Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals


Division of Endocrinology/Andrology, Research Institute of Endocrinology, Metabolism, and Reproduction, Hospital Vrije Universiteit, 1007 MB Amsterdam; and Department of Chronic Disease and Environmental Epidemiology, National Institute of Public Health and Environmental Protection, 3720 BA Bilthoven, The Netherlands

Elbers, J. M. H., H. Asscheman, J. C. Seidell, and L. J. G. Gooren. Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E317–E325, 1999.—We investigated prospectively the effect of sex steroids on regional fat depots and thigh muscle mass in adult transsexuals. Ethinyl estradiol in combination with cyproterone acetate, a progestational antiandrogen, was given to 20 male-to-female (M-F) transsexuals, and parenteral testosterone esters were given to 17 female-to-male (F-M) transsexuals. Before and after 12 mo of cross-sex hormone administration, several anthropometric measurements (weight, skinfolds, body circumferences, and bioimpedance) were performed, and transverse magnetic resonance images were obtained at the level of the abdomen, hip, and thigh to quantify fat depots (subcutaneous and visceral) and muscle areas. We observed that treatment with ethinyl estradiol in M-F transsexuals induced a significant increase in all subcutaneous fat depots, with a lesser but proportional and significant increase in the visceral fat depot and a decrease in thigh muscle area. Testosterone administration in F-M transsexuals markedly increased thigh muscle area, reduced subcutaneous fat deposition at all levels measured, but slightly increased the visceral fat area. We conclude that sex steroid hormones are important determinants of the sex-specific localization of body fat.

Estradiols; androgens; sex differences; regional fat distribution; thigh muscle mass

The regional localization of body fat is a better predictor of health risks in obesity than the total amount of body fat. In particular, an increased amount of intra-abdominal or visceral fat is associated with an increased risk for cardiovascular disease and non-insulin-dependent diabetes mellitus (9, 20). The underlying mechanism of the observed associations is not yet clear (41), but it is hypothesized that the higher turnover rate of visceral fat compared with subcutaneous fat and its specific localization in the abdominal cavity (“portal fat”) contribute to the metabolic disturbances observed in abdominal obesity (1, 7, 27). It is, therefore, of clinical importance to identify factors that regulate site-specific accumulation of body fat.

Regional fat distribution differs between men and women (22, 23, 38, 39, 46). Compared with men, premenopausal women have more subcutaneous fat, and their body fat is preferentially stored in fat depots in breasts, hips, and thighs. These typical “female” sites for fat storage are generally referred to as peripheral or gynoid. In men, fat is predominantly accumulated in the abdominal subcutaneous and visceral depots, with less fat storage in the hip and thigh regions compared with women, and is known as central or android fat distribution. After correction for total body fat mass, men generally have larger visceral fat areas than women, and it has been suggested that this is an important correlate of sex differences in cardiovascular disease. Because body fat distribution is regarded as a secondary sex characteristic, it is conceivable that sex steroid hormones are important determinants of regional fat deposition. Evidence suggests that female sex hormones are involved in the preferential accumulation of subcutaneous fat in the lower body regions. This typical female fat storage may be essential for normal reproductive function (35, 46). The exact role of androgens in body fat distribution is controversial, because the relation between endogenous testosterone levels and the amount of visceral fat and the effects of exogenous testosterone on the visceral fat depot seem to differ between men and women. Abdominal obesity in men has been found to be associated with testosterone levels relatively low for men (40, 42), and administration of testosterone resulted in a reduction of abdominal fat (29, 36), although not in all studies (25). By contrast, cross-sectional studies in women have shown that a “male” type of abdominal fat localization, as assessed by the waist-to-hip ratio, is associated with increased circulating testosterone levels (15, 19). We previously have shown that, in a small group of female-to-male (F-M) transsexuals experiencing a wide range of weight changes, testosterone administration decreased subcutaneous fat depots at the levels of the abdomen, hip, and thigh but increased visceral fat deposition (11). To further investigate the role of sex steroids on regional localization of body fat, we now extend these observations in a new and larger group of F-M transsexuals and in a group of male-to-female (M-F) transsexuals undergoing sex reassignment after a standard protocol of cross-sex hormone administration. Therefore, we studied prospectively for 12 mo the effect of administration of ethinyl estradiol combined with cyproterone acetate, an antiandrogen with progestational activity, in M-F transsexuals and of parenteral testosterone treatment in F-M transsexuals on body weight, regional fat depots, and thigh muscle mass, with the use of anthropometric methods and magnetic resonance imaging.

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SUBJECTS AND METHODS

Subjects

This study was conducted in transsexuals undergoing sex reassignment following a standard protocol of cross-sex hormone administration. Transsexuals are not different from nontranssexual men and women in their endocrine or metabolic functions. Twenty M-F transsexuals, with a mean age of 26 ± 6 (SD) yr (range of age: 18–37 yr) and a mean body mass index (BMI) of 20.8 ± 2.6 kg/m² (range of BMI: 16.1–24.5 kg/m²), participated. They were studied before and during 12-mo treatment with 100 µg ethinyl estradiol (Lynoral; Organon, Oss, The Netherlands) and 100 mg cyproterone acetate, a 17α-hydroxyprogesterone derivative with progestogenic and antianabolic properties (31) (Androcur; Schering AG, Berlin, Germany) daily.

Seventeen F-M transsexuals, with a mean age of 23 ± 5 yr (range of age: 16–34 yr) and a mean BMI of 21.7 ± 3.5 kg/m² (range of BMI: 16.6–29.0 kg/m²), were studied before and during 12-mo treatment with intramuscular injections of 250 mg testosterone esters every 2 wk (Sustanon 250; Organon). All subjects were eugonadal and healthy as assessed by medical history, physical examination, and biochemical criteria. Before the start of the study, they had not been treated with sex steroid hormones or other medication, and F-M transsexuals had normal regular menstrual cycles. All subjects gave their informed consent, and the study was approved by the ethical review board of the Hospital Vrije Universiteit in Amsterdam.

Anthropometric Measurements

Height was measured to the nearest 0.1 cm, and weight was recorded to the nearest 0.1 kg, with subjects wearing only underwear. Body circumferences were measured in duplicate with a flexible plastic tape at the level of the lower left upper arm (midway between tip of the acromion and the olecranon), the abdomen (midway between the lower rib margin and the iliac crest), the hip (over the great trochanters), and the left thigh (just below the gluteal fold). From these data, the waist-to-hip circumference ratio (WHR) was calculated.

Skinfold thicknesses were measured in triplicate by a Harpenden calliper at the left side of the body with subjects in upright position. Measurements were performed at the level of the triceps, biceps, subscapula, suprailliac, and paravumbilical, and the average of three measurements was taken. Percentage of body fat was calculated using the sum of four skinfold thicknesses (triceps, biceps, subscapula, and suprailiac) according to the method of Durnin and Womersley (%BF-SF). Also, the bioelectrical impedance (BIA) method was used to measure the percentage of body fat (%BF-BIA). Whole body resistance (Rz in Ω) and reactivity (Xc in Ω) of an electric current (50 kHz and 800 µA) were assessed using a tetrapolar portable BIA 101 analyzer (RJL Systems, Detroit, MI). Subjects were in supine position with the limbs abducted from the body, and the electrodes were placed as described by Lukaski et al. (26). Percentage of body fat was calculated with use of the manufacturer’s equation. The BIA method is based on the conductivity of an electric current by the electrolytes present in the lean tissues. Electric conductivity is determined by the size, the electrolyte content, and the hydration of the lean tissues. Because steroid hormone treatment is known to be associated with changes in the hydration status of tissues, the BIA measurements during cross-sex hormone treatment can be confounded by water being part of the fat-free compartment and should therefore be interpreted with caution. Another limitation of anthropometric methods, similar to skinfolds and BIA during cross-sex hormone administration, is the use of sex-specific equations. Before and during treatment, equations corresponding to the genetic sex of all subjects were used to calculate the percentage of body fat. However, these sex-specific equations are based on assumptions about body composition and distribution of fat over the body in men and women. Because exogenous sex hormone treatment induced significant changes in overall body composition, it is expected that no precise estimates were obtained by use of these sex-specific equations, and the estimations of percentage body fat by both BIA and skinfolds after 12 mo will be presented within parentheses.

All anthropometric measurements were performed by the same experienced investigator in the morning between 0900 and 1000 after an overnight fast before and again after 2, 4, and 12 mo of cross-sex hormone treatment.

Before and after 12 mo of cross-sex hormone administration, subjects were asked about their food intake with the use of a 24-h food frequency questionnaire, the Dutch EPIC food frequency questionnaire (32, 33), which was validated for use in epidemiological studies. The subjects’ energy intake in kilojoules per day was calculated.

Magnetic Resonance Imaging

Image acquisition. The imaging technique (MRI) based on magnetic resonance (MR) was used to quantify regional fat deposition (subcutaneous and visceral fat areas) and muscle area at thigh level. For image acquisition, an inversion recovery pulse sequence was used. Parameters were selected to obtain good image contrast between adipose tissue, which has a relatively short longitudinal relaxation time (T1), and other tissues. During the study, we used three different MR imagers with varying magnetic field strengths. However, in all subjects, repeated MR acquisition after 12 mo of treatment was performed on the same MR imager with the same scanning parameters. No MR images were made in one M-F transsexual, who did not want to participate in this part of the study. In three M-F transsexuals, images were obtained on a 0.6 Tesla imager [Teslonco II, Technicare, Solon, OH, USA; repetition time (TR) 524 ms, echo time (TE) 24 ms, and inversion time (TI) 150 ms]. Slice thickness was 10 mm, and the field of view (FOV) was 410 mm. In the other subjects, a 1.5 Tesla imager (SP 64, Siemens, Erlangen, Germany; TR 900 ms, TE 20 ms and TI 400 ms) or a 1.0 Tesla imager (Magnetom Impact Expert, Siemens; TR 700 ms, TE 20 ms, and TI 300 ms) was used for image acquisition. Slice thickness was 12 mm, and FOV was 400–500 mm, depending on the size of the subject.

 Sagittal and coronal localizers were obtained to determine precisely the anatomic sites for image acquisition. Transverse MR images were made at the level of the abdomen (lower edge of the umbilicus, comparable to the vertebral L4-L5 level), the hip (upper margin of the great trochanters), and the thigh (just below the gluteal fold). At the abdominal level, three images were obtained: one image at the level of the anatomic marker, one above and one below this position. Two images were obtained at the level of the hip (at the anatomic marker and one above) and two at thigh level (at the anatomic marker and one below).

Image analysis. Image analysis was performed on a Sparc10 workstation (Sun Microsystems, Palo Alto, CA) by use of an image-analyzing computer program developed at the Department of Biomedical Engineering of our hospital. The procedure of image analysis used by this computer program has been described in detail elsewhere (13). In short, the program is based on a seed-growing procedure. After a seed point is
placed in a fat depot, this depot can be circumscribed by selection of a pixel intensity range. The intensity range is selected for each image separately according to the pixel intensity histogram. The area of the circumscribed fat depot is quantified by converting the number of pixels to squared centimeters. All measurements were performed by the same experienced investigator, and the average area measurement of the different images per level was used in the statistical analysis.

Subcutaneous fat areas were measured at all three levels; and at the abdominal level, the visceral fat area was also obtained. For the visceral fat area, it is not possible to quantify separately the various subcutaneous fat depots, i.e., omental, mesenteric, and retroperitoneal fat. Muscle area was calculated by subtracting the areas of subcutaneous fat, bone, and connective tissue from the total area on the image below the marker at thigh level. Total areas at all body sites were measured at the imaging levels that relate to the levels for measurements of body circumferences. At the abdominal body site, total areas were measured on the image above the abdominal marker (upper image). For the hip and thigh, total areas were quantified on the images at the level of the respective anatomic markers. The ratio of the total area at the abdominal level to the total area at the level of the hip was calculated (WHR-area).

Blood and Urine Analyses

In all subjects, venous blood samples were taken in the morning between 0900 and 1030 after an overnight fast, and 24-h urine was collected (during the 24 h before blood sampling) at baseline, and again after 2, 4, and 12 mo of cross-sex hormonal administration. Radioimmunoassays were used to determine serum testosterone levels (Coat-A-Count, DPC, Los Angeles, CA; in nmol/l), serum levels of 5α-dihydrotestosterone (DHT; after extraction and oxidation, Intertech, Strassen, Luxembourg; in nmol/l), serum 17β-estradiol levels (Double antibody, Sorin Biomedica, Saluggia, Italy; in pmol/l), and free cortisol levels in 24-h urine samples (after extraction; Coat-A-Count, DPC; in nmol/24 h). Immuno-radiometric assays were used to measure serum levels of sex hormone-binding globulin (SHBG; Orion Diagnostica, Espoo, Finland; in nmol/l), serum insulin levels (Biosource Diagnostics, Fleurus, Belgium), and serum levels of growth hormone (GH; color, Sorin Biomedica, Saluggia, Italy; in μg/l). Immuno-radiometric assays (luminescence) were used to determine levels of follicle-stimulating hormone (FSH; Amerlite, Amersham, UK; in U/l) and luteinizing hormone (LH; Amerlite; in U/l).

Statistical Analysis

Values are presented as means ± SD. Differences from baseline within the groups were tested using the paired sample t-test or ANOVA for repeated measurements. Energy intake measurements were compared by use of the paired Wilcoxon test. Differences between groups or body fat regions were measured by unpaired sample t-tests. Pearson correlation coefficients were used to describe relations between variables. Levels of 17β-estradiol (in F-M transsexuals) were log-transformed to normalize the distribution. To study potential site-specific localization of body fat, the relation between baseline and final subcutaneous fat area measurements was compared between different fat locations (abdomen vs. thigh; abdomen vs. hip). Analysis of covariance (ANCOVA) was used, with final absolute fat area as the dependent variable and baseline absolute fat area as covariate, to correct for the differences in fat areas at baseline. Interaction factors between location (abdomen vs. thigh; abdomen vs. hip) and baseline fat areas were introduced in the model. Statistical analyses were performed with SPSS for MS Windows (Release 6.0; SPSS, Chicago, IL), and P values below 0.05 were considered significant.

RESULTS

Baseline Sex Differences in Anthropometry and Fat Distribution

In Table 1, anthropometric measurements at baseline and after 12 mo of cross-sex hormone administration are summarized for both M-F and F-M transsexuals. When the initial anthropometric values of both F-M Transsexuals (n = 20) and M-F Transsexuals (n = 17) are compared, no significant sex differences were observed. After 12 mo of treatment, there was a significant increase in weight and BMI in both groups. The increase in weight was not different between the two groups. However, the increase in BMI was significantly higher in F-M transsexuals compared to M-F transsexuals.

Values are means ± SD. M-F, male-to-female; F-M, female-to-male; BMI, body mass index; SF, skinfolds; BIA, biopsychometric method; %BF, percentage body fat; NS, not significant. *Values of %BF-SF and %BF-BIA after 12 months of treatment are presented in parentheses because these results should be interpreted with caution (see METHODS).
groups are compared, the sex difference in body fat distribution is obvious. With a similar BMI, F-M transsexuals at baseline had higher skinfold thicknesses and consequently a higher percentage of body fat than M-F transsexuals at baseline. Quantification of regional fat depots (Table 2) showed larger subcutaneous fat areas at all levels in F-M transsexuals compared with M-F transsexuals before treatment. The same absolute visceral fat area was found in both groups. Before the start of the hormone administration, M-F transsexuals had a larger muscle area at the level of the thigh, as assessed by MRI, than F-M transsexuals.

Changes in Anthropometric and MRI Measurements During Treatment in M-F Transsexuals

In M-F transsexuals, a gradual and significant increase in body weight was observed during treatment (Table 1). Mean body weight had increased by 3.8 ± 2.7 kg after 12 mo (P < 0.001). A gradual increase was also observed in all skinfold thicknesses, leading to a mean increase in the sum of four skinfolds of 17.0 ± 11.8 mm after 12 mo (P < 0.001). The observed significant mean increases in circumference measurements at the abdominal level of 3.8 ± 3.0 cm and at the level of the hip of 4.1 ± 2.5 cm after 12 mo of treatment did not result in a change in WHR (Table 1). MRI measurements in the estrogen-treated M-F transsexuals show that all subcutaneous fat areas had increased significantly after 12 mo (P < 0.001, Table 2). The largest fat deposition was observed at the level of the hip and thigh; the mean subcutaneous fat area increased by 60 ± 26 cm² (84 ± 46% from baseline) at the level of the hip and by 80 ± 33 cm² (84 ± 52% from baseline) at thigh level. The subcutaneous abdominal fat area increased by 50 ± 23 cm² (66 ± 33% from baseline), and the absolute increase in the visceral fat area by 7 ± 11 cm² (24 ± 30% from baseline) was significantly different from baseline (P = 0.01). When increases in subcutaneous fat areas were compared among the three levels, the absolute increase (in cm²) in subcutaneous abdominal fat was significantly smaller than the increase in body fat in the thigh region (by ANCOVA, P < 0.01), but not compared with the hip region (P = 0.116). After 12-mo treatment with estrogens and androgens in M-F transsexuals, thigh muscle area had decreased significantly by 29 ± 19 cm², which is a decrease of 9 ± 5% compared with baseline (P < 0.001). Significant correlations were observed between percent changes in body weight, on the one hand, and percent changes in abdominal subcutaneous fat areas (r = 0.70, P = 0.001), visceral fat areas (r = 0.66, P < 0.01), hip fat areas (r = 0.64, P < 0.01), and thigh fat areas (r = 0.53, P < 0.05) on the other hand. No significant correlation was present between percent changes in body weight and percent changes in thigh muscle areas (r = 0.24, P = NS). The results on energy intake with the use of a standardized food frequency questionnaire showed large interindividual variability. In M-F transsexuals, the baseline 25th, 50th, and 75th percentiles for the energy intake in kilojoules per day were, respectively: 10,567, 14,478, and 16,587 kJ/day, with a wide range of 4,633–21,738 kJ/day. After 12 mo of treatment, M-F transsexuals generally reported a lower energy intake: 7,282, 10,785, and 14,086 kJ/day for the 25th, 50th, and 75th percentiles, respectively. Again, a wide range of energy intake was reported: 4,756–23,957 kJ/day. When the results on energy intake were compared between baseline and after 12 mo with the use of the paired Wilcoxon test, the observed decrease in energy intake was close to significance (P = 0.06).

Changes in Anthropometric and MRI Measurements During Treatment in F-M Transsexuals

After 12 mo of testosterone treatment in F-M transsexuals, mean body weight had increased significantly by 2.7 ± 2.8 kg (Table 1, P < 0.001). All measured skinfold thicknesses had significantly decreased after 12 mo of treatment, except for the subscapular skinfold (Table 1), resulting in a significant decrease in the sum of four skinfolds after 12 mo of treatment (P = 0.001). WHR had increased significantly after 12 mo of treatment (P = 0.01), mainly as a result of a significant decrease in hip circumference after 12 mo (P = 0.01).

Table 2. MRI measurements at baseline and after 12 mo of cross-sex hormone administration in M-F and F-M transsexuals

<table>
<thead>
<tr>
<th>Subcutaneous fat, cm²</th>
<th>M-F Transsexuals</th>
<th>F-M Transsexuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 12 mo</td>
</tr>
<tr>
<td><strong>Abdomen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87 ± 43</td>
<td>137 ± 52</td>
<td>(39; 61)</td>
</tr>
<tr>
<td>91 ± 48</td>
<td>151 ± 49</td>
<td>(48; 73)</td>
</tr>
<tr>
<td><strong>Thigh</strong></td>
<td>123 ± 58</td>
<td>204 ± 57</td>
</tr>
<tr>
<td><strong>Visceral fat, cm²</strong></td>
<td>40 ± 19</td>
<td>47 ± 18</td>
</tr>
<tr>
<td><strong>V/S</strong></td>
<td>0.50 ± 0.18</td>
<td>0.37 ± 0.14</td>
</tr>
<tr>
<td><strong>Muscle area, cm²</strong></td>
<td>307 ± 47</td>
<td>270 ± 37</td>
</tr>
<tr>
<td><strong>Total area, cm²</strong></td>
<td>374 ± 74</td>
<td>416 ± 87</td>
</tr>
<tr>
<td><strong>Hip</strong></td>
<td>538 ± 88</td>
<td>594 ± 95</td>
</tr>
<tr>
<td><strong>Thigh</strong></td>
<td>441 ± 92</td>
<td>497 ± 93</td>
</tr>
<tr>
<td><strong>WHR area, cm²/cm²</strong></td>
<td>0.69 ± 0.07</td>
<td>0.70 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SD. CI, confidence interval; V/S, ratio of visceral to subcutaneous abdominal fat area; WHR, waist-to-hip ratio; NS, not significant.
The circumference at the level of the abdomen did not change significantly.

Testosterone treatment in F-M transsexuals resulted in a marked decrease in all subcutaneous fat depots as assessed by MRI (Table 2). After 12 mo, the subcutaneous abdominal fat area had decreased by 27 ± 34 cm² (P < 0.001), the subcutaneous hip area by 46 ± 22 cm² (P < 0.001), and the subcutaneous fat area at the level of the thigh by 56 ± 27 cm² (P < 0.001). The visceral fat area showed a significant absolute increase of 5 ± 7 cm² (P < 0.01).

When expressed as percentage from baseline, decreases in body fat areas seemed larger in both hip and thigh regions compared with the subcutaneous abdominal region (hip -27 ± 6% and thigh -23 ± 7% vs. abdomen -14 ± 14%). However, absolute changes in subcutaneous fat measurements were not statistically significantly different between the various fat depots. Testosterone administration in F-M transsexuals induced a significant increase in thigh muscle area (r = 0.57, P < 0.001), the subcutaneous hip area by 46 ± 22 cm² (r = 0.31, P = 0.22). A significant correlation was present between percent changes in body weight and percent changes in abdominal subcutaneous fat areas (r = 0.41, P = 0.11), visceral fat areas (r = 0.03, P = 0.91), hip fat areas (r = 0.15, P = 0.57), and thigh fat areas (r = 0.31, P = 0.22).

Changes in body fat areas seemed larger in both hip and thigh regions compared with the subcutaneous abdominal region (hip -27 ± 6% and thigh -23 ± 7% vs. abdomen -14 ± 14%). However, absolute changes in subcutaneous fat measurements were not statistically significantly different between the various fat depots. Testosterone administration in F-M transsexuals induced a significant increase in thigh muscle area (P < 0.001). No significant correlations were observed between percent body weight and percent changes in abdominal subcutaneous fat areas (r = 0.41, P = 0.11), visceral fat areas (r = 0.03, P = 0.91), hip fat areas (r = 0.15, P = 0.57), and thigh fat areas (r = 0.31, P = 0.22). A significant correlation was present between percent changes in body weight and percent changes in thigh muscle areas (r = 0.51, P < 0.05). No significant changes in energy intake in kilojoules per day were reported by F-M transsexuals, when baseline energy intake was compared with reported energy intake after 12 mo of testosterone administration. In F-M transsexuals, the baseline 25th, 50th, and 75th percentiles for the energy intake in kilojoules per day were, respectively: 7,329, 8,893, and 12,688 kJ/day, with a range of 5,147–21,674 kJ/day. Parents reported a comparable energy intake with 25th, 50th, and 75th percentiles of 7,615, 8,327, and 11,700 kJ/day, with a range of 5,147–18,772 kJ/day. After 12 mo of treatment, F-M transsexuals, parenteral testosterone esters increased serum testosterone levels and levels of DHT during treatment to supraphysiological levels for female subjects (Table 3). Serum levels of LH decreased significantly during treatment, but levels of FSH were not significantly suppressed. Levels of LH increased serum testosterone levels and levels of DHT during treatment to supraphysiological levels for female subjects (Table 3). Serum levels of LH decreased significantly during treatment, but levels of FSH were not significantly suppressed. Levels of LH decreased from 26 ± 15 pmol/l at baseline to 13 ± 5 pmol/l after 12 mo, but this decrease during 12 mo was not significant as assessed by ANOVA for repeated measurements. Free cortisol excretion in 24-h urine showed a significant increase during treatment as assessed by ANOVA for repeated measurements, but levels after 12 mo were not significantly different from baseline (P = 0.07, paired sample t-test).

**Blood and Urine Analyses in M-F Transsexuals**

In M-F transsexuals, parenteral testosterone esters increased serum testosterone levels and levels of DHT during treatment to supraphysiological levels for female subjects (Table 3). Serum levels of LH decreased significantly during treatment, but levels of FSH were not significantly suppressed. Levels of LH increased serum testosterone levels and levels of DHT during treatment to supraphysiological levels for female subjects (Table 3). Serum levels of LH decreased significantly during treatment, but levels of FSH were not significantly suppressed. Levels of LH decreased from 26 ± 15 pmol/l at baseline to 13 ± 5 pmol/l after 12 mo, but this decrease during 12 mo was not significant as assessed by ANOVA for repeated measurements. Free cortisol excretion in 24-h urine showed a significant increase during treatment as assessed by ANOVA for repeated measurements, but levels after 12 mo were not significantly different from baseline (P = 0.07, paired sample t-test).

**Blood and Urine Analyses in M-F Transsexuals**

Fig. 1. Relative changes in subcutaneous fat areas at level of abdomen (abd), hip, and thigh as assessed by MRI. Relative changes in subcutaneous fat areas at level of abdomen (abd), hip, and thigh as assessed by MRI. *P < 0.05.
treatment. GH levels showed a significant decrease (\(P < 0.05\)), and free cortisol excretion in 24-h urine did not change significantly during testosterone treatment in F-M transsexuals.

**DISCUSSION**

**General Findings of the Present Study**

In the present study, the effects of sex steroid hormones on fat distribution and muscle mass were prospectively investigated in adult transsexual subjects receiving cross-sex steroid hormones. Profound effects on weight and fat distribution were observed, evidencing the significant role that sex steroids play in the male and female types of fat distribution and muscle mass.

**Effects of Estrogens and Antiandrogens in M-F Transsexuals**

After administration of estrogens in combination with cyproterone acetate, a progestational antiandrogen, in M-F transsexuals, a marked increase in subcutaneous fat deposition was observed, with a small but significant decrease in GH levels (\(P < 0.05\)).

**Table 3. Levels of sex steroid hormones and SHBG before and during 12 mo of cross-sex hormone administration in M-F and F-M transsexuals**

<table>
<thead>
<tr>
<th></th>
<th>M-F Transsexuals</th>
<th>F-M Transsexuals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 2 mo</td>
</tr>
<tr>
<td>(17\beta)-Estradiol, pmol/l</td>
<td>96 ± 12</td>
<td></td>
</tr>
<tr>
<td>Testosterone, nmol/l</td>
<td>22 ± 6</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>DHT, nmol/l</td>
<td>2.7 ± 1.2</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>SHBG, nmol/l</td>
<td>35 ± 13</td>
<td>214 ± 47</td>
</tr>
<tr>
<td>LH, U/l</td>
<td>2.8 ± 2.0</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>FSH, U/l</td>
<td>2.9 ± 2.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>Insulin, pmol/l</td>
<td>41 ± 13</td>
<td>53 ± 31</td>
</tr>
<tr>
<td>GH, (\mu)g/l</td>
<td>3.3 ± 4.2</td>
<td>8.0 ± 6.6</td>
</tr>
<tr>
<td>Cortisol excretion, nmol in 24-h urine*</td>
<td>163 ± 70</td>
<td>252 ± 141</td>
</tr>
</tbody>
</table>

* Values are means ± SD. DHT, 5α-dihydrotestosterone; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; GH, growth hormone; NS, not significant. *n = 16 for M-F and n = 14 for F-M transsexuals.
proportional increase in visceral fat area and a significant decrease in thigh muscle area. Although a substantial amount of fat was also stored in the subcutaneous abdominal fat depot, significantly more body fat was accumulated in the typical female subcutaneous fat depot at the level of the thigh, which is generally not a primary site for fat storage in men (46). This agrees with findings in elderly men treated with estrogens for prostate carcinoma (21) and in one case of M-F transsexualism (43). However, the gain in fat also reflects the baseline distribution of the lean male subjects in our study, more fat in the thigh region than in the abdominal region.

The effects of hormonal treatment in M-F transsexuals on regional fat deposition and muscle mass are likely to be the combined action of ethinyl estradiol, a potent synthetic estrogen, and cyproterone acetate, a 17α-hydroxyprogesterone with potent antiandrogenic properties (31). The latter blocks effectively the effect of testosterone through competition with the androgen receptor and reduces testosterone synthesis by inhibiting gonadotropin secretion (31). The androgen deprivation itself may have affected body composition occurring in our subjects. Men with a deficient testosterone production or action show a feminine body habitus (8), with decreased muscle mass. Androgen administration to hypogonadal men or adolescents with delayed puberty reduces body fat and increases muscle mass (2, 5).

In addition to its antiandrogenic action, cyproterone acetate is also a potent progestin (31); progesterone may be involved in determining the glucocorticosteroid pattern of fat distribution typically seen in women (34). The glucocorticosteroid (40) in subcutaneous fat on administration of ethinyl estradiol in M-F transsexuals seems to contrast with the effects of estrogen administration observed in postmenopausal women. The relative estrogen-deficient state after menopause is associated with a gain in body weight and fat content, and postmenopausal women receiving hormone replacement therapy compared with placebo have been shown to gain less body weight (6, 14, 17, 37). This discrepancy is perhaps explained by the fact that the type and dose of estrogens given to the M-F transsexuals were more potent and the transsexuals were also more lean at baseline than most perimenopausal women. However, similar to our findings in M-F transsexuals, the observations in postmenopausal women provide evidence for the significance of female sex steroids in female fat distribution. In the postmenopausal state, a shift toward a more male type of body fat distribution in the absence of female sex steroids can be seen, which can be (partially) reversed on hormone replacement therapy (6, 14, 17, 18, 37). Moreover, the effects in our young, lean male subjects show similarities with the effects of the rising female sex steroids on body composition in young girls during puberty. From several studies using the imaging technique (computed tomography or MRI), it was observed that premenopausal women can accumulate more body fat before reaching amounts of visceral fat that are similar to amounts in men, possibly by a “protective” effect of female sex hormones (7, 22, 23). It seems that women have different fat depots and a larger capacity for fat storage in the presence of female sex hormones than men, who have the abdominal regions as predominant sites of fat accumulation in the presence of testosterone.

**Effects of Testosterone Administration in F-M Transsexuals**

In agreement with our earlier study (11), we found that testosterone administration to F-M transsexuals resulted in a significant reduction of subcutaneous fat, with a selective retention and even slight increase in visceral fat area. Our observations on testosterone treatment in female subjects are consistent with the findings of Lovejoy et al. (24) in postmenopausal women but differ from the observations in middle-aged, abdominally obese men, with testosterone levels relatively low for men (29, 36). The female subjects of our studies presented with a typical female fat distribution: relatively small amounts of visceral fat in relation to the large subcutaneous fat storage. The results of testosterone treatment in young F-M transsexuals show similarities with the events during adolescence in men, when there is evidence of an association between an increase in upper body fat predominance and male sex hormone levels (3, 10). Prolonged exposure to testosterone (over decades as occurs in middle-aged men) may further promote storage of excess fat in abdominal and peripheral depots. The changes observed may be a direct or indirect effect of testosterone. Estrogens per se do not seem to be an important factor, as no significant decrease in serum estrogen levels was observed in testosterone-treated F-M transsexuals (due to peripheral aromatization of testosterone in estradiol). After testosterone administration there were no longer cyclic changes in estrogens and progesterone, and thus a lack of the hormonal drive of fat to the gluteo-femoral depots may have played a role in the selective increase in visceral fat area. Moreover, the route of administration of testosterone (transdermally, orally, intramuscularly) may matter for the influence on the visceral fat depot. The large increase in circulating testosterone levels in F-M transsexuals also led to a significant increase in thigh muscle mass of 20%. Androgens increase muscle mass by increasing the size of the muscle fibers. Recent studies showed that administration of androgens to hypogonadal men and of supraphysiological doses of testosterone to normal men increased muscle mass (2, 4, 5, 16).

**Secondary Effects of Cross-Sex Hormone Treatment on Body Fat Distribution**

The administration of sex steroids may affect levels and/or actions of other hormones, which in turn influence body fat accumulation (7). Obesity is negatively associated with GH secretion and insulin-like growth factor I (IGF-I) levels and positively associated with serum insulin levels (and insulin resistance) and free cortisol excretion in 24-h urine (28, 30). GH and testosterone may act synergistically on inhibition of fat uptake and stimulation of fat mobilization (7). Administration of cross-sex hormones in transsexuals affected GH levels. IGF-I levels are significantly reduced in M-F
transsexuals on administration of estrogens in combination with antiandrogens and are increased in F-M transsexuals on testosterone administration (45). The relative contribution of changes in these hormones, secondary to cross-sex hormone administration in transsexuals, to the changes in body fat accumulation is difficult to assess.

Unfortunately, our data collected on changes in food intake were inadequate to assess whether changes in energy intake induced by cross-sex hormone administration may have influenced the observed changes in fat deposition. Leptin has a possible role in the regulation of body weight via actions on food intake and energy expenditure. We recently reported on changes in circulating leptin levels upon cross-sex hormone administration in transsexuals that induced a reversal of the sex difference in leptin levels (12). It could be shown that, in addition to the amount of body fat, the circulating sex steroid milieu is an important determinant of leptin levels.

Possible Long-Term Risks and/or Benefits

Longer follow-up data (more than 12 mo) are needed for information on the effects of long-term cross-sex hormone administration on body fat distribution and associated health risks. Long-term treatment of female subjects with testosterone may be detrimental to lipo-protein and glucose homeostasis via effects on fat distribution or via direct effects on different organs and tissues. Similarly, long-term treatment with ethinyl estradiol and cyproterone acetate in male subjects may have some favorable and unfavorable effects on metabolism that are independent of their effects on body composition and fat distribution (44).

We conclude that sex steroid hormones are important determinants of sex-specific localization of body fat in adult subjects. Estrogen and antiandrogen treatment in M-F transsexuals induced marked increases in subcutaneous fat deposition, resulting in a more female type of fat localization. A shift toward a male type of body fat distribution was observed during testosterone administration in F-M transsexuals, as this treatment resulted in a small but significant increase in the visceral fat area whereas subcutaneous deposits were significantly reduced. Apparently, the “typical” female body composition (a larger body fatness and less muscle mass than men) is the result of an abundance of female sex hormones in the absence of androgens. An increase in visceral fat area was observed in testosterone-treated F-M transsexuals, even in the presence of estradiol. However, it is unclear how the elimination of the progesteragenic activity in these subjects affected fat distribution.

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Address for reprint requests: H. Asscheman, Div. of Endocrinology/Andrology, Hospital Vrije Universiteit, PO Box 7057, 1007 MB Amsterdam, The Netherlands.

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