0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 83, No. 9
Printed in U.S.A.

Inverse Correlation between Serum Testosterone and Leptin in Men*

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ABSTRACT

Besides its role in the regulation of energy balance, leptin seems to be involved in linking energy stores to the reproductive system. A gender-dependent difference exists in plasma leptin concentration and leptin messenger ribonucleic acid expression in rodents and humans. This difference does not seem to be explained simply by differences in the amount of body fat between genders. To elucidate the relationship of endogenous testosterone and leptin, we studied the serum leptin concentrations in 269 elderly nondiabetic men. In addition, to assess whether exogenously administered testosterone could influence leptin production, we followed the serum levels of leptin in 10 healthy men during a 12-month treatment with 200 mg testosterone enanthate, im, weekly for contraceptive purposes. We found that the serum leptin concentration correlated inversely ($r = -0.39$; $P < 0.001$) with that of testosterone in elderly men. This inverse correlation was still present when body mass index and plasma insulin were included in the analysis. The administration of testosterone to young men suppressed serum leptin from the pretreatment level of 3.4 ± 1.4 to 1.9 ± 0.6 μg/L during the therapy. After cessation of testosterone injections, serum leptin concentration returned back to the pretreatment level. It is concluded that testosterone has a suppressive effect on leptin production, as reflected by circulating levels of this hormone. (*J Clin Endocrinol Metab* **83:** 3243–3246, 1998)

LEPTIN is a hormonal product of the *ob* gene secreted by adipocytes. It plays an important role in the regulation of energy balance by reducing food intake and increasing energy expenditure (1, 2). Leptin seems to signal metabolic information to the reproductive system, as leptin treatment results in earlier onset of puberty in normal female mice (3) and prompt return of fertility in congenitally infertile female *ob/ob* mice (4). Male *ob/ob* mice treated with leptin have elevated serum levels of FSH, increased testicular and seminal vesicle weights, greater seminal vesicle epithelial cell height, and elevated sperm counts compared to controls (5).

In both humans and rodents, males have lower plasma leptin concentrations than their female counterparts at any level of adiposity (6, 7). Leptin messenger ribonucleic acid expression is lower in obese men than in obese women (8) and is lower in obese boys than in obese girls (9). The gender difference in serum leptin concentration is well established (10–12), and *in vitro* results suggest that gonadal hormones, such as testosterone, may be important regulators of leptin secretion. A strong inverse association between serum levels of leptin and testosterone was recently reported in untreated and testosterone-treated hypogonadal men (13, 14). The effect of testosterone treatment on circulating leptin has been studied previously in adolescents with delayed puberty (15), but to date has not been examined in eugonadal men. To further elucidate the role of androgens on leptin production, we performed two studies in men. First, the relationship between circulating testosterone and serum leptin was studied in a cross-sectional survey of elderly nondiabetic men. Second, the effect of testosterone treatment on serum leptin was determined in a longitudinal study in eugonadal young men.

Subjects and Methods

Cross-sectional study in elderly men

A total of 269 men participated in a cross-sectional study investigating the health of elderly inhabitants of the city of Turku, Finland. Subjects were a representative 73% cohort of 71- to 72-yr-old men living in this area. The subjects visited local health care centers for routine medical examination and an oral glucose tolerance test and provided serum samples. At the time of the visit, the mean age of the men was 71.3 ± 0.5 yr (range, 71–72 yr). Their mean weight was 78.2 ± 10.7 kg, mean height was 1.75 ± 0.05 m, and mean body mass index (BMI) was 25.9 ± 3.6 kg/m^2. A 75-g oral glucose tolerance test was performed according to the WHO criteria on the morning after an overnight fast. Blood samples were collected at 0 and 120 min for the determination of plasma glucose and insulin concentrations. Men in the survey with diabetes mellitus or with an abnormal glucose tolerance test according to the WHO criteria were excluded from the present study. The study was approved by the local institutional ethical review committee.

Testosterone treatment in young men

The serum samples of 10 men participating in the WHO Task Force on Methods for the Regulation of Male Fertility were used to study the

Received February 26, 1998. Revision received June 3, 1998. Accepted June 12, 1998.

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* This work was supported by the Jahnsson Foundation (Helsinki, Finland).

effect of exogenous testosterone on serum leptin levels (16). The mean age was 31.3 ± 7.7 yr (range, 25–41 yr), the mean weight was 75.2 ± 10.7 kg, the mean height was 1.81 ± 0.06 m, and the mean BMI was 22.8 ± 2.1 kg/m^2. The participants had no concomitant diseases. Routine medical examinations were performed, and the men provided blood samples for testosterone, LH, and FSH measurements. The samples were also used for leptin and insulin determinations. The subjects received weekly injections of testosterone enanthate (200 mg, im, as 0.8 mL Testoviron-Depot 250, Schering, Berlin, Germany). Testosterone injections continued for 12 months after the subjects had become azoospermic, which usually occurred within 2–4 months. Before the treatment and every 3 months after the initiation of the testosterone injections, the participants were examined by a physician. Blood samples were taken twice before treatment (~1 month apart), every 3 months during treatment, and once after treatment. The protocol was approved by the WHO Toxicology Group, scientific and ethical review group, and the Secretariat committee on research involving human subjects. The study was also approved by the joint ethical committee of the University of Turku and Turku University Hospital. The men and their partners provided witnessed written informed consent.

Laboratory methods

Blood samples were collected after an overnight fast, and serum was separated after centrifugation and stored at −20 C until analyzed. In the study of the elderly men, blood glucose was determined by the hexokinase method. Serum leptin was measured with a human leptin RIA kit (Linco Research, St. Charles, MO), using a polyclonal antibody raised in rabbits against highly purified human leptin. The assay had a sensitivity of 0.5 μg/L. The interassay coefficient of variation (CV) was 6.5% at a mean leptin concentration of 2.9 μg/L and 9.9% at a mean concentration of 14.5 μg/L. Serum insulin was determined with a Phadeseph insulin RIA kit (Pharmacia, Uppsala, Sweden), with a detection limit of 2.5 mU/L. The intraassay CV was 0.5% at a serum insulin concentration of 13.3 mU/L, and the interassay CV was 6.4% at an insulin concentration of 20.1 mU/L. The assay procedures for the immunofluorometric assays of LH and FSH were performed as previously described (16). Testosterone was measured as previously described by Huhtaniemi *et al.* (17).

Statistical analyses

In the study of the elderly men, Pearson correlation coefficients and multiple linear regression analysis were used. In the study of young men, the statistical comparisons were performed with one-way ANOVA for repeated measurements and with Student's *t* test. $P < 0.05$ was considered statistically significant. The values given below are means with SDs. BMI was calculated by dividing body weight (kilograms) by square height (meters). Statistical analyses were performed with Statistical Analysis System software (SAS version 6.12, SAS Institute, Cary, NC).

Results

The cross-sectional study in elderly men

The mean serum leptin, testosterone, and insulin concentrations in the study population were 8.5 ± 5.5 μg/L, 20.7 ± 8.0 nmol/L, and 11.1 ± 6.8 IU/L, respectively. Pearson correlation coefficients among serum leptin, testosterone, insulin, and BMI are presented in Table 1. A significant inverse correlation was found between leptin and testosterone (r = −0.39; $P < 0.001$; Fig. 1). There were significant positive correlations between leptin and insulin and between leptin and BMI (r = 0.60; $P < 0.001$ and r = 0.50; $P < 0.01$, respectively). Furthermore, significant correlations were found between insulin and testosterone and between insulin and BMI (Table 1). To determine the independent contributions of testosterone, insulin, and BMI to the serum leptin concentration, they all were included in multiple linear regression analysis. These variables jointly explained a very high proportion (85%) of the total variability in serum leptin concentrations. Testosterone had a significant inverse (r = −0.176; SE = 0.034; $P < 0.001$), and both insulin and BMI had a significant positive effect on serum leptin (r = 0.389; SE = 0.054; $P < 0.001$ and r = 0.318; SE = 0.040; $P < 0.001$, respectively). A similar conclusion was reached when the partial correlation between testosterone and leptin concentrations, adjusted for insulin concentration or BMI, was calculated (r = −0.27; $P < 0.0001$ and r = −0.33; $P < 0.001$, respectively).

TABLE 1. Pearson correlation coefficients between hormone concentrations in plasma or serum and BMI in elderly men

	Testosterone	Insulin	BMI
Leptin	r = −0.39[a] n = 269	r = 0.60[a] n = 262	r = 0.50[a] n = 187
Testosterone		r = −0.30[a] n = 262	r = −0.19[b] n = 187
Insulin			r = 0.33[a] n = 182

[a] $P < 0.001$.
[b] $P < 0.01$.

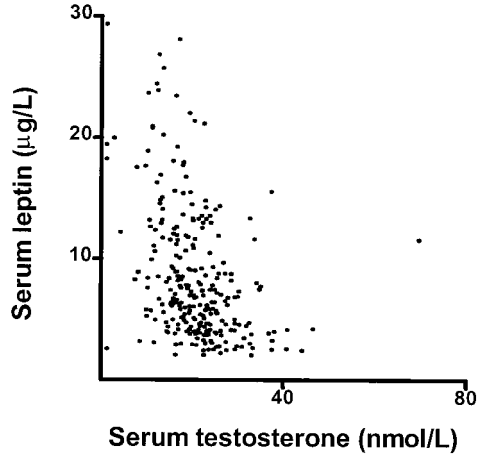

FIG. 1. The relationship between serum leptin and testosterone in elderly men (r = −0.39; $P < 0.001$; n = 269).

Testosterone treatment in young men

There was a significant correlation between serum leptin and testosterone concentrations (r = −0.48; $P < 0.001$; Fig. 2). Testosterone injections significantly reduced serum leptin ($P = 0.002$, by ANOVA for repeated measurements), LH ($P < 0.001$), and FSH ($P < 0.001$) concentrations, whereas BMI ($P = 0.071$) and serum insulin ($P = 0.195$) remained unchanged (Fig. 2 and Table 2). There was a 44% decrease in the mean serum leptin concentration from 3.4 ± 1.4 μg/L before treatment to 1.9 ± 0.6 μg/L during testosterone treatment ($P < 0.005$, by paired *t* test). Three months after the cessation of testosterone treatment, plasma leptin levels had returned to the basal level. There was a 2.7-fold increase in the mean serum testosterone concentration from 25.1 ± 7.9 to 67.0 ± 18.4 nmol/L during treatment ($P < 0.001$). Pretreatment concentrations of serum testosterone were also reached 3 months after discontinuing testosterone injections. Reduced serum

LH and FSH concentrations were only partially normalized, especially in the case of LH, after the cessation of therapy.

Discussion

In the cross-sectional study of elderly men, we found an inverse correlation between serum leptin and testosterone, suggesting that testosterone may down-regulate serum leptin. However, because of the concomitant correlations among testosterone, insulin, and BMI, no firm conclusions on causalities could be drawn. Nevertheless, all of these variables were significant predictors of the serum leptin concentration when included in the multivariate analysis model, and the partial correlation between leptin and testosterone was significant even after adjusting for BMI or serum insulin.

The existence of such a relationship between testosterone and leptin was further strengthened by the prospective study in young males in whom serum leptin was reduced after testosterone injections and increased again after the discontinuation of the treatment. Our findings suggest that testosterone, either directly or indirectly, suppresses serum leptin in the normal male population.

This is in keeping with earlier findings in subgroups of male patients. Intramuscular testosterone treatment decreased plasma leptin levels in female to male transsexuals (18). Testosterone supplementation has been reported to normalize otherwise elevated plasma leptin levels in both young and old hypogonadal men (19, 20) without concomitant changes in body habitus, body fat content, or BMI (20). An inverse association of leptin and testosterone has also been reported in both untreated and testosterone-treated hypogonadal men (14) and in young men with insulin-dependent diabetes mellitus (21). Similarly, besides confirming a strong correlation between leptin and BMI and a gender difference in the levels of leptin, a recent population-based study reported an inverse correlation between leptin and testosterone that was independent of BMI (22).

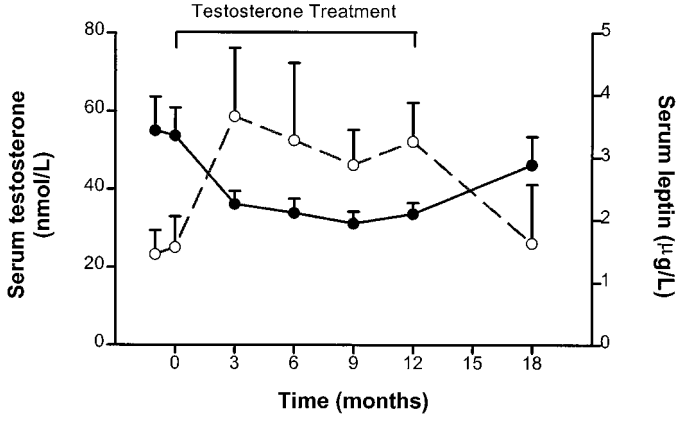

FIG. 2. Serum leptin (●) and testosterone (○) concentrations during testosterone treatment in young men. The first measurements were performed at admission, 3 wk before the basal samples (time zero) were collected. The *error bars* present the SD.

Significant correlations between serum testosterone and leptin have not been found in all published studies. After adjusting for BMI, Haffner *et al.* found no correlation between leptin and sex hormones in a cross-sectional study of middle-aged normoglycemic men with asymptomatic atherosclerosis (23). In a study of elderly hypogonadal men, circulating leptin levels correlated strongly with BMI and body fat, but not with age, testosterone, or bioavailable testosterone (20). The reason for the diverging results is not known, but considering the complexity of factors affecting the regulation of leptin secretion, the heterogeneity of the populations studied may be a contributing factor.

One confounding factor in determining the testosterone-leptin relationship is the concentration of sex hormone binding globulin (SHBG), which may influence both total and free testosterone levels. SHBG increases with age (24). In our study, the effect of age has been controlled by using subjects in a narrow age range. As SHBG is reduced in obese individuals (24, 25), obese men with the highest leptin levels may also have relatively low SHBG concentrations. SHBG is known to be positively correlated with total testosterone and inversely correlated with the percent free testosterone (24). Therefore, the fraction of free testosterone might have been greater than anticipated in obese subjects, and the correlation between testosterone and leptin might be even steeper than that presented in Fig. 1.

Old age in men is accompanied by a decrease in the testosterone concentration (26) and changes in body composition. Therefore, age might also be an important secondary regulator

TABLE 2. Testosterone treatment: a) serum hormone concentrations and BMI before testosterone administration, b) maximal effect during testosterone treatment, and c) hormone concentrations and BMI 3 months after discontinuation of testosterone treatment

	a	b	c	Paired *t* test
Leptin (μg/L)	3.4 ± 1.4	1.9 ± 0.6	3.7 ± 2.2	a *vs.* b, $P < 0.005$ a *vs.* c, $P = 0.35$ b *vs.* c, $P < 0.009$
Testosterone (nmol/L)	25.1 ± 7.9	67.0 ± 18.4	26.2 ± 13.7	a *vs.* b, $P < 0.001$ a *vs.* c, $P = 0.93$ b *vs.* c, $P < 0.001$
LH (IU/L)	7.3 ± 2.8	0.5 ± 0.7	3.9 ± 1.5	a *vs.* b, $P < 0.001$ a *vs.* c, $P < 0.01$ b *vs.* c, $P < 0.001$
FSH (IU/L)	4.1 ± 3.0	0.5 ± 0.1	1.9 ± 1.5	a *vs.* b, $P < 0.005$ a *vs.* c, $P = 0.10$ b *vs.* c, $P < 0.028$
BMI (kg/m^2)	22.8 ± 2.1	24.0 ± 1.8	23.3 ± 1.5	[a]
Insulin (IU/mL)	8.3 ± 2.8	6.22 ± 1.5	7.5 ± 1.7	[a]

Values are presented as the mean ± SD.
[a] Changes in BMI and insulin concentration were nonsignificant during the testosterone treatment (by ANOVA for repeated measurements).

of plasma leptin. In rats, leptin gene expression increases with age, independently of increasing adiposity (27). In humans, there are contradicting results of age dependence of the plasma leptin concentration. Ostlund *et al.* reported circulating leptin to be inversely related to age even after adjustment for percent body fat and gender (28). In contrast, Roberts *et al.* found no effect of age on the relationship between circulating leptin and body fat mass in weight-stable nonobese men and women aged 18–81 yr (29). It is noteworthy that any possible effect of age was eliminated in our study due to the narrow age range of the elderly subjects.

In our study, neither BMI nor serum insulin changed in testosterone-treated young men. As BMI does not discriminate between fat-free and fat mass, it cannot separate a muscular person from a fatty one. Nevertheless, BMI is easy to measure, it is usually also available retrospectively, and it is the most precise measure of short term longitudinal changes in body fat in a single person. In an earlier study in which weekly testosterone injections were used for contraceptive purposes in young men, fat mass decreased by 16% after 6 months of treatment (30). Therefore, the decline in serum leptin levels observed after repeated testosterone injections in our study could also be a phenomenon secondary to a decrease in body fat.

Adipocytes specifically bind androgens (31) and appear to carry androgen receptors (32). Administration of testosterone increases lipolysis and the number of β-adrenoceptors in male rat adipocytes (33). Recently, it was reported that testosterone and its active metabolite dihydrotestosterone were able to suppress leptin secretion and leptin messenger ribonucleic acid in primary culture of human adipocytes, suggesting a direct effect of testosterone at the level of adipocytes (9). The mechanism by which testosterone exerts this negative effect is currently unclear.

In summary, we found an inverse association between leptin and testosterone both in a population-based study of elderly men and in young eugonadal men. Furthermore, the plasma leptin concentration was clearly suppressed during testosterone treatment and returned to the pretreatment level after cessation of testosterone treatment without concomitant changes in either BMI or serum insulin. The association between serum testosterone and leptin was still significant after adjusting for BMI and insulin. The mechanism underlying this effect of testosterone remains to be elucidated. Testosterone is probably one of the most important factors contributing to the lower serum leptin levels in men compared with women.

Acknowledgments

We are grateful to Ms. Raija Kaartosalmi, Ms. Elina Kahra, and Ms. Taina Lehti for their skillful technical assistance.

References

1. **Matson CA, Wiater MF, Weigle DS.** 1996 Leptin and the regulation of body adiposity. Diabetes Rev. 4:488–508.
2. **Bray GA, York DA.** 1997 Leptin and clinical medicine: a new piece in the puzzle of obesity. J Clin Endocrinol Metab. 82:2771–2776.
3. **Chebab FF, Mounzih K, Lu R, Lim ME.** 1997 Early onset of reproductive function in normal female mice treated with leptin. Science. 275:88–90.
4. **Chehab FF, Lim ME, Lu R.** 1996 Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet. 12:318–320.
5. **Barash IA, Cheung CC, Weigle DS, et al.** 1996 Leptin is a metabolic signal to the reproductive system. Endocrinology. 137:3144–3147.
6. **Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS.** 1995 Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat Med. 1:1311–1314.
7. **Saad MF, Damani S, Gingerich RL, et al.** 1997 Sexual dimorphism in plasma leptin concentration. J Clin Endocrinol Metab. 82:579–584.
8. **Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S.** 1996 Depot- and sex-spesific differences in human leptin mRNA expression. Diabetes. 46:342–347.
9. **Wabitsch M, Blum WF, Muche R, et al.** 1997 Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J Clin Invest. 100:808–813.
10. **Rosenbaum M, Nicolson M, Hirsch J, et al.** 1996 Effects of gender, body composition, and menopause on plasma concentrations of leptin. J Clin Endocrinol Metab. 81:3424–3427.
11. **Hickey MS, Israel RG, Gardiner SN, et al.** 1996 Gender differences in serum leptin levels in humans. Biochem Mol Med. 59:1–6.
12. **Kennedy A, Gettys TW, Watson P, et al.** 1997 The metabolic significance of leptin in humans: gender-based differences in relationship to adiposity, insulin sensitivity, and energy expenditure. J Clin Endocrinol Metab. 82:1293–1300.
13. **Bhasin S, Storer TW, Berman N, et al.** 1997 Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. J Clin Endocrinol Metab. 82:407–413.
14. **Behre HM, Simoni M, Nieschlag E.** 1997 Strong association between serum levels of leptin and testosterone in men. Clin Endocrinol (Oxf). 47:237–240.
15. **Arslanian S, Suprasongsin C.** 1997 Testosterone treatment in adolescents with delayed puberty: changes in body composition, protein, fat, and glucose metabolism. J Clin Endocrinol Metab. 82:3213–3220.
16. **WHO task force on methods for the regulation of male fertility.** 1990 Contraceptive efficacy of testosterone-induced azoospermia in normal men. Lancet. 336:955–959.
17. **Huhtaniemi I, Nikula H, Rannikko S.** 1985 Treatment of prostatic cancer with a gonadotropin-releasing hormone agonist analog: acute and long term effects on endocrine functions of testis tissue. J Clin Endocrinol Metab. 61:698–704.
18. **Elbers JMH, Asschemann H, Seidell JC, Frölich M, Meinders AE, Gooren LJG.** 1997 Reversial of the sex difference in serum leptin levels upon cross-sex hormone administration in transsexuals. J Clin Endocrinol Metab. 82:3267–3270.
19. **Jockenhövel F, Blum WF, Vogel E, et al.** 1997 Testosterone substitution normalizes elevated serum leptin levels in hypogonadal men. J Clin Endocrinol Metab. 82:2510–2513.
20. **Sih R, Morley JE, Kaiser FE, Perry III HM, Patrick P, Ross C.** 1997 Testosterone replacement in older hypogonadal men: a 12-month randomized controlled trial. J Clin Endocrinol Metab. 82:1661–1667.
21. **Tuominen JA, Ebeling P, Stenman U-H, Heiman ML, Stephens TW, Koivisto VA.** 1997 Leptin synthesis is resistant to acute effects of insulin in insulin-dependent diabetes mellitus patients. J Clin Endocrinol Metab. 82:381–382.
22. **Nyström F, Ekman B, Österlund M, Lindström T, Öhman KP, Arnqvist HJ.** 1997 Serum leptin concentrations in a normal population and in GH deficiency: negative correlation with testosterone in men and effects of GH treatment. Clin Endocrinol (Oxf). 47:191–198.
23. **Haffner SM, Miettinen H, Karhapää P, Mykkänen L, Laakso M.** 1997 Leptin concentrations, sex hormones, and cortisol in nondiabetic men. J Clin Endocrinol Metab. 82:1807–1809.
24. **Longcope C, Goldsfield SRW, Brambilla DJ, McKinlay J.** 1990 Androgens, estrogens, and sex hormone-binding globulin in middle-aged men. J Clin Endocrinol Metab. 71:1442–1446.
25. **Jarow JP, Kirkland J, Koritnik DR, Cefalu WT.** 1993 Effect of obesity and fertility status on sex steroid levels in men. Urology. 42:171–174.
26. **Vermeulen A.** 1990 Androgens in the aging male. J Clin Endocrinol Metab. 73:221–224.
27. **Li H, Matheny M, Nicolson M, Tumer N, Scarpace PJ.** 1997 Leptin gene expression increases with age independent of increasing adiposity in rats. Diabetes. 46:2035–2039.
28. **Ostlund REJ, Yang JW, Klein S, Gingerich R.** 1996 Relation between plasma leptin concentration and body fat, gender, diet, age and metabolic covariates. J Clin Endocrinol Metab. 81:3909–3913.
29. **Roberts SB, Nicholson M, Staten M, et al.** 1997 Relationship between circulating leptin and energy expenditure in adult men and women aged 18 years to 81 years. Obesity Res. 5:459–463.
30. **Young NR, Baker HW, Liu G, Seeman E.** 1993 Body composition and muscle strength in healthy men receiving testosterone enanthate for contraception. J Clin Endocrinol Metab. 77:1028–1032.
31. **Xu X, De Pergola G, Björntorp P.** 1990 The effects of androgens on the regulation of lipolysis in adipose precursor cells. Endocrinology. 126:1229–1234.
32. **Sjögren J, Li M, Björntorp P.** 1995 Androgen hormone binding to adipose tissue. Biochim Biophys Acta. 1244:117–120.
33. **Xu X, De Pergola G, Björntorp P.** 1991 Testosterone increases lipolysis and the number of β-adrenoceptors in male rat adipocytes. Endocrinology. 128:379–382.