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Reversal of the Sex Difference in Serum Leptin Levels upon Cross-Sex Hormone Administration in Transsexuals*

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ABSTRACT

Women have higher circulating leptin levels than men. This sex difference is not simply explained by differences in the amount of body fat and is possibly influenced by their different sex steroid milieus. This prompted us to study prospectively the effects of cross-sex steroid hormones on serum leptin levels in 17 male to female transsexuals and 15 female to male transsexuals. Male to female transsexuals were treated with 100 μg ethinyl estradiol and 100 mg cyproterone acetate (antiandrogen) daily, and female to male transsexuals received testosterone esters (250 mg/2 weeks, im). Before and after 4 and 12 months of cross-sex hormone treatment, serum leptin levels and measures of body fatness were assessed. Before treatment, female subjects had higher serum leptin levels than male subjects independently of the amount of body fat ($P < 0.01$). Cross-sex hormone administration induced a reversal of the sex difference in serum leptin levels. Estrogen treatment in combination with antiandrogens in male subjects increased median serum leptin levels from 1.9 ng/mL before treatment to 4.8 ng/mL after 4 months and 5.5 ng/mL after 12 months of treatment ($P < 0.0001$). Testosterone administration in female subjects decreased median serum leptin levels from 5.6 to 2.6 ng/mL after 4 months and to 2.5 ng/mL after 12 months ($P < 0.0001$). Analysis of covariance revealed that the changes in serum leptin levels were independent of changes in body fatness in both groups ($P < 0.01$).

In conclusion, these results indicate that sex steroid hormones, in particular testosterone, play an important role in the regulation of serum leptin levels. The prevailing sex steroid milieu, not genetic sex, is a significant determinant of the sex difference in serum leptin levels. (*J Clin Endocrinol Metab* **82:** 3267–3270, 1997)

LEPTIN IS the product of the *ob* gene, which is expressed in adipocytes (1, 2). Several studies in rodents suggest that leptin acts as a signaling factor from the adipose tissue to the central nervous system, regulating food intake and energy expenditure (2, 3). It is hypothesized that via this leptin feedback loop, homeostasis of body weight and a constant amount of body fat are achieved (4). In humans, a strong positive correlation is observed between serum leptin levels and the amount of body fat (5, 6). However, a large variation in serum leptin levels exists between individuals with the same degree of adiposity. This suggests that factors other than body fat also regulate leptin levels. Furthermore, women have higher circulating leptin levels than men (5–12). This sex difference in serum leptin levels is not simply explained by differences in the amount of body fat between the sexes (7–13). To investigate whether sex steroid hormones influence serum leptin levels, we studied prospectively 32 young and nonobese transsexuals before and during cross-sex hormone treatment.

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Subjects and Methods

Subjects

The study was conducted in transsexuals undergoing sex reassignment following a standard protocol of cross-sex hormone administration. Transsexuals are not different from nontranssexual men or women in their endocrine or metabolic functions. All subjects were eugonadal and healthy, as assessed by medical history, physical examination, and relevant laboratory data. They had not been treated with sex steroid hormones before the start of the study, and no other medication was used.

In this study, 17 male to female (M-F) transsexuals participated, with a mean (±SD) age of 26 ± 7 yr (range, 18–37 yr) and a mean body mass index of 20.5 ± 2.7 kg/m^2 (range, 16.1–24.5 kg/m^2). They were treated with 100 μg ethinyl estradiol (Lynoral, Organon, Oss, The Netherlands) and 100 mg cyproterone acetate (an antiandrogen; Androcur, Schering, Berlin, Germany) daily.

Fifteen female to male (F-M) transsexuals, with a mean age of 23 ± 5 yr (range, 16–34 yr) and a mean body mass index of 21.1 ± 3.3 kg/m^2 (range, 16.6–29.0 kg/m^2), were treated with im injections of 250 mg testosterone esters/2 weeks (Sustanon 250, Organon). All subjects were studied before and during 12 months of cross-sex hormone administration. This study was approved by the ethical review board of the Hospital Vrije Universiteit in Amsterdam, and all subjects gave their informed consent.

Anthropometry and body fat distribution

Height was measured to the nearest 0.1 cm, and weight was recorded to the nearest 0.1 kg with subjects wearing only underwear. Four skinfold thicknesses (triceps, biceps, subscapula, and suprailiac) were measured in triplicate using a Harpenden caliper at the left side of the body

with subjects in the upright position. Body fat (in kilograms) was calculated using the sum of four skinfolds according to the method of Durnin and Womersley (14). The bioelectrical impedance method was used to estimate the amount of body fat. Whole body resistance of an electric current (50 kHz and 800 μA) was assessed using a tetrapolar portable BIA 101 analyzer (RJL Systems, Detroit, MI). Subjects were in the supine position with the limbs abducted from the body, and the percentage of body fat was calculated with use of the manufacturer's equation.

Anthropometric measurements were performed in the morning between 0900–1000 h after an overnight fast before and after 4 and 12 months of cross-sex hormone treatment by the same experienced observer.

Before and after 12 months of treatment, the imaging technique based on magnetic resonance was used to quantify fat depots. An inversion recovery pulse sequence was used, and parameters were selected to obtain good image contrast between fat and other tissues. In all subjects, image acquisition before and after 12 months of treatment was performed on the same imager using the same parameters. Transverse magnetic resonance images were obtained at the level of the abdomen (lower edge of the umbilicus, three images), the hip (upper margin of the great trochanters, two images), and the thigh (just below the gluteal fold, two images). Image analysis was performed using an image-analyzing computer program, as described in more detail by Elbers *et al.* (15). The average of the two or three images per body region was used in the statistical analysis. The sum of the sc fat areas (in square centimeters) at the level of the abdomen, hip, and thigh was calculated.

Serum analyses

In all subjects, venous blood samples were taken in the morning between 0900–1000 h after an overnight fast at baseline and again after 4 and 12 months of cross-sex hormonal treatment. Serum leptin levels were measured in samples stored at −20 C using a recently developed RIA (Linco Research, St. Charles, MO; in nanograms per mL), as described by Ma *et al.* (12). RIAs were used to determine serum testosterone levels (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA; in nanomoles per L; lower limit of detection, 1.0 nmol/L) and 17β-estradiol levels (double antibody; Sorin Biomedica, Saluggia, Italy; in picomoles per L; lower limit of detection, 90 pmol/L). Sex hormone-binding globulin levels were measured by an immunoradiometric assay (Orion Diagnostica, Espoo, Finland; in nanomoles per L).

Statistics

Values are presented as the mean ± SD or medians and ranges. Analysis of covariance was performed using the mixed procedure by SAS Statistical Systems (version 6.11 for Windows, SAS Institute, Cary, NC). We used a statistical model with log-transformed serum leptin levels as the dependent variable, measures of adiposity or body fat distribution as independent variables, and factors defined as genetic sex (0 or 1; same before and during treatment) and hormonal sex (0 or 1; varying before and after the start of treatment according to circulating sex steroid hormone levels). ANOVA for repeated measurements was used to test changes within groups. $P < 0.05$ was considered statistically significant.

Results

Table 1 shows the subjects' characteristics and the changes in different variables during cross-sex hormone administration. Before hormonal treatment, M-F transsexuals and F-M transsexuals were similar in body mass index, but differed significantly in the amount of body fat. A sex difference in serum leptin levels was observed; at baseline, female subjects had higher serum leptin levels than male subjects even after adjusting for differences in the amount of body fat (by analysis of covariance, sum of skinfolds: $F = 133.8$; $P < 0.0001$; genetic sex: $F = 8.7$; $P = 0.006$; body fat in kilograms, sc fat areas and visceral fat areas by magnetic resonance imaging, and abdominal and gluteal fat cell diameters, $P < 0.01$; data not shown). The slopes of the regressions between serum leptin levels and all measures of body fatness were significantly higher in female subjects than in male subjects ($P < 0.001$; data not shown).

Estrogen and antiandrogen treatment in M-F transsexuals resulted in gradual increases in body weight and the amount of body fat (Table 1). Serum leptin levels increased during treatment in M-F transsexuals (Table 1; $F = 56.8$; $P < 0.0001$, by ANOVA for repeated measurements). Compared to baseline, the median increase in serum leptin levels was 180% after 4 months and 164% after 12 months of treatment.

Through its anabolic action, testosterone administration in F-M transsexuals resulted in a marked increase in body weight, with significant decreases in sc fat depots after 12 months of treatment (Table 1). Using both the skinfold method and bioimpedance, no significant change in the amount of body fat was observed after 4 months of treatment in F-M transsexuals. The decrease in the amount of body fat after 12 months of treatment was significant when measured by bioimpedance ($P < 0.05$ paired sample *t* test *vs.* baseline).

TABLE 1. Characteristics of 17 M-F transsexuals and 15 F-M transsexuals before, after 4 months, and after 12 months of cross-sex hormone administration

Variable	M-F transsexuals			F-M transsexuals		
	Before	4 months	12 months	Before	4 months	12 months
Wt (kg)	64.8 ± 12.2	67.6 ± 11.6	68.7 ± 11.8[a]	59.3 ± 11.8	63.2 ± 11.8	62.3 ± 11.4[a]
BMI (kg/m^2)	20.5 ± 2.7	21.4 ± 2.7	21.7 ± 2.8[a]	21.1 ± 3.3	22.5 ± 3.2	22.2 ± 3.0[a]
Body fat (skinfolds, kg)	9.6 ± 4.7	12.4 ± 4.2	13.4 ± 4.5[a]	16.8 ± 7.1	17.7 ± 6.7	16.2 ± 6.4[b]
Body fat (bioimpedance, kg)	9.1 ± 2.9	11.4 ± 3.1	12.6 ± 3.2[a]	16.3 ± 6.3	16.3 ± 5.9	15.2 ± 6.0[b]
Sc fat area (cm^2)[c]	294 ± 149		481 ± 156[a]	549 ± 218		436 ± 180[a]
Sum of skinfolds (mm)	29.4 (18.2–55.7)	45.1 (26.8–58.1)	48.8[a] (26.1–76.3)	54.2 (24.6–105.3)	54.0 (20.5–96.9)	41.2[a] (24.0–85.2)
Leptin (ng/mL)	1.9 (1.2–3.6)	4.8 (2.4–10.1)	5.5[a] (2.0–11.3)	5.6 (1.9–23.5)	2.6 (1.6–11.2)	2.5[a] (1.3–7.6)
Testosterone (nmol/L)	21 ± 6	1 ± 1	1 ± 0[a]	2 ± 1	27 ± 11	30 ± 11[a]
17β-Estradiol (pmol/L)[d]	96 ± 12	92 ± 7	90 ± 0	157 ± 53	120 ± 32	126 ± 30[b]
SHBG (nmol/L)	35 ± 14	242 ± 47	258 ± 40[a]	60 ± 29	27 ± 12	26 ± 10[a]

Values are expressed as the mean ± SD or median, the range is *in parentheses.*
[a] $P < 0.001$ (changes within groups by ANOVA for repeated measurements).
[b] $P < 0.05$ (changes within groups by ANOVA for repeated measurements).
[c] Sum of the sc fat areas at the level of the abdomen, hip, and thigh, as assessed by MRI.
[d] M-F transsexuals were treated with ethinyl estradiol, which could not be measured by our 17β-estradiol assay.

Testosterone administration in F-M transsexuals decreased median leptin levels by 50% after 4 months and by 61% after 12 months of treatment compared to the baseline (Table 1; $F = 40.8$; $P < 0.0001$, by ANOVA for repeated measurements). Cross-sex hormone administration induced a reversal of the sex difference in serum leptin levels (Fig. 1) and in the relation between serum leptin levels and measures of body fatness (see Fig. 2 for sum of skinfolds). In contrast to the relation at baseline, testosterone-treated F-M transsexuals had significant lower serum leptin levels than estrogen-treated M-F transsexuals with the same sum of skinfolds. Compared to baseline, the slope of the linear regression between the sum of skinfolds and serum leptin levels was significantly smaller in the testosterone-treated F-M transsexuals after 12 months of treatment and *vice versa* (Fig. 2). Results were essentially the same for all other measures of adiposity (body fat in kilograms, percentage of body fat measured by skinfold method and bioimpedance, abdominal and gluteal fat cell diameters, sc fat areas, and visceral fat areas; data not shown). Statistical analysis revealed that the most important determinants of serum leptin levels in our study were measures of adiposity and the prevailing sex hormone milieu, and not genetic sex (by analysis of covariance for repeated measurements, sum of skinfolds: $F = 176.7$; $P < 0.0001$; hormonal sex: $F = 81.0$; $P < 0.0001$; and genetic sex: $F = 0.0$; $P = 0.96$). The changes in sex steroid milieus upon cross-sex hormone administration in M-F transsexuals and F-M transsexuals are presented in Table 1.

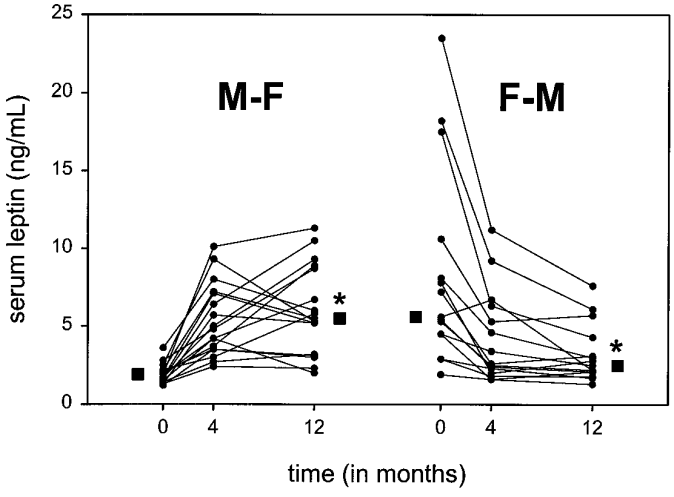

FIG. 1. Individual serum leptin levels before and after 4 and 12 months of cross-sex hormone administration in M-F transsexuals (*left graphic*; n = 17) and F-M transsexuals (*right graphic*; n = 15). The *solid squares* in each graphic represent the median values before and after 12 months of treatment. *, $P < 0.0001$, by ANOVA for repeated measurements (changes within groups).

Discussion

The present study indicates that sex steroid hormones account for a sex difference in serum leptin levels independently of body fat. Cross-sex hormone administration in transsexual subjects showed that the prevailing sex steroid milieu is an important determinant of serum leptin levels; subjects with high circulating testosterone, whether male or female, had significantly lower serum leptin levels at a certain degree of body fatness compared to subjects (male or female) with high estrogen and low testosterone levels.

There are differences in body fat accumulation between men and women that emerge in puberty. This suggests that sex steroid hormones are involved in the sex-specific localization of body fat. From the observations in earlier studies (5–12) it is unresolved whether the sex difference in circulating leptin levels is codetermined by sex steroid hormones. Cross-sectional (7, 8) and prospective (16) studies in women showed no effect of menopausal estrogen decline or of estrogen replacement therapy in postmenopausal women on leptin levels. Schwartz *et al.* (9) reported that women had

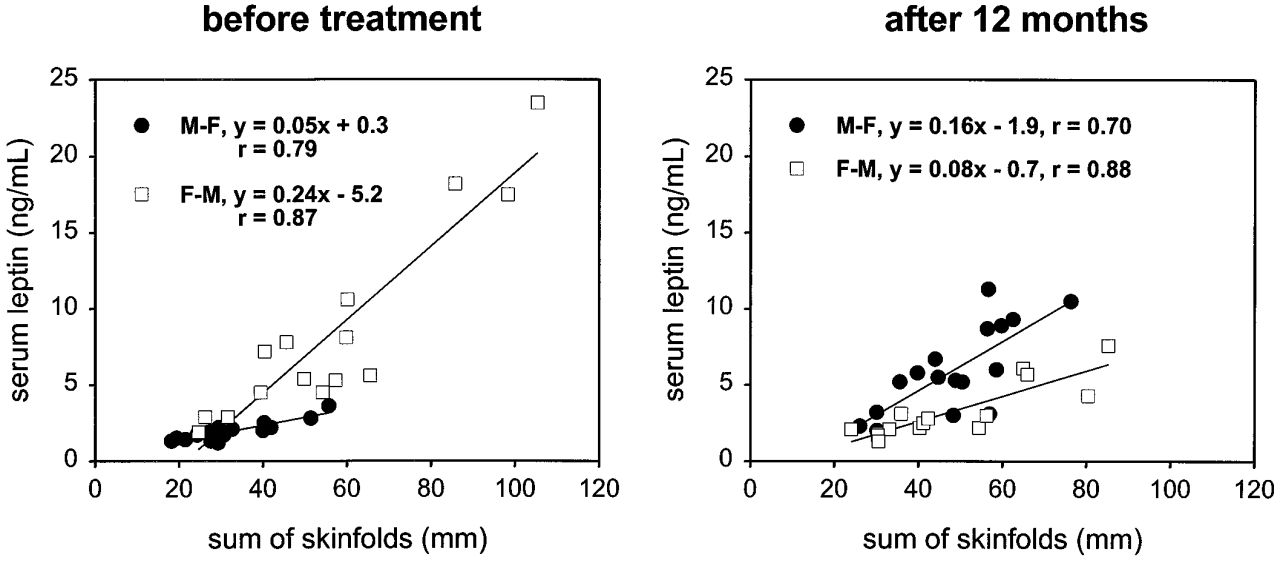

FIG. 2. Relationship between serum leptin levels (*y*-axis) and sum of four skinfolds (*x*-axis) before and after 12-months cross-sex hormone administration in M-F transsexuals (*solid circles*; n = 17) and F-M transsexuals (*open squares*; n = 15). Lines and equations represent the least squares linear regression for each group separately. All regressions were significant at the $P < 0.001$ level. Relations between the variables were assessed by Spearman rank correlations (all *P* values below 0.01).

significantly higher leptin levels in cerebrospinal fluid than men even after adjusting for the significant higher plasma leptin levels in women. Because the study was performed in postmenopausal women, the researchers postulated that sex steroid hormones were unlikely to be important regulators of leptin levels. By contrast, in a study by Rosenbaum *et al.* (10), leptin levels, corrected for the amount of body fat, were significantly lower in postmenopausal women than those in premenopausal women, but a consistent finding of most studies is that women have higher adiposity-corrected leptin levels than men regardless of menopausal status. In F-M transsexuals, testosterone administration led to a strong decrease in serum leptin levels while still biologically significant levels of estradiol were present due to peripheral aromatization of testosterone in estradiol. The most likely interpretation of these observations is that testosterone lowers serum leptin levels. In our study, it is not immediately clear whether the observed increase in serum leptin levels in M-F transsexuals is due to estrogenic or antiandrogenic actions. Upon administration of this combination, serum testosterone fell to an undetectable level. It is therefore possible that the lack of testosterone, rather than the increase in estradiol levels, is responsible for the increase in serum leptin levels. In support of this assumption is a recent cross-sectional study by Saad *et al.* (8). These researchers found a difference in plasma leptin levels between men and women, whereas no difference was observed between premenopausal and postmenopausal women. Consequently, differences in testosterone concentrations, rather than estradiol, could account for the sex difference in serum leptin levels.

In conclusion, sex steroid hormones, in particular testosterone, play an important role in the regulation of serum leptin levels. The prevailing sex steroid hormone milieu, not genetic sex, is a significant determinant of the sex difference in serum leptin levels.

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