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Plasma Adrenal, Gonadal, and Conjugated Steroids before and after Long Term Overfeeding in Identical Twins*

JANET PRITCHARD, JEAN-PIERRE DESPRÉS, JACQUES GAGNON, ANDRÉ TCHERNOF, ANDRÉ NADEAU, ANGELO TREMBLAY, AND CLAUDE BOUCHARD

Department of Physiology, Faculty of Medicine, Dentistry, and Health Sciences, University of Melbourne (J.P.), Victoria, Australia; Lipid Research Center (J.-P.D.) and the Diabetes Research Unit (A.N.), CHUL Research Center and Laval University, and the Physical Activity Sciences Laboratory, Laval University (J.G., A.T., C.B.), Quebec, Canada G1K 7P4; and the Department of Medicine, University of Vermont (A.T.), Burlington, Vermont 05405

ABSTRACT

An analysis of the data collected in the Quebec Overfeeding Study of identical twins was undertaken to determine any evidence of a genotype effect on plasma levels of adrenal and gonadal steroids arising from long term positive energy balance. Plasma levels of sex hormone-binding globulin (SHBG), testosterone, dihydrotestosterone (DHT), dehydroepiandrosterone sulfate (DHEA-S), androsterone glucuronide, androstane-3α,17β-diol glucuronide (3α-DIOL-G), and cortisol were measured in 12 pairs of young, sedentary, male monozygotic twins before and after 100 days of overfeeding. The dietary energy excess of 4.2 MJ/day (1000 Cal), 6 days a week, resulted in a total positive energy balance of 353 MJ (84,000 Cal). Overfeeding induced significant changes ($P < 0.0001$) in body weight and other measures of body composition. Within-twin pair resemblance was observed at baseline in all steroids, except cortisol [intraclass correlation range: DHEA-S, 0.50 ($P < 0.05$); DHT, 0.77 ($P < 0.001$)] and was lost with overfeeding, except for DHT and SHBG ($P < 0.05$). SHBG levels fell and 3α-DIOL-G rose with the gain in body fatness. The change in testosterone was a significant correlate of the change in upper body fat ($r = -0.48$; $P < 0.05$). The change in 3α-DIOL-G correlated positively with increases in all measures of central adiposity ($r = 0.52$; $P < 0.01$). A decrease in DHEA-S occurred with a higher, but not with a lower, gain in abdominal visceral fat ($P < 0.05$). Thus, analysis of adrenal and gonadal steroids and of conjugated metabolites before and after overfeeding in monozygous twins supports the idea that there is a genotype effect on steroid circulating steroid levels and that these blood levels are correlated with the pattern of body fat distribution. Moreover, the baseline within-twin pairs similarity in steroid levels was attenuated by prolonged positive energy balance and body fat gain. (*J Clin Endocrinol Metab* **83:** 3277–3284, 1998)

THE ASSOCIATION among body weight, patterns of fat distribution, and plasma steroid levels has attracted much scientific interest (1, 2). Cross-sectional studies of the metabolic profile and body composition in spontaneous human obesity have identified high correlations between adipose tissue mass and the levels of specific steroids in both women (3, 4) and men (5–8).

Dehydroepiandrosterone (DHEA), which has been found to be inversely associated with body fatness, has also been described as an antiobesity agent in rodents (9, 10). Androstenediol (Δ5-DIOL) was recently reported as the best single steroid correlate of body fatness and abdominal fat deposition in men (11). Sex hormone-binding globulin (SHBG) and testosterone (TESTO) have been shown to be reduced (8) in men and elevated (12) in women with increasing central adiposity. In men, plasma estrone, estradiol, and 3α-DIOL and its glucuronide metabolite (3α-DIOL-G) were positively associated (13, 14), and DHEA and its sulfated metabolite (DHEA-S) (15, 16) were negatively associated with total and regional body fatness. The high levels of cortisol found in obese women (4) and men (17) have been postulated to induce an insulin-related lipid-accumulating effect (18).

In an overfeeding study of Vermont state prisoners (17), cortisol was the only adrenal steroid measured. The observed increase was not significant when adjusted for the gain in body weight (19). No other overfeeding studies have reported the effect of altered body composition on gonadal or adrenal steroid and conjugated metabolite levels.

Interest in the influence of genes on blood steroid levels has arisen from reports based on twin and family studies. From a cross-sectional study of adrenal steroid levels in twins, Meikle and colleagues quantified the genetic influence on plasma steroid concentrations and tissue production (20). Familial factors were found to account for more than 50% of the variation in plasma hormone levels in identical twins, with between 1–76% accounted for by genetic effects on specific hormones. Whether these relationships are maintained when body composition phenotypes are controlled for has not been reported.

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Address all correspondence and requests for reprints to: Claude Bouchard, Ph.D., Physical Activity Sciences Laboratory, Division of Kinesiology, Department of Social and Preventive Medicine, Faculty of Medicine, Pavillon de l'Education Physique et des Sports, Laval University, Ste-Foy, Quebec, Canada G1K 7P4. E-mail: claude.bouchard@kin.msp.ulaval.ca.

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TABLE 1. Effect of 100 days of overfeeding on body composition in 12 pairs of male twins

Variable	Before overfeeding	After overfeeding
Body composition		
BW (kg)	60.3 ± 8.0	68.4 ± 8.2[a]
% Fat	11.3 ± 5.0	17.8 ± 5.7[a]
Fat mass (kg)	6.9 ± 3.5	12.3 ± 4.5[a]
Fat-free mass (kg)	53.4 ± 6.6	56.1 ± 6.7[a]
BMI	19.7 ± 2.0	22.4 ± 2.0[a]
Skinfold thickness		
Total (mm)	75.9 ± 21.1	129.4 ± 32.9[a]
Trunk (mm)	42.5 ± 15.1	79.4 ± 24.5[a]
Extremities (mm)	33.4 ± 7.4	50.0 ± 11.8[a]
CT-assessed fat distribution		
Abdominal		
Total (cm^2)	106 ± 46	199 ± 50[a]
sc (cm^2)	72 ± 40	141 ± 46[a]
Visceral (cm^2)	34 ± 9	58 ± 15[a]
Femoral		
Total (cm^2)	87 ± 36	151 ± 42[a]

Values are expressed as the mean ± SD.
[a] $P < 0.0001$.

Previous reports of the Quebec long term overfeeding study of 12 pairs of monozygous twins have described the changes in body weight, fat distribution, lean body mass, adipose tissue lipolysis, energy expenditure, and plasma levels of thyroid hormones, glucose, insulin, and glucagon (21–27). Here, further analysis of the data collected in this intervention study was undertaken to describe the changes in adrenal and gonadal steroids and their associations with changes in body mass and composition. An important goal was to test the hypothesis that individual differences in steroid levels in response to chronic overfeeding are compatible with a genetic model.

Subjects and Methods

Subjects

Twelve pairs of young, sedentary, male twins (aged 21 ± 2 yr) gave written consent to participate in an overfeeding study approved by the Laval University medical ethics committee and the Office for the Protection from Research Risks of the NIH (Bethesda, MD). The specific aims, study design, and methodology have been previously described in detail (21, 22).

The twins were established as monozygous and had no recent illness or history of obesity, diabetes, hyperlipidemia, hypertension, or endocrinopathy. They were fed an energy surplus for 6 days/week for 100 days while maintaining a sedentary, controlled level of energy expenditure. Subjects were housed at the Laval University campus for 120 days under 24-h supervision, such that diet and physical activity were closely monitored to ensure compliance. The first 14 days constituted a baseline period involving 3 days of testing and the establishment of the energy cost of weight maintenance while subjects ate freely from prepared diets. During the intervention, the subjects consumed a total excess energy of 353 MJ (84000 Cal) above baseline maintenance energy needs on 84 of the 100 days [4.2 MJ (1000 Cal)/day]. The nutrient contribution to the energy content of the diet was 50% carbohydrate, 35% lipid, and 15% protein. The subjects' schedule of sedentary activities included playing video games, reading, playing cards, watching television, and walking 30 min/day. There was a postoverfeeding testing period of 6 days.

Testing before and after the overfeeding period

Dietary analysis. All of the foods selected and the plate waste remaining during the baseline period were recorded and weighed for each subject, and the nutrient and energy composition were derived from a computerized analysis using the Canadian food composition tables (28) to establish energy required for weight maintenance. During the overfeeding period, daily energy intakes were monitored, and top-up portions were provided to ensure that individual energy intakes provided the excess 4.2 MJ (1000 Cal)/day required.

Body composition. Observations before and after overfeeding as well as daily measurements during the intervention involved body weight measured at the same time daily with subjects wearing light exercise shorts, body density determined before and after overfeeding by underwater weighing (29) using the helium dilution technique to measure pulmonary residual volume (30) and the Siri equation to estimate percent body fat (31), fat mass and fat-free mass obtained from percent body fat and body weight (kilograms), and skinfold thickness measurements (millimeters) at 10 sites [5 trunk (subscapular, suprailiac, abdominal, midaxillary, and chest) and 5 extremities (biceps, triceps, front midthigh, suprapatellar, and medial calf)] according to standardized procedures (32). Computed tomography (CT) was performed before and after overfeeding with a Siemens Somatom DRH scanner (Erlangen, Germany) according to the method described by Sjöström *et al.* (33) to determine abdominal visceral fat, abdominal subcutaneous (sc) fat, total abdominal fat, and total femoral fat areas (square centimeters).

Biochemical analyses. Blood samples were obtained 24 h after termination of the overfeeding treatment after an overnight fast between 0730–0800 h in the morning for the determination of plasma steroid levels and for fasting glucose, insulin, and glucagon levels. Plasma insulin levels were determined by RIA, as described by Oppert *et al.* (27). The insulin assay reliability was analyzed in a batch at completion of the study, and the coefficient of variation was 10.8%. The steroids were measured by RIA after separation of conjugated and unconjugated steroids by C_{18} column chromatography, as described previously by Bélanger *et al.* (34). Sulfate derivatives were submitted to hydrolysis. Glucuronide conjugates were also submitted to hydrolysis with β-glucuronidase. Steroids from each fraction were further separated by elution on LH-20 columns. Levels of the steroids were measured by RIA as previously described (35). Plasma SHBG levels were measured by the direct immunoradiometric method using a commercial kit from Farmos Diagnostic (Turku, Finland).

Among all the steroids measured, six have been identified as having high assay reproducibility. Their analytical errors ranged between 6.6–11.9% (except for 3α-DIOL-G and ADT-G with 19.4% and 21.5%, respectively) and an intraclass correlation coefficient for repeated assays greater than 0.96 (except for 3α-DIOL-G and ADT-G with coefficients of 0.80% and 0.81%, respectively; Gagnon, J., et al., personal communication). The day to day variability for three of the six steroids ranged from 8.8–13.5% (3α-DIOL-G and ADT-G had coefficients of variation of 22.3% and 23.4%, respectively), with intraclass correlation coefficients above 0.94 (except for 3α-DIOL-G and ADT-G with coefficients of 0.73 and 0.77). Cortisol had a high day to day variation (26.0%), but its analytical error was low (6.6%) with an intraclass correlation coefficient for repeated assays of 0.98.

The baseline plasma levels of these steroids were within the normal range for adult men (36). The postoverfeeding level of TESTO of one subject was 5 SD above the mean for all subjects. It was removed from the statistical analysis to avoid an excessive contribution to the results from this particular individual. Its removal did not alter the significance of changes in TESTO or the direction of correlations. One testosterone postoverfeeding level was also missing.

Statistical analysis. The effects of overfeeding on the body fat phenotypes and the interaction between genotype- and intervention-induced changes were assessed with a two-way ANOVA for repeated measures on one factor (time) (21). The twins were considered nested within the pair, whereas the treatment effect was considered a fixed variable. The intraclass correlation coefficient was computed from the between-pairs and within-pairs means of squares and was used to quantify the similarity within pairs for plasma adrenal and gonadal steroids. Correlation analysis was undertaken to estimate the association between overfeeding-induced changes in body fat with 1) the preoverfeeding (baseline) plasma adrenal and gonadal steroid levels, and 2) the overfeeding-induced changes in plasma adrenal and gonadal steroid levels, with 24 subjects (22 for TESTO) considered as independent individuals. Analyses were conducted with and without adjustment for changes in total fat mass whenever appropriate. Statistical analyses were performed with the SAS statistical package (version 6.12 for Windows, SAS Institute, Cary, NC).

TABLE 2. Effect of overfeeding on plasma adrenal, gonadal, and conjugated steroid levels and within-twin pair resemblance in response to the intervention in 12 pairs of male twins

Steroid	Before (nmol/L)[a]	After (nmol/L)[a]	Change (nmol/L)[b]	Intrapair resemblance in response	
				F ratio	Intraclass coefficient
SHBG	18.4 ± 3.6	15.3 ± 2.6	−3.1 ± 0.4[c]	2.62	0.45
TESTO	13.7 ± 2.7	14.5 ± 2.4[d]	0.9 ± 0.6[d]	0.95	0.0
DHT	3.0 ± 0.9	3.2 ± 0.9	0.2 ± 0.3	2.84	0.48[e,f]
Cortisol	229 ± 69	201 ± 68	−28 ± 18	0.77	0.0
DHEA-S	3456 ± 1293	3432 ± 1494	−24 ± 34	1.40	0.17
ADT-G	56 ± 18	61 ± 26	4.3 ± 4.2[f]	1.96	0.32
3α-DIOL-G	8.1 ± 2.8	11.3 ± 4.9	3.1 ± 0.9[g]	1.48	0.19

From a two-way ANOVA with repeated measurements on one factor (time) and twins nested.
[a] Mean ± SD.
[b] Mean ± SEM.
[c] $P < 0.0001$.
[d] n = 22.
[e] $P < 0.05$.
[f] Significant after adjustment for gain in fat mass.
[g] $P < 0.01$.

Results

Body composition

Changes in body weight and measures of body composition with overfeeding in the Quebec Overfeeding Study have been reported previously (21). Overfeeding induced significant changes ($P < 0.0001$) in body weight, measures of body composition, and fat distribution (Table 1).

Plasma adrenal and gonadal steroids and SHBG

Effects of overfeeding. Plasma levels of the sex hormone transport protein SHBG fell significantly (15.9 ± 1.6%; $P < 0.0001$), whereas levels of 3α-DIOL-G rose (41.3 ± 9.7%; $P < 0.01$). There was no change in DHEA-S, TESTO, DHT, ADT-G, or cortisol (Table 2). After adjustment for change in fat mass, the changes in SHBG and 3α-DIOL-G were no longer significant, and the change in ADT-G became statistically significant ($P < 0.05$). The percent changes in the steroids after overfeeding are illustrated in Fig. 1.

Twin resemblance. Table 3 shows the within-twin pair resemblance for the absolute plasma levels of the steroids before and after overfeeding. The similarity within twin pairs at baseline was highest for SHBG and DHT levels (intraclass coefficient, 0.76 and 0.77, respectively; $P < 0.001$). There was more than 7 times the variance between pairs as within pairs for these steroids. Concentrations of DHEA-S, TESTO, ADT-G, and 3α-DIOL-G also showed significant within-pair resemblance. For these steroids, the variance between pairs was 2.7- to 3.8-fold higher than that within pairs. Cortisol was the only steroid that showed no significant within-pair resemblance at baseline.

After overfeeding, only SHBG and DHT levels were characterized by a significant within-pair resemblance (intraclass coefficient, 0.55 and 0.48, respectively; $P < 0.05$), with the variance between pairs being 3.5 and 2.8 times greater than the variance within pairs for these steroids. After adjustment for gain in body fat mass, only postoverfeeding DHT retained a significant within-pair resemblance (intraclass coefficient, 0.48; $P < 0.05$) as shown in Table 3. The within-twin pair resemblance in the response of plasma TESTO, as an example of a steroid not exhibiting twin-pair similarity, and of plasma DHT (the only steroid characterized by an intrapair resemblance and response) levels to the overfeeding treatment is illustrated in Fig. 2.

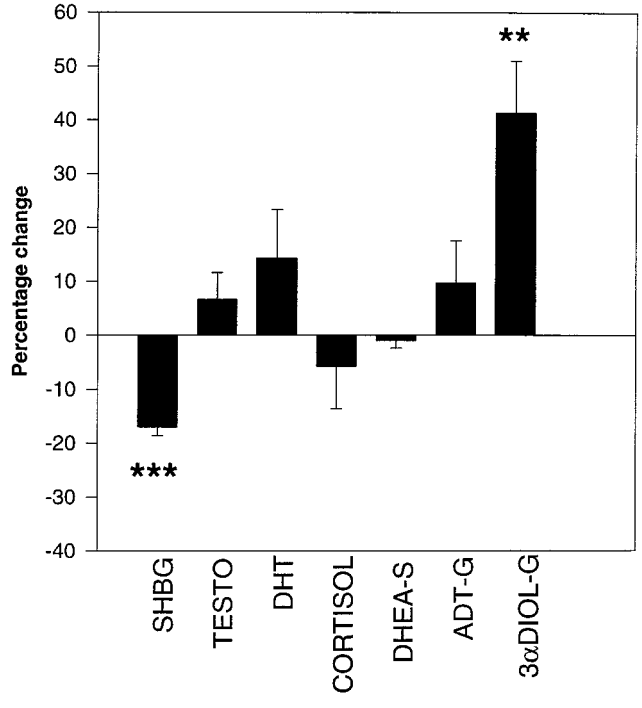

FIG. 1. Mean percent change (±SEM) in plasma steroid levels with overfeeding. **, $P < 0.01$; ***, $P < 0.0001$.

Association between plasma steroid levels and body fat phenotypes

Body composition. The associations between body composition phenotypes and plasma steroid levels were investigated by calculating the correlation coefficients with all men considered as independent subjects before and after overfeeding, with and without adjustment for the changes in fat mass after the intervention. Positive correlations were found between baseline TESTO and baseline body weight (r = 0.40) and between baseline 3α-DIOL-G and body weight and fat mass (r = 0.52 for both), whereas correlations were negative between baseline DHEA-S and body weight (r = −0.59) and

TABLE 3. Similarity within-twin pairs in plasma levels of adrenal, gonadal, and conjugated steroids before and after overfeeding

Variable	Twin resemblance before overfeeding			Twin resemblance after overfeeding		
	F ratio	Intraclass coefficient	*P* level	F ratio	Intraclass coefficient	*P* level
SHBG	7.28	0.76	<0.001	3.48	0.55	<0.05
TESTO	2.74	0.47	<0.05	1.39	0.16	0.31
DHT	7.64	0.77	<0.001	2.83	0.48	<0.05[a]
Cortisol	0.59	0.0	0.81	0.73	0.0	0.70
DHEA-S	2.99	0.50	<0.05	1.40	0.17	0.29
ADT-G	3.75	0.58	<0.05	1.61	0.23	0.22
3α-DIOL-G	3.80	0.58	<0.05	0.85	0.0	0.60

Statistical significance was determined by a two-way ANOVA for repeated measures on one factor (time). The F ratio was the ratio of the variance between pairs to that within pairs.

[a] Significant after adjustment for gain in fat mass.

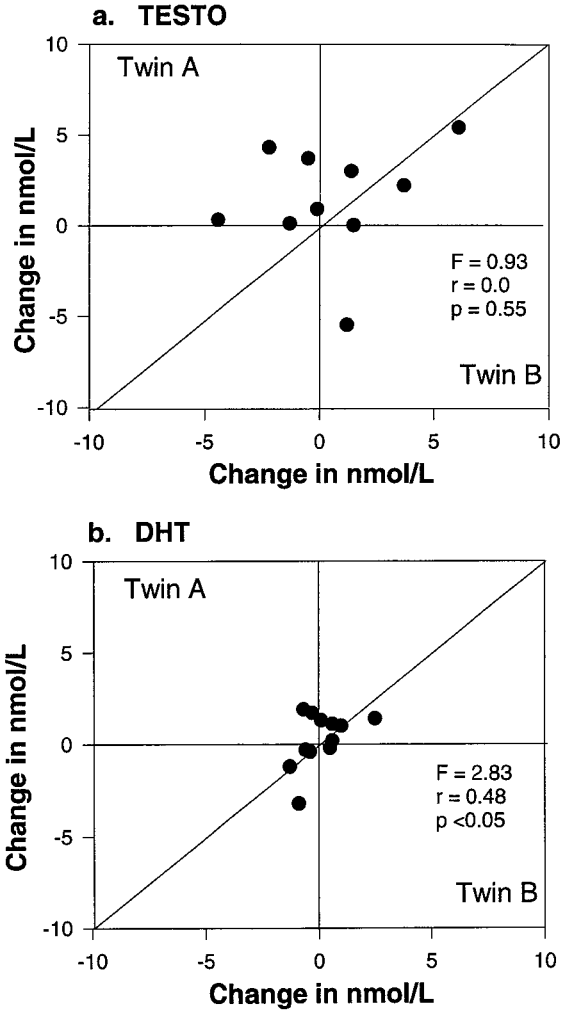

FIG. 2. Within-twin pair resemblance for the changes with overfeeding in TESTO (a; n = 10 pairs) and DHT (b; n = 12 pairs).

fat mass (r = −0.46) and between baseline ADT-G and body weight (r = −0.41; Table 4a). Baseline cortisol showed a negative correlation (r = −0.57) with change in body weight with overfeeding, whereas the baseline level of 3α-DIOL-G was positively correlated with the postoverfeeding changes in body weight and fat mass (r = 0.52 for both; Table 4b). Changes in levels of cortisol and ADT-G were negatively correlated with the change in body weight with overfeeding (r = −0.48 and r = −0.46, respectively). Conversely, the change in 3α-DIOL-G was positively correlated with the change in fat mass with overfeeding (r = 0.52; Table 4c).

Body fat distribution. Baseline DHEA-S was negatively correlated with baseline CT measures of abdominal and femoral fat, and baseline ADT-G was negatively correlated with baseline trunk to extremities skinfold ratio (Table 4a), but the significance of these associations was lost after adjustment for fat mass. However, baseline levels of TESTO and cortisol correlated negatively with increases in measures of abdominal fat after overfeeding (Table 4b), and baseline 3α-DIOL-G, which correlated positively with changes in total abdominal fat and trunk skinfolds, retained significance after adjustment for fat mass (Table 4b; TESTO with trunk to extremity ratio of skinfolds, r = −0.52; TESTO with CT assessed visceral fat, r = −0.44; cortisol with trunk skinfolds, r = −0.43).

Correlations between changes in plasma steroid levels with changes in body fat distribution phenotypes after overfeeding were significant and negative for TESTO with visceral fat (r = −0.46), for cortisol with abdominal sc and visceral fat (r = −0.49 and r = −0.52, respectively), for DHEA-S with trunk to extremity skinfold ratio (r = −0.48), and for ADT-G with abdominal sc fat (r = −0.48; Table 4c). Conversely, correlations were positive for 3α-DIOL-G with abdominal sc fat and total abdominal fat (r = 0.51 and r = 0.44, respectively). The change in femoral fat was negatively correlated with the change in ADT-G (r = −0.41) and positively with the change in 3α-DIOL-G (r = 0.59). It is noteworthy that only the correlations of TESTO and 3α-DIOL-G retained significance after adjustment for total fat gain.

Postoverfeeding levels of TESTO showed significant negative correlation with the postoverfeeding CT measure of abdominal visceral fat (r = −0.46) retained after adjustment for fat mass (Fig. 3a). Similarly, plasma levels of DHEA-S after overfeeding were inversely correlated with abdominal sc fat (r = −0.42, with adjustment for fat mass; Fig. 3b), whereas postoverfeeding levels of 3α-DIOL-G showed a positive correlation with the postoverfeeding skinfold trunk to extremity ratio (r = 0.52, with adjustment for fat mass; Fig. 3c). Plasma SHBG was not correlated with body fat phenotypes at baseline or after overfeeding or with postoverfeeding values.

In summary, these correlations indicate that high TESTO levels were associated with a low level of abdominal fat. The steroid precursor DHEA-S and the metabolite ADT-G were also negatively correlated with abdominal fat, whereas the andro-

TABLE 4a. Correlations between baseline plasma steroid levels and baseline body composition and fat distribution

Baseline steroid	Baseline measures							
	BW	FM	SKF trunk	SKF-TER	AVF	ASF	TAF	TFF
TESTO	0.40[a]							
DHEA-S	−0.59[b]	−0.46[a]				−0.56[b]	−0.56[b]	−0.52[b]
ADT-G	−0.41[a]			−0.49[a]				
3α-DIOL-G	0.52[b]	0.52[b]						

4b. Correlations between baseline plasma steroid levels and changes in body composition and fat distribution in response to overfeeding

Baseline steroid	Changes in response to overfeeding							
	BW	FM	SKF trunk	SKF-TER	AVF	ASF	TAF	TFF
TESTO				−0.52[b]	−0.44[a]			
DHT				0.41[a]				
Cortisol	−0.57[a]		−0.43[a]					
3α-DIOL-G	0.52[b]	0.52[b]	0.65[c]					

4c. Correlations between changes in plasma steroids and changes in body composition and fat distribution in response to overfeeding

Changes in steroid	Changes in response to overfeeding							
	BW	FM	SKF trunk	SKF-TER	AVF	ASF	TAF	TFF
TESTO					−0.46[a]			
Cortisol	−0.48[a]					−0.49[b]	−0.52[b]	
DHEA-S				−0.48[a]				
ADT-G	−0.46[a]	−0.46[a]				−0.48[a]		−0.41[a]
3α-DIOL-G		0.52[b]				0.51[b]	0.44[a]	0.59[a]

FM, Fat mass; SKF trunk, sum of five trunk skinfold measurements; SKT-TER, ratio of sum of five trunk to five extremities skinfold measurements; AVF, abdominal visceral fat; ASF, abdominal sc fat; TAF, total abdominal fat; TFF, total femoral fat.

[a] $P < 0.05$.

[b] $P < 0.01$.

[c] $P < 0.001$.

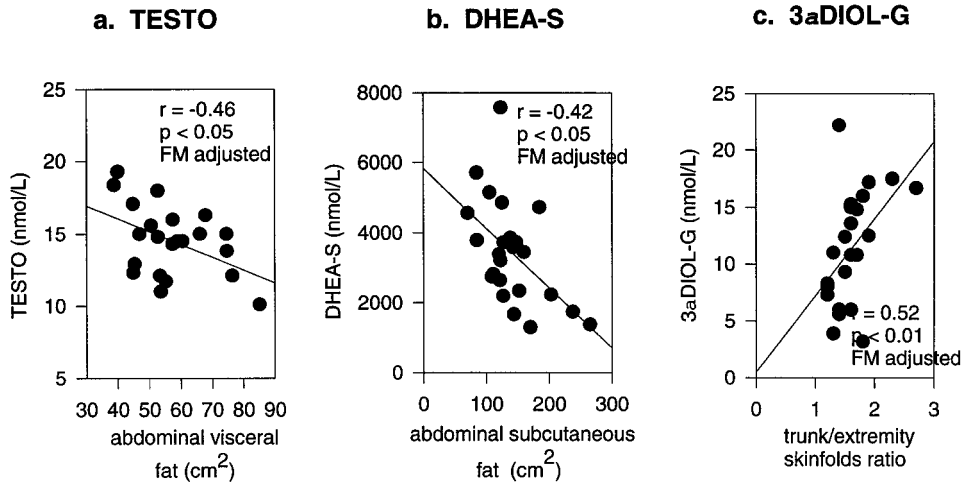

FIG. 3. Correlations between plasma steroid levels and measures of abdominal fat after overfeeding. Values have been adjusted for the level of fat mass.

gen metabolite 3α-DIOL-G was in positive correlation with total and regional fat measures before and after overfeeding.

Individual subjects were subdivided into high gainers and low gainers of body fat phenotypes (n = 6 in each group, without two members of the same pair) in an attempt to assess differences with respect to baseline steroid levels or changes with overfeeding. High fat gainers had lower postoverfeeding change in TESTO levels than low fat gainers ($P < 0.01$). ADT-G showed a similar pattern ($P < 0.05$), whereas 3α-DIOL-G differences almost reached the significance level ($P < 0.06$), but DHEA-S levels did not. This relationship is shown in Fig. 4a. Subjects were also subdivided on the basis of high and low abdominal visceral fat gainers with overfeeding. The postoverfeeding changes in DHEA-S in the high visceral fat gainers were significantly different from those in the low gainers ($P < 0.01$). There was no difference between these two subgroups for the overfeeding-induced changes in TESTO, ADT-G, and 3α-DIOL-G (Fig. 4b).

Relationship between insulin and steroids levels. It has been proposed that sex hormone levels are related to hyperinsulinemia through obesity (14). Whether there was any relationship between insulin levels and steroid levels before and after overfeeding was examined. Baseline fasting insulin was not

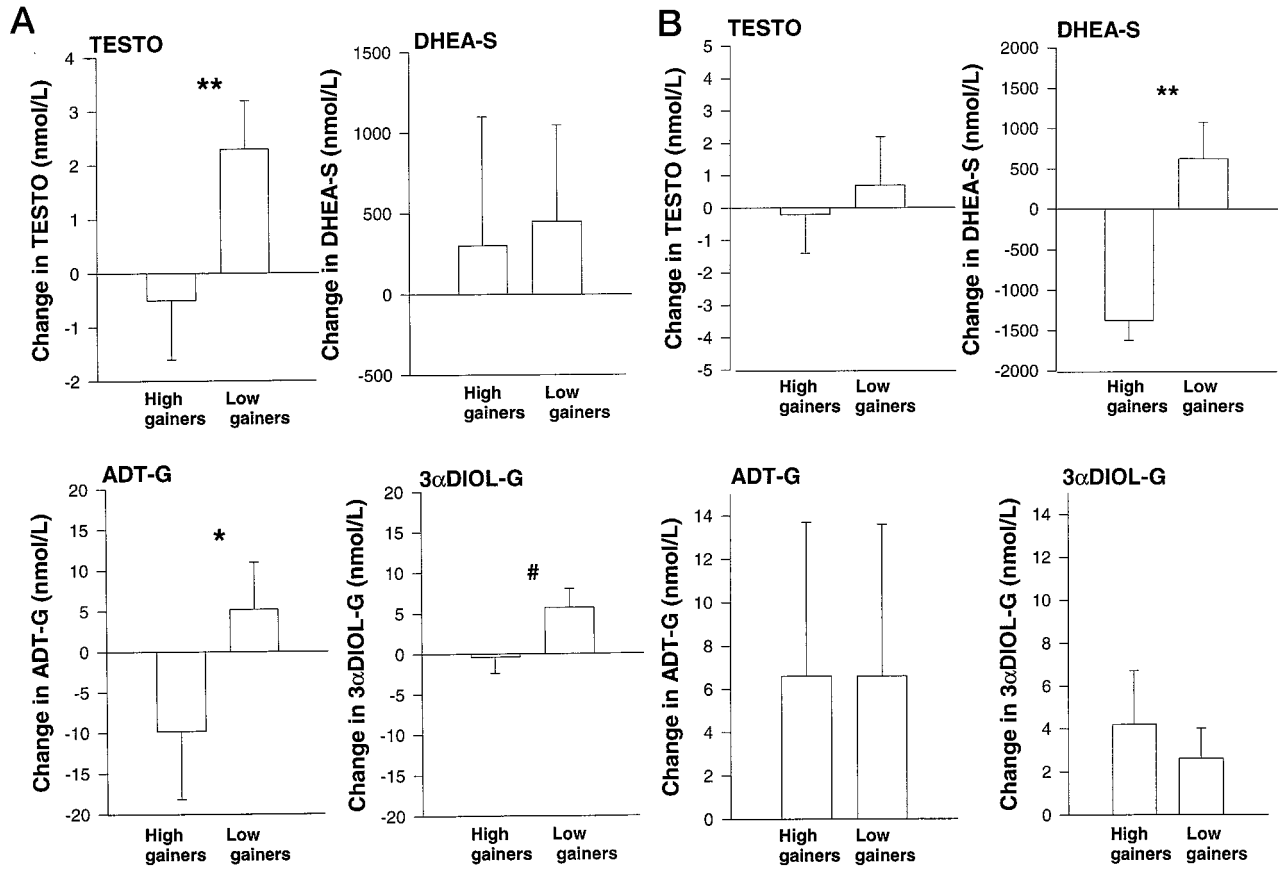

FIG. 4. a, Mean changes (±SEM) in plasma steroids (TESTO, DHEA-S, ADT-G, and 3α-DIOL-G) between high gainers (n = 6) and low gainers (n = 6) of fat mass with overfeeding. b, Mean changes (±SEM) in plasma steroids (TESTO, DHEA-S, ADT-G, and 3α-DIOL-G) between high gainers (n = 6) and low gainers (n = 6) of abdominal visceral fat with overfeeding. *, $P < 0.05$; **, $P < 0.01$; #, $P = 0.06$.

significantly correlated with baseline DHEA-S, DHT, ADT-G, or 3α-DIOL-G, but was negatively correlated with the changes in TESTO with overfeeding (r = −0.48, adjusted for change in fat mass). A two-way ANOVA for repeated measures on time in fasting insulin and TESTO levels indicated a significant interaction effect (F ratio = 22.7; $P < 0.0001$ after adjustment for change in fat mass). Those subjects with higher baseline insulin showed a greater overfeeding-induced decrease in TESTO (r = −0.54; Fig. 5). However, the correlation between the changes in insulin and the changes in TESTO with overfeeding was not significant (r = 0.27, after adjustment for change in fat mass).

Discussion

All plasma adrenal and gonadal steroids, except cortisol, showed significant within-twin pair resemblance at baseline. The sex hormone carrier SHBG and the active androgen DHT showed the highest indexes of intrapair resemblance (intraclass correlation, 0.77 and 0.76, respectively), whereas TESTO, the sulfated precursor DHEA-S, and the androgen-conjugated metabolites ADT-G and 3α-DIOL-G were all characterized by intraclass coefficients of about 0.50 or better. With overfeeding, the twin resemblance remained significant for SHBG and DHT. These results suggest that genetic differences play a role in the overfeeding-associated changes in plasma steroid levels. A high genetic effect on the plasma levels of 3α-DIOL-G and a moderate influence on TESTO and DHT have been reported in a previous study of MZ and DZ twins (20). Earlier work with nontwin families had identified significantly less variability within brother siblings than among nonbrothers for SHBG and TESTO, but not DHT (37), although fathers and sons in the same sample of families were significantly correlated for DHT levels (38). A genetic effect has also been described for blood levels of DHEA-S in a study of steroid concentrations in 26 families (39). The analysis of the baseline steroid levels in the Quebec Overfeeding Study confirms these earlier reports.

Overfeeding substantially decreased the twin resemblance, particularly for those steroids with high baseline intrapair resemblance. Increases in measures of abdominal fat were correlated with decreasing TESTO levels even after adjustment for fat mass. Although there was high within-pair resemblance in abdominal fat after overfeeding (21), with the exception of DHT, the within-twin pair resemblance for the steroids, which were also highly correlated with abdominal fat measures, was lost after overfeeding. It is as if the putative genotype effect on steroid levels observed at baseline (from the intrapair resemblance) was disrupted or overwhelmed by the overfeeding or obscured by the body mass, body fat, and abdominal fat gains.

The present results also confirm previously reported relationships between SHBG, DHEA-S, TESTO, and 3α-DIOL-

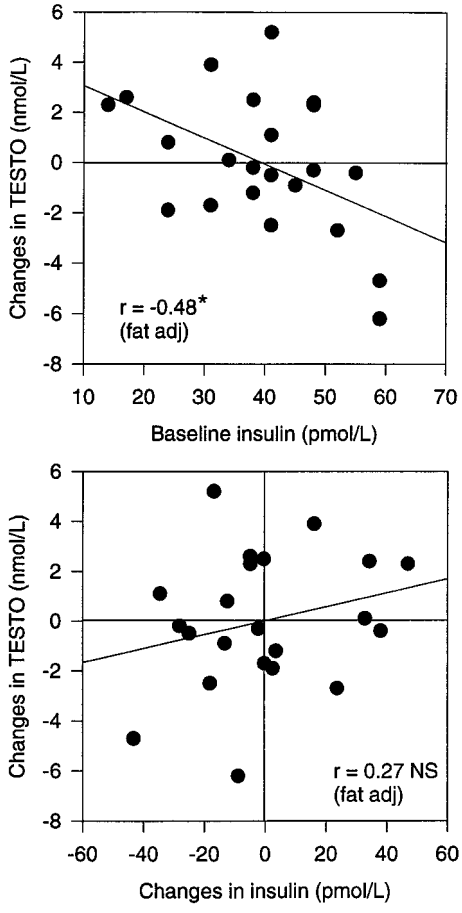

FIG. 5. Changes in TESTO (nanomoles per L) in response to overfeeding plotted against baseline fasting insulin levels (a) and change in fasting insulin levels in response to overfeeding (b).

G steroids and body fatness (7, 8, 11, 13, 15). In a cross-sectional study of middle-aged men, Seidell and colleagues (8) found that overweight men with high levels of body fat and visceral fat had lower concentrations of SHBG and TESTO. Accordingly, in the Quebec Overfeeding Study, SHBG levels fell in proportion to the gain in fat mass. Overall TESTO and DHEA-S levels did not change with overfeeding, but they were well correlated with the postoverfeeding body fat levels. In a cross-sectional analysis of 1241 randomly sampled middle-aged US men, Field *et al.* (15) showed decreased plasma adrenal steroid and sex hormone levels with increasing relative weight. Pasquali *et al.* (7) confirmed this relationship in a comparison of obese men with normal weight controls. In the Quebec Overfeeding Study, those who gained the least amount of body fat did not decrease their TESTO or ADT-G levels. Similarly, those who gained most abdominal fat had greater increases in plasma 3α-DIOL-G and greater decreases in plasma DHEA-S.

In a multivariate analysis of TESTO and the adrenal C_{19} steroid precursor Δ5-DIOL in a cross-sectional study of a sample of 80 middle-aged men, a greater association between steroid hormones and total fatness than between steroids and visceral fat accumulation was reported (11). The intervention design of the Quebec Overfeeding Study provided new insights into the relationships of the sex steroids to body fat distribution. Changes in TESTO were a significant correlate of changes in abdominal fat. A fall in DHEA-S was accompanied by a gain in abdominal visceral fat. The increase in 3α-DIOL-G levels with overfeeding was highly correlated with all measures of total and abdominal fat. Hence, the present study confirmed the positive associations recently described in a cross-sectional study of obese men (13). It also identified the baseline level of this steroid as a strong correlate to the changes in body mass, fat mass, and trunk skinfold thickness. However, the proposal of an association of elevated cortisol with an increased lean body mass accompanying the gain in body weight (1, 4) was not supported by the present study.

In men, secretion of TESTO and DHT from the testis contributes approximately 40–50% of active DHT, whereas a significant contribution to DHT formation comes from synthesis within the adrenal gland and peripheral conversion of sex hormone precursors such as DHEA-S (40). On the other hand, glucuronidation has been postulated as the major pathway for steroid elimination (34). Major glucuronide steroid conjugates in men (ADT-G and 3α-DIOL-G) have no intrinsic activity but, as they are more soluble, return to plasma for excretion through the urine. It has been suggested that glucuronide levels are markers of androgen metabolism (34, 41). The decrease in the sulfated DHEA precursor of active steroids with increasing adiposity suggests either a decline in the activity of the adrenal steroid synthetic pathway or a higher metabolic clearance of this water-soluble steroid with higher body fatness. The negative correlation of TESTO and the positive correlation of 3α-DIOL-G with increasing adipose tissue with overfeeding is in accordance with the proposal that the enzymatic control of active metabolism of TESTO may occur in adipose tissue (42). This correlation is also concordant with the recent finding of the expression of UDP-glucuronosyltransferase enzymes responsible for metabolism and elimination of androgens in omental and sc abdominal adipose tissue (43).

Hyperinsulinemia has been described as an endocrine correlate of high levels of body fat (44) and is consistently correlated with specific adrenal steroid levels (10). Variation in insulin levels in response to overfeeding in the present set of twins is characterized by a significant intrapair resemblance (27). It has also been postulated that hyperinsulinemia may be involved in the regulation of steroid metabolism (7). TESTO, which is known to stimulate lipid mobilization and to have an anabolic effect on lean body mass, has been reported to be negatively associated with the hyperinsulinemia and the insulin resistance state accompanying abdominal obesity in men (8, 14). The present study extends these findings to the demonstration of a relationship between the baseline insulin level and both the changes in TESTO and the changes in abdominal fat under the influence of overfeeding.

It should be pointed out that the subjects in the Quebec Overfeeding Study were young lean adults. Thus, even after a mean weight gain of about 8 kg, these young men were still normal weight at the end of the overfeeding period, with a mean body mass index of 22.4 kg/m^2 and a low level of CT-measured visceral fat (average area, 58 cm^2). Thus, the correlations reported herein are derived from men at the low end of the adiposity spectrum. It cannot be excluded that an overfeeding study conducted in overweight patients may

have yielded different correlation patterns with the changes in body fat or abdominal fat. This study cannot rule out the hypothesis that some of the observed plasma steroid changes are actually associated in part with the overconsumption of calories rather than with the changes in body mass, body composition, or fat topography. It would be useful to design a study to specifically address the latter issue.

In conclusion, the long term Quebec Overfeeding Study in identical twins has identified a significant within-twin pair resemblance for C_{19} steroids and conjugated metabolites before the overfeeding intervention. The twin resemblance was, however, attenuated with overfeeding for all steroids except DHT. DHT retained its within-twin pair resemblance after overfeeding independently of the gain in fat mass. Overfeeding-induced changes in fat mass and fat distribution were related inversely to cortisol, DHEA-S, and TESTO, but positively to 3α-DIOL-G. The change in plasma TESTO was a significant correlate of abdominal fat gain with overfeeding. The study has also confirmed an inverse relationship between hyperinsulinemia and TESTO with body fat gain. The pattern of plasma adrenal and gonadal steroid levels before and after overfeeding in these monozygous twins suggests that the genotype could be an important determinant of the sex hormones that influence the pattern of fat distribution in healthy young men. However, this apparent genetic determinism can be overcome by prolonged positive energy balance and the ensuing gain in body fat.

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