Changes in Fat Cell Size and In Vitro Lipolytic Activity of Abdominal and Gluteal Adipocytes After a One-Year Cross–Sex Hormone Administration in Transsexuals

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We prospectively studied the effects of cross–sex hormone administration on fat cell size and in vitro lipolytic activity in subcutaneous abdominal and gluteal fat biopsies obtained from 19 male-to-female (M-F) transsexuals and 17 female-to-male (F-M) transsexuals. The amount of subcutaneous fat at the abdominal and gluteal levels was quantified with the use of magnetic resonance imaging (MRI). Before cross–sex hormone administration, M-F transsexuals had less subcutaneous fat with smaller fat cells compared with F-M transsexuals, with a higher baseline in vitro lipolytic activity expressed as glycerol release per milligram of triglyceride (TG) in the abdominal region ($P < .05$). Before cross–sex hormone treatment, no differences in lipolytic activity stimulated with arterenol (ART), isoproterenol (ISO), or ISO + insulin (INS) were observed between groups or regions. After a 1-year treatment with estrogens and antiandrogens in M-F transsexuals, subcutaneous fat areas on MRI and fat cell size were increased ($P < .001$) and reductions were observed in the basal lipolytic activity of gluteal and abdominal fat biopsies ($P < .05$). Following administration of testosterone to F-M transsexuals, subcutaneous fat and fat cell size at the gluteal and abdominal depots were decreased ($P < .01$) and basal lipolysis was increased significantly at the abdominal level ($P < .05$) but not at the gluteal level. In both M-F and F-M transsexuals, no effect of sex hormone administration was observed on stimulated lipolytic activities. In conclusion, regional sex differences in the amount of subcutaneous fat, adipocyte size, and in vitro basal lipolytic activity were demonstrated that could be largely reversed by cross–sex hormone treatment in adult subjects, providing evidence for their dependence on the sex steroid milieu.

THE DISTRIBUTION OF FAT over the body differs between men and women and may therefore be considered a secondary sex characteristic. Women in the reproductive period of life have more subcutaneous body fat than men and seem to store body fat in specific "female" subcutaneous fat depots such as the hips and thighs. This type of fat distribution is often referred to as peripheral or gynoid. Men tend to have more fat in the abdominal region, and this male type of fat storage is known as central or android fat distribution.[1-3]

The amount of fat in a particular depot is dependent on the number and size of adipocytes. The processes of fat storage in adipocytes, mainly regulated by the enzyme lipoprotein lipase (LPL), and mobilization of triglyceride (TG) from the adipocytes by lipolysis (hydrolysis of TG in free fatty acids and glycerol) are dependent on energy balance and modulated by several endocrine and genetic factors.[2,3] Site-specific variations in the effects of these factors are likely to determine the regional amount of body fat, and regional differences in LPL activity and fat cell adrenoceptors have been found.[4-8] An increased deposition of fat in the abdominal region is an important risk factor for the metabolic disorders observed in obesity.[1-3] It is therefore of important to identify factors that regulate regional adipocyte metabolism. Observations from several cross-sectional studies have shown that differences in sex steroid hormone concentrations between the sexes[2,5-12] and, furthermore, variations in sex steroid levels in different phases of (reproductive) life[13-16] parallel regional differences in fat storage and mobilization, but only a few prospective data are available.

We recently demonstrated that administration of estrogen in combination with antiandrogens in male subjects (male-to-female [M-F] transsexuals) significantly increased subcutaneous fat, whereas testosterone administration in female subjects (female-to-male [F-M] transsexuals) significantly reduced subcutaneous fat.[17] It is unknown to what extent adipocyte metabolism changed upon cross–sex hormone administration in these adult transsexuals. For this purpose, we studied fat cell size and in vitro lipolytic activity (basal and stimulated) in abdominal and gluteal adipocytes before and after a 1-year cross–sex hormone administration, and compared these findings with the subcutaneous abdominal and gluteal fat area measurements obtained by magnetic resonance imaging (MRI).

SUBJECTS AND METHODS

Subjects

Nineteen M-F transsexuals participated in the study, with a mean age (mean ± SD) of 26 ± 6 years (range, 18 to 36) and a mean body mass index (BMI) of 20.6 ± 2.6 kg/m^2 (range, 16.1 to 24.5). They were studied before and after a 1-year treatment with 100 μg ethinyl estradiol (Lynoral; Organon, Oss, The Netherlands) and 100 mg cyproterone acetate (an antiandrogen, Androcur; Schering, Berlin, Germany) daily. Seventeen F-M transsexuals with a mean age of 23 ± 5 years (range, 16 to 34 y) and a mean BMI of 21.7 ± 3.5 kg/m^2 (range, 16.6 to 29.0) were studied before and after a 1-year treatment with intramuscular injections of 250 mg testosterone esters every 2 weeks (Sustanon 250; Organon). All subjects were eugonadal and healthy as assessed by medical history, physical examination, and biochemical criteria. They were not treated with sex steroid hormones prior to the start of the study, and no other medication was used. All subjects provided informed consent, and the study was approved by the ethics review board of Hospital Vrije Universiteit in Amsterdam.

Anthropometry and MRI

Before and after a 1-year cross–sex hormone administration, body weight was measured. MRI was used to quantify subcutaneous fat depots.[17] An inversion recovery pulse sequence was used, and parameters were selected to obtain good image contrast between fat and other

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tissues. In all subjects, repeated image acquisition was performed on the same imager using the same scanning parameters. One M-F transsexual did not participate in this part of the study. The procedure of image acquisition has been described in detail elsewhere.[18] Three transverse images were obtained at the level of the abdomen: one at the level of the anatomical marker (lower edge of the umbilicus) and one above and one below this position (slice thickness, 10 to 12 mm depending on the imager). Two images were taken of the hip region: one at the upper margin of the greater trochanters and one above this marker. Image analysis was performed using an image-analyzing computer program based on a seed-growing procedure. The average subcutaneous fat area (in cm^2) of the two or three images per body site was used in statistical analysis.

Fat Biopsies and In Vitro Lipolytic Activity

Subjects were studied between 9:30 and 10:30 AM after a 12-hour overnight fast. The needle aspiration technique (14-gauge × 51-mm needle, Abbocath-T; Abbott Ireland, Sligo, Ireland; and a 20-mL syringe) was used to obtain subcutaneous adipose tissue biopsies from the abdominal region (lateral to the umbilicus) and the gluteal region (upper lateral quadrant) after local anesthesia of the skin (Lidocaine 1%; Braun, Melsungen, Germany). Care was taken not to infiltrate the adipose tissue with Lidocaine. Aspirated adipose tissue was immediately transferred to a vial with Parker solution (medium 199 with Hanks salts and L-glutamine, containing 4% bovine albumin Fraction V powder, pH 7.4; Sigma Chemical, St Louis, MO). In the laboratory, the adipose tissue was washed with Parker solution to remove blood and was incubated in 5 mL Parker solution with 4 mg collagenase (Clostridiopeptidase A; Boehringer, Mannheim, Germany) for 60 minutes at 37°C in a shaking water bath to isolate adipocytes from the stroma. All material was siliconized (Aquasil siliconizing fluid; Pierce Chemical, Rockford, IL; 1:200 in demineralized water using a Milli-Q UF Plus water purification system (Millipore, Milford, MA) to prevent the loss of adipose tissue. After filtration through a polyamide mesh (250 μm), adipocytes were carefully washed four times and a cell suspension with a lipocrit of approximately 50% in fresh medium was obtained. From this cell suspension, aliquots of 100 μL were added to vials containing 2 mL Parker solution without (basal lipolysis) or with (stimulated lipolysis) 20 μL 10^{-3} mol/L arterenol ([ART] final concentration, 10^{-5} mol/L; Sigma Chemical), 10^{-3} mol/L isoproterenol ([ISO] final concentration, 10^{-5} mol/L; Sigma Chemical) or 10^{-3} mol/L ISO in combination with human insulin 100 mU/mL ([ISO + INS] final INS concentration, 1,000 μU, Velosulin; Novo Nordisk, Bagsvaerd, Denmark). After a 2-hour incubation period at 37°C in a gently shaking water bath, the cell suspension in medium was transferred to soft plastic vials and 0.8 mL silicone oil (melting-point bath oil; Sigma Chemical) was added to each vial. These were centrifuged for 3 minutes at 2,800 rpm to separate the incubation medium from the cells. The glycerol content of the incubation medium was taken as an index of lipolytic activity and was determined enzymatically (Glycerol kit; Boehringer Mannheim; lower detection limit 7 μmol/L and upper limit of linearity 1,000 μmol/L). Measurement of the TG content of the cell suspension was based on TG extraction according to Dole and Meinertz[19] and determined as described by Carlson.[20] Lipolytic activity was expressed as glycerol release per milligram of TG or per 10^5 cells per cell surface area ([CSA] $\pi(d^2 + SD^2)$) per 2 hours. Fat cell diameter was measured by assessing the diameter of 200 adipocytes from a sample of the cell suspension after staining with crystal violet (Merck, Darmstadt, Germany; 0.25% in demineralized water using a Milli-Q UF Plus water purification system; Millipore).

Baseline fat biopsies in F-M transsexuals were obtained between day 5 and day 9 of the menstrual cycle, presumably in the follicular phase. Following hormone treatment, menstrual activity ceased and fat biopsies after 1 year were taken within 5 to 9 days after the preceding testosterone injection. In all subjects, the fat cell diameter in regional fat biopsies was assessed, but it was not always possible to perform all measurements of lipolytic activity, because the amount of aspirated adipose tissue was not always sufficient to obtain reliable measurements.

Statistics

Values are expressed as the mean ±SD or the median and range. To test for differences between groups at baseline (unpaired) or within groups during treatment (paired), Student's *t* test or nonparametric tests were used when appropriate. For measurements of lipolytic activity, nonparametric tests were used: Mann-Whitney *U* test for differences between groups (M-F and F-M transsexuals) or body sites (abdominal and gluteal) or Wilcoxon signed-rank test for differences within groups. Spearman rank correlations were used to describe relations between variables when appropriate. *P* values less than .05 were considered statistically significant.

RESULTS

Anthropometry and Body Fat Distribution

Table 1 shows the results for body weight, MRI fat area measurements of subcutaneous abdominal and gluteal fat depots, fat cell diameter, and CSA. Before cross–sex hormone administration, F-M transsexuals had a lower body weight but larger subcutaneous fat depots than M-F transsexuals. Following estrogen and antiandrogen treatment in M-F transsexuals, there was a significant gain in body weight accompanied by an increase in subcutaneous body fat ($P < .001$). Testosterone administration in F-M transsexuals significantly increased body weight, while subcutaneous fat area measurements as assessed by regional MRI were decreased ($P < .01$).

Fat Cell Size

At baseline, F-M transsexuals had larger subcutaneous fat cells in both abdominal and gluteal regions as compared with M-F transsexuals ($P < .001$; Table 1). Both M-F and F-M transsexuals had larger adipocytes in the gluteal region than in the abdominal region. Administration of ethinyl estradiol in combination with antiandrogens in M-F transsexuals induced an enlargement of subcutaneous fat cells in both regions ($P < .001$), and testosterone administration for 1 year in F-M transsexuals led to a significant decrease in both subcutaneous abdominal and gluteal fat cell size ($P < .01$). In each group (M-F or F-M transsexuals), the magnitude of the change in fat cell size was similar between subcutaneous abdominal and gluteal fat depots.

Relation Between Fat Cell Diameter and MRI Fat Area Measurements

MRI was used at body sites corresponding to the regions where fat biopsies were taken. MRI fat area measurements at the level of the umbilicus and the greater trochanter are shown in Table 1. Correlations between fat cell diameters and MRI fat area measurements assessed at baseline and after the 1-year treatment are presented in Table 2.

The median and range for the percentage change in MRI fat area measurements and cross-sectional adipocyte areas were calculated. For both M-F and F-M transsexuals, these figures are of a similar magnitude for the abdominal level. For M-F transsexuals, the increase was 57% (−13% to 112%) for abdominal adipocytes and 66% (11% to 132%) for abdominal

Table 1. Body Weight, MRI Fat Area Measurements, Fat Cell Diameter, and CSA of Subcutaneous Abdominal and Gluteal Fat Depots at Baseline and After 12 Months of Cross-Sex Hormone Administration in Transsexual Subjects

Variable	M-F Transsexuals (n = 19)		F-M Transsexuals (n = 17)	
	Baseline	After 1 Year	Baseline	After 1 Year
Body weight (kg)	65.4 ± 11.6	69.3 ± 11.3*	60.7 ± 11.8	63.4 ± 11.4*
MRI fat area measurements (cm^2)‡				
Abdominal	82 ± 38	124 ± 53*	164 ± 90	136 ± 71†
Hip	86 ± 44	149 ± 50*	175 ± 70	129 ± 54*
Fat cell diameter (μm)				
Abdominal	71 ± 13	86 ± 10*	85 ± 14	80 ± 1†
Gluteal	84 ± 13	100 ± 9*	102 ± 10	94 ± 8†
CSA ($\times 10^3$ μm^2)				
Abdominal	17.0 ± 5.9	24.0 ± 5.4*	23.5 ± 7.3	21.0 ± 5.9†
Gluteal	23.4 ± 6.8	32.7 ± 5.6*	33.7 ± 6.6	29.1 ± 5.3†

NOTE. Values are the mean ± SD.
*$P < .001$.
†$P < .01$.
‡Obtained from 18 M-F transsexuals.

MRI fat area measurements. For F-M transsexuals, there was a change of −11% (−36% to 7%) for abdominal adipocytes and −14% (−42% to 7%) for abdominal MRI fat area measurements. For measurements at the gluteal level, the percentage changes in the fat areas as measured by MRI were generally larger than the percentage changes in the cross-sectional adipocyte area. For M-F transsexuals, it was 46% (0% to 223%) for gluteal adipocytes versus 86% (17% to 190%) for gluteal MRI fat area measurements, and for F-M transsexuals, it was −18% (−32% to 18%) for gluteal adipocytes and −27% (−35% to −12%) for gluteal MRI fat area measurements, also on an individual basis (data not shown).

Lipolytic Activity

Results on basal and stimulated in vitro lipolytic activity (expressed as nanomoles of glycerol per milligram of TG) are graphically presented as box plots in Fig 1 for abdominal adipocytes and in Fig 2 for gluteal adipocytes. In our relatively lean study population, it was sometimes difficult to obtain enough fat aspirates for measurements, and in the figures, the number of subjects for whom measures on basal or stimulated lipolysis both before and after a 1-year treatment could be obtained are given in brackets. Within the two groups, no significant baseline differences were observed in basal lipolysis or stimulated lipolysis between both regions. Before hormone treatment, basal lipolytic activity in the abdominal region was significantly higher and the range of measurements was much wider in M-F transsexuals compared with F-M transsexuals ($P < .05$), while no significant differences were present in the basal lipolysis of gluteal adipocytes in the two groups (Table 3). No baseline differences in stimulated lipolytic activity expressed per milligram of TG were observed between groups or between body sites after maximal stimulation with ISO (a pure β-adrenergic agonist) or ART (a mixed α_2- and β-adrenergic agonist) or after a combination of stimulation with ISO and partial inhibition of this effect by INS (ISO + INS).

After a 1-year treatment (estrogens + antiandrogens) in M-F transsexuals, a significant reduction in basal lipolytic activity was observed in both abdominal and gluteal regions ($P < .05$ *v* baseline). Stimulated lipolysis (ISO, ART, or ISO + INS) did not change significantly after a 1-year treatment in M-F transsexuals. Testosterone administration for 1 year in F-M transsexuals increased basal lipolysis in the abdominal region, without a significant change in basal lipolysis at the gluteal level. Also in this group, no changes were observed in the stimulated lipolytic activities.

Significant negative correlations (baseline and after a 1-year treatment) were observed between fat cell size and in vitro basal glycerol release (expressed per milligram of TG) for both regions in both M-F and F-M transsexuals ($-.52 \leq r \leq -.74$, $P < .05$), except for a nonsignificant correlation of −.44 in abdominal adipocytes of F-M transsexuals at baseline.

A calculation of the percent stimulation of lipolysis over basal lipolysis could only be performed for subjects in whom sufficient fat tissue was available for all lipolytic measurements (both basal and stimulated). These data are available in a limited number of subjects, as the amount of aspirated fat was not sufficient in all subjects. Median values for absolute lipolytic activity in this subgroup of transsexuals were not different from those of the whole group (data not shown). Results on the percent stimulation of lipolysis over basal lipolysis are presented in Table 4. After a 1-year treatment with estrogens + cyproterone acetate in M-F transsexuals, the percent stimulation over basal lipolysis in the abdominal region was significantly larger for ART, ISO, and ISO + INS ($P = .05$), whereas this percentage was not different for the gluteal region before and

Table 2. Spearman Rank Correlation Coefficients Between Fat Cell Diameters and MRI Fat Areas at the Abdominal and Gluteal Fat Depots at Baseline and After a 1-Year Cross-Sex Hormone Administration in Transsexuals

Variable	MRI Fat Area Measurements (cm^2)			
	M-F Transsexuals		F-M Transsexuals	
	Baseline	After 1 Year	Baseline	After 1 Year
Fat cell diameters (μm)				
Abdominal	.71†	.31	.94†	.92†
Gluteal	.85†	.46	.89†	.59*

*$P < .05$.
†$P < .01$.

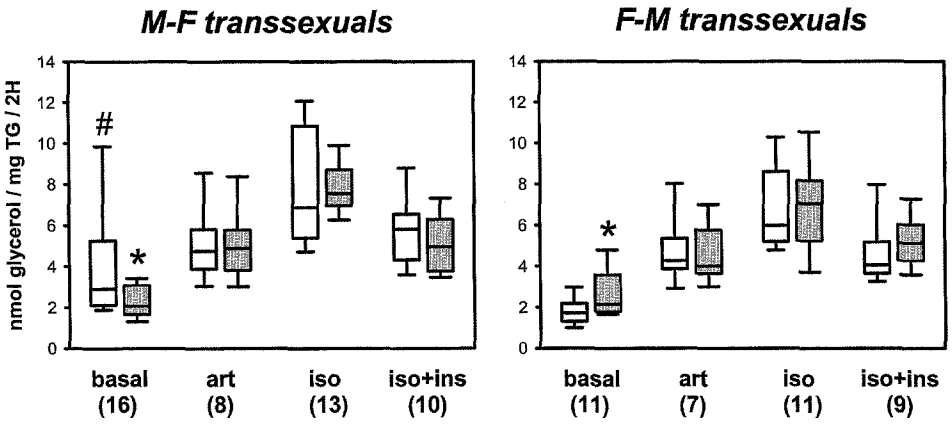

Fig 1. Box plots of basal and stimulated in vitro lipolytic activity of abdominal adipocytes at baseline (□) and after a 1-year cross–sex hormone administration (▩) in M-F and F-M transsexuals. *$P < .05$ *v* before cross–sex hormone administration; #$P < .05$, M-F *v* F-M transsexuals at baseline (before cross–sex hormone administration).

after treatment. After a 1-year testosterone administration in F-M transsexuals, the percent stimulation of lipolysis by ART over baseline was significantly reduced versus pre–hormone administration in both the abdominal and gluteal regions ($P < .05$).

It is known from the literature that fat cell size and fat cell density are both significant determinants of lipolytic activity.[21-23] Because changes in fat cell size and therefore changes in the number of fat cells per milligram of TG occurred during cross-sex treatment, lipolytic activity was also expressed as glycerol release per 10^5 cells per CSA (a measure of fat cell size), and essentially the same results were obtained (data not shown).

DISCUSSION

The present study shows that cross–sex steroid administration in transsexual subjects affects in vitro basal lipolytic activity, expressed as glycerol release per milligram of TG, and fat cell size in both abdominal and gluteal fat depots, accompanying changes in the amount of body fat in these regions as assessed by MRI.

Fat Cell Size

In the present study, female subjects had larger fat cells in the subcutaneous depots before treatment than male subjects, in accordance with the general observation that women have larger subcutaneous fat stores compared with men. In both our female and male subjects, gluteal fat cells were consistently larger than abdominal fat cells. In previous studies, it was shown that only non-obese premenopausal women had a specific enlargement of fat cells in the gluteofemoral region as compared with the abdominal depot, while fat cells in these regions were not different in size in young non-obese men and postmenopausal women.[9,11,12,14,22] However, Mauriège et al[24] also reported larger fat cells in the gluteal region in comparison to the abdominal region in young non-obese men. Increased body fatness in men has been shown to be associated with larger fat cells at the abdominal level. Treatment with estrogens in combination with cyproterone acetate in M-F transsexuals increased the mean cell size of both abdominal and gluteal adipocytes, and no regional difference was observed in fat cell enlargement. This latter observation is not consistent with a study by Krotkiewski and Björntorp,[25] in which 22 older men (mean age, 72 ± 9 years) treated with estrogens for prostate carcinoma showed a specific increase in gluteal, but not abdominal, adipocyte size without a significant change in body weight.

In M-F transsexuals treated with estrogens and antiandrogens, the increase in subcutaneous fat areas assessed by MRI was generally larger than expected on the basis of the increase in the cross-sectional areas of the adipocytes, in particular at the level of the hip. Although we did not measure it, this may suggest that besides the hypertrophy of adipocytes, an increase in the number of fat cells occurred. Adipocytes are capable of accumulating TG until a certain degree of filling, after which recruitment of new fat cells from preadipocytes can occur at any

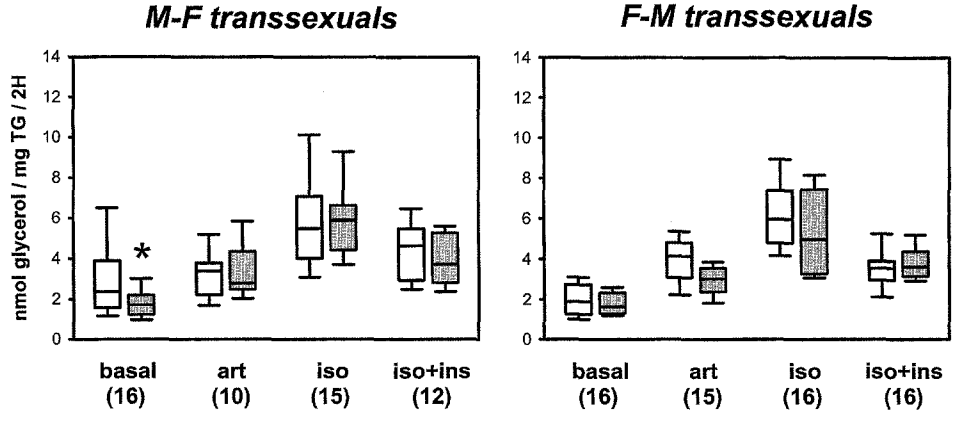

Fig 2. Box plots of basal and stimulated in vitro lipolytic activity of gluteal adipocytes at baseline (□) and after a 1-year cross–sex hormone administration (▩) in M-F and F-M transsexuals. *$P < .05$ *v* baseline.

Table 3. Basal Lipolytic Activity in Abdominal and Gluteal Adipocytes Before and After a 1-Year Cross–Sex Hormone Administration in Transsexual Subjects

Basal Lipolytic Activity (nmol glycerol/mg TG)	M-F Transsexuals			F-M Transsexuals		
	No.	Before	After 1 Year	No.	Before	After 1 Year
Abdominal	16	2.9 (1.7-11.9)	2.1 (0.9-5.9)*	11	1.7 (1.0-3.5)	2.2 (1.6-5.5)*
Gluteal	16	2.4 (0.8-6.8)	1.7 (0.9-3.3)*	16	1.9 (0.8-3.8)	1.6 (0.8-4.7)

NOTE. Values are the median and range. Number of subjects per assessment with measurements before and after 12 months' treatment are shown.

*$P < .05$.

stage of life.[22] The factors that trigger preadipocyte differentiation in vivo have yet to be determined. In vitro studies using cell culture systems demonstrated that 17β-estradiol promotes preadipocyte replication.[26] In a prospective study by Vague et al[27] in one M-F transsexual treated with estrogens for 34 months, an increase in the trochanter adipocyte size and number was demonstrated.

After a 1-year testosterone administration in F-M transsexuals, MRI fat area measurements showed a large decrease at the hip region, and this decrease can only be explained in part by the observed reduction in gluteal fat cell size. This may suggest that the number of fat cells at the gluteal level decreased in female subjects following testosterone treatment. This is in accordance with an early observation by Vague et al[28] that testosterone administration in three eunuchoids induced a considerable decrease in the number of adipocytes in the trochanter area, whereas it had little effect on adipocyte volume. Moreover, Vague et al[27] observed in one F-M transsexual that long-term testosterone administration led to an important decrease of trochanter adipocyte number and volume.

Lipolytic Activity

Regional differences in lipolysis are largely determined by the relative number of β and α_2-adrenoceptors on fat cells.[4,6,8] Sex steroid hormones are probably involved in the regulation of lipolysis by modulating receptor expression.[8,29] Before cross–sex hormone administration in the present study, a sex difference in in vitro basal lipolysis of abdominal adipocytes was observed, with a higher basal lipolytic activity in male versus female subjects. An interesting finding is that after a 1-year exposure to cross–sex hormones in transsexual subjects, significant changes occurred in in vitro basal lipolysis and the direction of these changes is in accordance with the observed changes in fat deposition as assessed by MRI measurements and fat cell size. This is also in line with the observed negative correlations between fat cell size and in vitro basal lipolytic activity both before and after a 1-year cross–sex hormone treatment; in other words, the higher the basal lipolysis and consequently fat mobilization, the smaller the fat cells (and vice versa). The reduction in basal lipolytic activity in both abdominal and gluteal adipocytes induced by estrogen + antiandrogen treatment in M-F transsexuals may have contributed to a reduced fat mobilization from these depots, leading to an enlargement of fat cells. Testosterone administration in female subjects significantly increased basal lipolytic activity in the abdominal region, which may partly explain the reduction in abdominal fat cell size. The effects of testosterone treatment in F-M transsexuals on gluteal adipocyte metabolism are less clear, as the amount of fat in this region was significantly reduced as shown by the mean MRI fat area measurements and fat cell size, but no appreciable effect on lipolytic activity was noted. These findings are comparable to those in middle-aged male subjects after testosterone treatment: an increase in basal lipolysis was observed in the abdominal region, while no change was found in the lipolytic activity of femoral cells.[30,31] No sex or regional differences in absolute lipolytic responsiveness to stimulatory agents were observed before hormone administration to our subjects. Cross–sex hormone administration to both M-F and F-M transsexuals did not significantly change the absolute stimulated lipolytic activity. This latter observation combined with a reduction in basal lipolysis resulted in a relative larger stimulation over basal lipolysis in

Table 4. Percent Increase in Lipolytic Activity by Stimulatory Agents Over Basal Lipolysis in Abdominal and Gluteal Adipocytes Before and After a 1-Year Cross–Sex Hormone Administration in M-F and F-M Transsexuals

	Increase in Lipolytic Activity by Stimulatory Agents Over Basal Lipolysis (%)					
	M-F Transsexuals			F-M Transsexuals		
Region	No.	Before	After 1 Year	No.	Before	After 1 Year
Abdominal	8			7		
ART		61 (17-247)	191 (48-346)*		156 (57-768)	83 (34-269)*
ISO		170 (0-318)	376 (163-517)*		328 (98-1,030)	285 (13-581)†
ISO + INS		78 (16-176)	158 (85-202)*		115 (56-841)	161 (38-356)
Gluteal	10			15		
ART		58 (2-191)	93 (21-492)		76 (14-462)	52 (16-201)*
ISO		104 (26-618)	330 (69-722)		188 (36-841)	244 (24-620)
ISO + INS		79 (35-303)	142 (39-387)		76 (16-372)	105 (37-321)

NOTE. Values are the median and range. Number of subjects per assessment with measurements before and after 12 months' treatment are shown.

*$P \leq .05$.

†$P = .06$.

the abdominal region by all stimulatory agents after a 1-year treatment with estrogens + antiandrogens in M-F transsexuals. The percent stimulation by ART (a mixed α_2- and β-adrenergic agonist) over basal lipolysis was significantly reduced after a 1-year testosterone administration in F-M transsexuals in both regions (abdominal and gluteal). In vivo studies in female and male hamsters showed that testosterone administration resulted in a specific increase in adipocyte α_2-adrenoceptor (antilipolytic) density, while no changes in the number of β-adrenoceptors were found.[32] Our human data are not supportive of an increase in the number of β-adrenergic receptors on adipocytes, as observed in rats.[29] However, it is unknown whether these animal data can provide an explanation for our observations in human adipocytes. Moreover, we investigated stimulated lipolysis only as the response to a maximally effective agonist concentration in only a limited number of subjects. In our young and lean study population, the amount of aspirated subcutaneous fat was often insufficient to perform all lipolytic measurements (both basal and stimulated). For the same reason, no dose-response measures with varying concentrations of the stimulatory agents could be performed.

In our study, we expressed lipolytic activity as glycerol release per unit of fat mass (milligrams of TG), as this objective method of expressing lipolysis data allows a better comparison between before and after treatment. During treatment, large changes occurred in the size and number of fat cells, which are both important determinants of lipolytic activity. Therefore, we also expressed lipolytic activity per 10^5 cells (to correct for the cell density at which the incubations were performed) per CSA, and essentially the same results were obtained (data not shown). Differences in the study populations and the methods of data expression render comparisons between studies difficult and may lead to contrasting findings. Basal lipolysis has repeatedly been shown to be higher in obese subjects compared with lean individuals both in vitro and in vivo,[33,34] but in vivo lipolysis in obesity is lower when expressed per unit of fat mass.[34,35]

In obesity, there are several reports on the positive correlations between the fat cell size and basal lipolytic rate,[22,36,37] whereas we found negative correlations between these variables in our lean individuals. Most of the obesity studies were performed in weight-stable obese adults, and the regulation of lipolysis may be different during dynamic phases of TG storage,[35] as also occurred in our subjects. Administration of estrogens in combination with antiandrogens in our M-F transsexuals induced an active period of changes in energy balance, reflected by the significant weight gain and body fat accumulation. This balance is also altered in several other conditions such as Cushing's syndrome[38] and during the process of aging,[39-41] with comparable effects on the lipolytic rate.

From our results, it seems reasonable to assume that exogenous sex steroids affected regional fat deposition by at least partly influencing basal lipolytic activity. Although this is a plausible mechanism, we only investigated the in vitro lipolytic activity of isolated adipocytes. In vitro results might not reflect the physiological situation, as blood flow and innervation are important for adipocyte metabolism in vivo.[35] Moreover, the in vitro technique is susceptible to measurement errors. Factors such as the fragility of isolated adipocytes and differences in the concentration of cell suspension added to the incubation medium or in the glycerol content at time zero may contribute to variability in measurements of lipolytic activity, but are not always easy to control. Besides affecting lipolytic activity, sex steroids might have induced changes in other aspects of regional adipocyte metabolism. For example, the observed reduction in gluteal fat cell size in the testosterone-treated F-M transsexuals might have been mainly due to a reduction in the activity of LPL, with is also reported by others.[30,31] Measuring LPL activity, fatty acid uptake, and glyceride synthesis might have provided additional explanations for the mechanism by which cross–sex hormone administration induces changes in fat cell size and in the amount of body fat.

In conclusion, cross–sex hormone administration in adult transsexual subjects affects the regional amount of body fat and fat cell size. The changes in regional fat deposition may be at least partly due to the observed changes in in vitro basal lipolytic activity. Estrogen administration in combination with cyproterone acetate in M-F transsexuals significantly reduced basal lipolytic activity in both the abdominal and gluteal regions with increases in fat cell size. Testosterone administration to F-M transsexuals increased basal lipolysis only at the abdominal level, while fat cell size increased in both the abdominal and gluteal regions.

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