The best-fit regression line for the 50th percentile was an exponential curve: $OB = 0.0237 \times e^{(0.1985 \times BMI)}$. The corresponding equations for the 5th and 95th percentiles were: $OB = 0.0237 \times e^{(0.1985 \times BMI - 1.0473)}$ and $OB = 0.0237 \times e^{(0.1985 \times BMI + 1.0473)}$, respectively. To adjust for BMI, OB levels were converted to SD scores by the following equation: OB SD score = [ln (OB) − ln (0.0237) − (0.1985 × BMI)]/0.6386, taking the log normal distribution of serum OB levels into account.

Statistical analysis

Results are reported as the mean ± SE. The androgen/estrogen ratio (A/E ratio) was derived by dividing the total androgen concentration (T plus DHT; nanomoles per L) by the estrogen concentration (picomoles per L). The area under the hormone curve was calculated by the trapezoidal rule. Statistical comparisons use a paired *t* test or repeated measures ANOVA as appropriate, with the level of significance set at $P < 0.05$ (by Student-Newman-Keuls test) using the SPSS (SPSS, Chicago, IL) software package. Changes in BMI (kilograms of body weight divided by height in meters squared) were tested with the Wilcoxon matched pairs sign test. Pearson's correlation analysis and multiple regression analysis were applied to assess the relation of hormone parameters and BMI to OB concentrations.

Results

Hormones

In both groups, serum T was normalized during substitution, with average T levels of 14.33 ± 2.63 nmol/L (days 21–189) in the TE group and 24.98 ± 1.64 nmol/L in the TPEL group (Fig. 1). Whereas implantation of T pellets produced large increases in DHT and E_2, this was less pronounced in the TE group: DHT, days 21–189: TE, 4.20 ± 0.57 nmol/L; TPEL, 5.11 ± 0.56; E_2, days 21–189: TE, 95.6 ± 7.3 pmol/L; TPEL, 102.1 ± 6.1; Fig. 1). Accordingly, the A/E ratio was elevated in both groups, and serum sex hormone-binding globulin levels decreased in response to T substitution in both groups (Fig. 1). Due to the study design, with the final TE injection on day 189, all changes were more prolonged in the TE group.

Leptin

At baseline, mean OB levels for all 22 hypogondal men were 12.39 ± 2.93 μg/L and significantly elevated ($P < 0.01$) compared to the BMI-dependent normal range for men (+1.20 ± 0.20 SD score). For normal men with similar BMI, a mean OB level of 4.28 ± 0.52 μg/L (95th percentile, 12.19 ± 1.48 μg/L) was calculated (Fig. 2). In 7 of 22 hypogonadal men, OB levels exceeded the 95th percentile of the normal range. Between treatment groups no significant differences

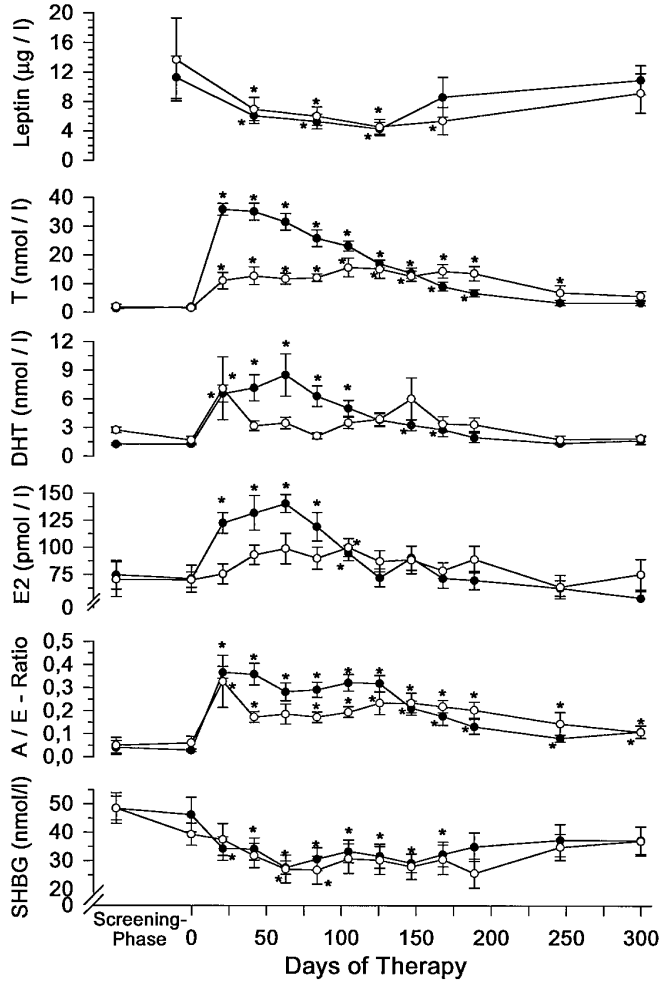

FIG. 1. Hormonal parameters (mean ± SEM) in the TE (*open symbols*) and TPEL (*closed symbols*) groups. The A/E ratio was calculated by dividing the sum of androgens (T plus DHT) by estradiol. *, Significantly different from baseline, by ANOVA ($P \leq 0.05$).

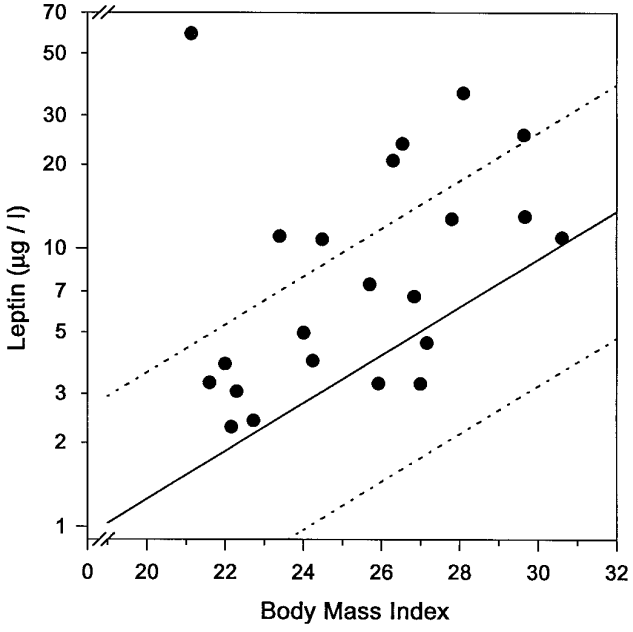

FIG. 2. Baseline serum OB levels in the 22 hypogonadal men compared to the normal range established with 393 normal men [mean (*solid line*) and 5th and 95th percentiles (*stippled lines*)].

in OB levels were observed (TE, 13.70 ± 5.62; TPEL, 11.30 ± 2.87 µg/L).

With substitution of T, a highly significant decrease in serum OB levels occurred in both groups ($P < 0.01$), which was more prolonged in the TE group (Fig. 1). Serum OB concentrations reached a nadir on day 126 with 4.61 ± 1.02 µg/L in the TE group and 4.34 ± 0.91 µg/L in the TPEL group, not different from their expected normal value. Parallel to the earlier decreases in serum T and the A/E ratio in the TPEL group compared to the TE group, OB levels started to increase in the TPEL group earlier than in the TE group (Fig. 1). After cessation of substitution, OB levels completely returned to the elevated baseline levels. There were now significant differences in OB levels between men with primary and secondary hypogonadism at any time point.

By single regression analysis, OB levels correlated significantly with all hormone parameters (Table 2). However, in multiple regression, the only factor significantly determining OB levels for both groups was the A/E ratio (TE, r = −0.30 and $P = 0.034$; TPEL, r = −0.34 and $P = 0.008$).

The BMI increased slightly during substitution in the TE group (day 0, 24.45 ± 0.85 kg/m^2; day 105, 24.86 ± 0.85; $P \leq 0.05$), but not in the TPEL group (day 0, 26.24 ± 0.80 kg/m^2; day 105, 26.54 ± 0.67; P = NS) and did return to baseline on day 300 (TE, 24.78 ± 0.83 kg/m^2; TPEL, 26.69 ± 0.72).

At baseline, serum OB levels (day 0) did not correlate with BMI on day 0 in either group (TE, r = −0.05 and $P = 0.89$; TPEL, r = 0.38 and $P = 0.22$) or when both groups were combined (r = 0.09; $P = 0.76$). Whereas the correlation was considerably improved in the TPEL group during and after substitution (OB on day 42 with BMI on day 105, r = 0.54 and $P = 0.085$; OB on day 300 with BMI on day 300, r = 0.60 and $P = 0.04$), in the TE group no significant correlation was observed.

TABLE 2. Correlation of OB serum concentrations (µg/L) with hormone parameters

Parameter	TE	T-Pellet
Testosterone	r = −0.32	r = −0.29
	$P = 0.016$	$P = 0.019$
DHT	r = −0.19	r = −0.26
	P = n.s.	$P = 0.048$
Androgens	r = −0.30	r = −0.30
	$P = 0.025$	$P = 0.017$
E2	r = −0.03	r = −0.14
	P = n.s.	P = n.s.
A/E Ratio	r = −0.30	r = −0.34
	$P = 0.014$	$P = 0.008$
SHBG	r = −0.17	r = −0.13
	P = n.s.	P = n.s.

Androgens refers to the sum of testosterone and DHT serum concentrations. The A/E ratio was calculated by dividing the androgen concentration by the estradiol concentration. r, coefficient of correlation; n.s., not significant.

Discussion

This is the first study demonstrating an influence of sex steroids on serum OB concentrations. With our assay system we established a BMI-dependent normal range for men. In this assay system, men with hypogonadism have 3-fold higher OB levels than normal men of similar BMI. The BMI is a good indicator of obesity and total amount of adipose tissue; therefore, the lack of information on the percent body fat in the hypogonadal men, which has not been determined in this study, does not degrade this observation. Furthermore, the finding of elevated OB levels in hypogonadal men compares with previous studies showing lower OB concentrations in men than in women of similar BMI, although the absolute OB levels were not reported (8, 9). This sex difference is maintained in obesity, as *ob* gene expression is 75% higher in obese women than in obese men (11).

The normalization of OB levels in hypogonadal men upon substitution of T indicates an important influence of sex steroids on the regulation of OB production. Due to its anabolic action, T increases lean body mass, but not adipose tissue, the source of OB (15, 16). Even extremely high doses of T do not change the percentage of body fat (15). Therefore, the 50% decline in serum OB levels observed during substitution of TE and TPEL cannot be accounted for by a possible decrease in the percent body fat. Thus, T substitution has lowered serum OB levels independently of its effects on the proportion of body fat, suggesting a regulatory role of T in OB production. The observation of similar pharmacodynamic effects on serum OB levels by the two T preparations with markedly different pharmacokinetics (12) is explained by the fact that the areas under the T *vs.* time curve might actually be quite similar for the two testosterone preparations when the initial supraphysiological levels of TE are considered. As blood was sampled only at the end of the injection interval, the serum T levels in the TE group are underestimated because they do not reveal the preceding high levels.

The mechanism by which T lowers OB production is unclear. Adipocytes specifically bind androgens (17) and appear to carry androgen receptors (18). Therefore, a direct suppressive effect of T on OB gene expression is possible. On

the other hand, indirect effects of androgens on adipocyte metabolism have been shown. *In vitro*, T enhances the lipolytic activity and the activity of adenylate cyclase of adipocytes by increasing the number of β-adrenergic receptors (17). The involvement of estrogens intracellularly derived from androgens is unlikely, because androgens and estrogens seem to have opposite effects on OB levels, and *in vitro* the effects of T on adipocytes are not influenced by aromatase inhibitors (17). Thus, T appears to directly cause the suppression of adipocyte OB production. The suppressive effect of T on OB might be the link between the frequent association of low serum T with visceral obesity (19, 20), which is an important risk factor for cardio-cerebrovascular disease and noninsulin-dependent diabetes mellitus (21, 22). The amount of intraabdominal fat mass is strongly negatively correlated with serum T levels (23), and T administration to men with visceral obesity is followed by an apparently region-specific decrease in abdominal adipose tissue (24). Possibly, hypogonadal men are in a state of OB resistance, which is corrected by the androgen-induced reduction of OB levels, thereby restoring responsiveness of the hypothalamus to OB action.

Impaired reproductive function due to hypothalamic dysfunction is a characteristic feature of all of the recessively transmitted obesities in rodents (25–27). Recent studies demonstrated that administration of recombinant OB during states of absent or low OB levels reverses or prevents the impairment of reproductive function (28, 29). Therefore, OB might be a modulator of reproductive function, signaling the central nervous system about the amount of fat tissue and thereby indicating reproductive adequacy, possibly by interaction with NPY (6). NPY has been shown to modulate GnRH release (30). In this case, sex steroids would be the natural corresponding part of the hypothalamic-pituitary-gonadal-adipose tissue axis, as is suggested by this study.

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