



Biochemistry/Physiology

Plasma steroids, body composition, and fat distribution: effects of age, sex, and exercise training

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Abstract

Background/objectives Plasma steroid hormone levels vary between men and women, but their associations with BMI and adiposity are controversial. Furthermore, little is known about the role of exercise programs on the relationship between steroid hormones and adiposity. This report evaluates these relationships for plasma levels of adrenal, gonadal, and conjugated steroids with body composition and fat distribution in sedentary men and women, aged 17–65 years, and their responses to an exercise program.

Subjects/methods In the sedentary state, 270 men (29% Blacks) and 304 women (34% Blacks) from the HERITAGE Family Study were available. Among them, 242 men and 238 women completed a 20-week fully standardized exercise program. Fourteen steroid hormones and SHBG concentrations were assayed in a fasted state and were compared for their associations with adiposity in men and women and in response to the exercise program. Covariates adjusted for in partial correlation analysis were age, ancestry, menopause status (women), and oral contraceptives/hormone replacement treatment status (women) at baseline, as well as baseline value of the trait for the training response. Differences among normal weight, overweight, and obese subjects were also considered. Statistical significance was set at $P < 0.0001$.

Results Baseline levels of dihydrotestosterone (DHT), 17 hydroxy progesterone (OHPROG), sex hormone-binding globulin (SHBG), and testosterone (TESTO) were negatively associated with fat mass and abdominal fat ($P < 0.0001$) in men and for SHBG in women ($P < 0.0001$). TESTO was not correlated with fat-free mass in men or women, but was significantly associated with % fat-free mass in men. No association was detected between baseline steroid hormone levels and changes in adiposity traits in response to 20 weeks of exercise.

Conclusion In men, low DHT, OHPROG, SHBG, and TESTO were associated with higher adiposity and abdominal and visceral fat. A similar adiposity profile was observed in women with low SHBG.

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Introduction

There is evidence that blood levels of steroid hormones and their binding globulins influence body composition and fat distribution, but the findings are controversial, and the magnitude of the associations is generally unknown.

In men [1–5], there are consistently inverse associations between testosterone (TESTO) and fat mass measures starting around 30 years of age [1–4]. In women, the relationship is positive in young [6] and middle-aged females adjusted for menopausal status [2] but negative after menopause [7]. The relation between lean mass and TESTO is rather controversial: while no associations were reported in some studies of men [2, 3, 8] and women [2, 6], other studies found a positive correlation in men [9, 10] and women [7] or an inverse association in men [5, 11, 12]. Declines in TESTO with age were not

associated with lean mass in either sex in late midlife [2].

Regarding body fat distribution, TESTO was shown to be negatively associated with various traits reflecting abdominal fat levels in men [1, 4, 12], whereas the relations were found to be nonsignificant [7, 13] or positive [14] in postmenopausal women. Previous findings are also equivocal for sex hormone-binding globulin (SHBG). In men, SHBG was negatively correlated with fat mass [1, 5, 11], indicators of abdominal fat accumulation [8, 10, 12], and lean mass [1, 3, 5, 11]. In women, SHBG was inversely associated with lean mass [2] and fat mass [15], whereas the association between SHBG and fat distribution was found to be nonsignificant [6, 16] or negative [15].

Some studies have reported positive associations between plasma levels of estradiol (E2) with lean mass [3], fat mass [1], body mass index (BMI), and waist circumference [17] in men, as well as with fat mass [18] and fat distribution in women [15]. Other reports have found no association with adiposity in men [19, 20]. Blood levels of DHT were negatively correlated with fat mass and abdominal fat accumulation traits in women [21], but the findings were not replicated in another study [7]. Dehydroepiandrosterone (DHEA) sulfate (DHEAS) was shown to be inversely [16, 22], positively [23], or not correlated [18, 21, 23, 24] with fat distribution in women.

One study dealt with the effects of regular exercise in older men. Baseline plasma levels of TESTO did not correlate with the beneficial effects of a 12-month, home-based, moderate-to-vigorous aerobic training program on body composition [25].

In summary, these conflicting observations may be caused by differences in age coverage (young [16], middle [2, 4], older [3, 10, 20], or mixed [1, 5, 9, 11, 12]), be specific to one sex [1, 3, 4], vary by ancestry (black [15], white, mixed [1], or undefined [26]), menopausal status (pre [2, 6, 15, 24], post [2, 7, 14, 18, 23, 27], early-post, late-post [13]), health status (health [6, 7], obesity [4, 16, 22], diseases [21]), physical activity level (activity [28], inactivity, sustained exercisers [8]), or methodology used to assess concentrations of steroid hormones, particularly androgens [29]. Furthermore, most reports are based on only one or a few hormones.

Here, we are taking advantage of the multicenter HERITAGE Family Study to examine the relationships between plasma steroid hormone levels with body composition and indicators of fat distribution in the sedentary state and after a standardized exercise training program in Blacks and Whites, men and women ranging in age from 17 to 65 years, using a panel of 14 steroid hormones plus SHBG.

Methods

Subjects

The HERITAGE Family Study cohort has been previously described [30]. Sedentary subjects aged 17–65 years took part in a standardized 20-week endurance exercise program. They were recruited at four institutions located in Austin (Texas), Phoenix (Arizona) (the site moved later to Indianapolis (Indiana)), Minneapolis (Minnesota), and Quebec City (Canada). Recruitment focused on nuclear families, and parents and their adult offspring were recruited. The study protocol had been previously approved by the institutional review board at each of the four clinical centers of HERITAGE. Informed written consent was obtained from each subject.

Anthropometric, body composition, and fat distribution measurements

Body weight, height, waist, and hip circumferences were measured following standardized procedures. Body density was measured using the hydrostatic weighing technique. The mean of the highest three (of 10) measurements was used in the calculation of percent body fat from body density using the equation of Siri for Caucasian men [31], Lohman for Caucasian women [32], Schutte et al. for Black men [33], and Ortiz et al. for Black women [34]. Fat mass was obtained by multiplying body weight by percent body fat (% body fat). These measurements have been shown to be highly reproducible with no difference among clinical centers or drift over time in the course of data collection [35]. Intraclass coefficients for repeated measurers and coefficients of variation ($SD/mean \times 100$) were calculated for all body composition and adiposity measures. Briefly, body fat percentage and fat mass calculated from body density as described above are characterized by intraclass correlations of 0.99 with CVs extending from 0.8% **for body fat percentage** to 0.6 kg **for fat mass** (based on three repeated tests over 3 weeks in 60 adults) [35].

The computed tomography (CT) scans were used to measure abdominal total fat (ATF), abdominal subcutaneous fat (ASF), and abdominal visceral fat (AVF) [36, 37]. The CT scans were obtained between the fourth (L4) and fifth (L5) lumbar vertebrae while subjects were supine with arms extended above the head. ATF and AVF areas were calculated by delineating the areas with a graph pen and then computing the surface areas by using an attenuation range of -190 to -30 Hounsfield units. The AVF area was measured by drawing a line within the inner portion of the muscle wall surrounding the abdominal cavity. ASF area was calculated by subtracting AVF from ATF. In a

recent paper, inter-raters and intra-rater intraclass coefficients for CT abdominal and visceral fat were of the order of 0.99 with CVs ranging from 0.5 to 2.2% [38]. Repeated measures based on a fatty phantom in HERITAGE generated test–retest correlations not different from 1.0 with a coefficient of variation around 1%.

Endurance exercise training program

Each subject in the HERITAGE study exercised three times per week for 20 weeks on cycle ergometers. The intensity of the training was customized for each individual on the basis of heart rate (HR) and VO_2 measurements taken at two baseline maximal exercise tests. Details of the exercise training protocol can be found elsewhere [30]. Briefly, subjects trained at the HR associated with 55% of baseline $\text{VO}_{2\text{max}}$ for 30 min per session for the first 2 weeks. The duration and intensity were gradually increased every 2 weeks, until reaching 50 min and 75% of the HR associated with baseline $\text{VO}_{2\text{max}}$. This level was maintained for the final 6 weeks of training. All training was performed on Universal Aerobicycles (Cedar Rapids, IA) and power output was controlled by direct HR monitoring using the Universal Gym Mednet (Cedar Rapids, IA) computerized system. The protocol was standardized across all four clinical centers and supervised to ensure that the equipment was working properly and that participants were compliant with the protocol. The present report is based on completers ($N = 480$) defined as having performed at least 57 of the required 60 exercise sessions and having all adiposity and steroid data.

Plasma steroid hormone and SHBG concentrations

The hormonal assays have been previously described [19, 39]. Fourteen steroid hormones or their derivatives and SHBG concentrations were assayed: adrenal androgen precursors: DHEA, androstenedione (DELTA4), DHEAS; active androgens: TESTO, and dihydrotestosterone (DHT); androgen metabolites: androstane $3\alpha,17\beta$ diol glucuronide (DIOLG), and androsterone glucuronide (ADTG); pregnenolone fatty acid ester (PREGE), progesterone (PROG), and E2; aldosterone (nmol/L), cortisol (nmol/L). Additionally, free androgen index (FAI) and E2/T were calculated as $\text{TESTO}/\text{SHBG} \times 100$ and as $\text{E2}/\text{TESTO} \times 100$, respectively. Supplementary Table 1 (Table S1) provides the full name of each hormone, the abbreviations used in the present report and a brief comment on the source of production or transformation of the hormones and their precursors.

On 2 consecutive days, two blood samples were obtained after a 12-h fast pre- and post-exercise program. For eumenorrheic women, all samples were obtained in the early follicular phase of the menstrual cycle. None of the

women of reproductive age had dramatically irregular menstrual cycles as determined by questionnaire and an interview. After centrifugation, aliquots were frozen at -80°C . For nonconjugated steroids, ALDO, DHEA, and TESTO were differentially extracted with hexane-ethyl acetate. Petroleum ether was used for the extraction of DELTA4 and DHT. In-house RIAs were used to measure levels of these four steroids. PROG, OHPRG, CORT, E2, and DHEAS were assayed using commercial kits (Diagnostics Systems Lab, Webster, TX, USA). For glucuronide (ADTG and DIOLG) and ester (DHEAE and PREGE)-conjugated steroids, ethanol extraction was performed, followed by C18 column chromatography. Glucuronide conjugates were submitted to hydrolysis with β -glucuronidase (Sigma Co., St Louis, MO, USA). Fatty acid derivatives were submitted to saponification. Steroids were then separated by elution on LH-20 columns and measured by radioimmunoassay (RIA). SHBG was determined with an IRMA-Count solid phase immunoradiometric assay using ^{125}I (Diagnostics Systems Laboratories, Inc.). All assays were performed in the laboratory of Dr. Alain Belanger, Molecular Endocrinology, Laval University Medical Center (CHUL), Québec, Canada.

Statistical analysis

The present study is based on sedentary adults from the HERITAGE Family Study who had complete hormonal and body composition and fat distribution data at baseline and who completed a standardized exercise training program. The mean of two measurements both before and after the exercise program has been used for all hormones and SHBG levels. Data of men and women were analyzed separately. Paired *t*-tests were used to compare variables of body composition and fat distribution at baseline and after the exercise program.

The baseline value of steroid hormones, SHBG, FAI, and E2/T in men and women are available in Table S2. Mean and median values are presented, as well as 95% confidence intervals. Testing for skewness of distributions revealed that there was no indication of major skewness problems (skewness < 3.0), with the exception of DIOLG in men (skewness = 4.7) and E2 in women (skewness = 4.4). Both were log transformed to reduce skewness.

In men, Pearson product–moment correlations were used to quantify the relationships between baseline body composition or fat distribution traits (after adjustment for age and ancestry) and their exercise training responses (after adjustment for baseline value, age, and ancestry) with each fasting plasma steroid hormone, SHBG concentration, FAI, and E2/T. One-way analysis of variance (ANOVA) was used to compare each steroid across quartiles of baseline body composition or fat distribution variables and their

training responses. Quartiles were defined for each age and ancestry group and then pooled.

In women, oral contraceptives (OCs) and hormone replacement therapy (HRT) status had a significant impact on some steroids of the hormonal panel at baseline (Table S3, S4 for OC and HRT, respectively) and post-exercise training (Table S5, S6). Pre-menopause and post-menopause status also influenced the level of some hormones at baseline (Table S7) and in response to the exercise program (Table S8). As a result, correlation coefficients were used to quantify the relationships between baseline body composition and fat distribution traits (after adjustment for age, ancestry, menopause status, and OC/HRT status) and their exercise training response (after adjustment for baseline value, age, ancestry, menopause status, and OC/HRT status) with fasting plasma steroid hormone, SHBG concentration, FAI, E2/T. The general linear model (GLM) procedure was used to compare each steroid, SHBG, FAI, and E2/T across quartiles of baseline body composition or fat distribution variables and their training responses with menopause status and OC/HRT status as covariates. Quartiles were defined taking into account age and ancestry. The GLM procedure was used to compare each steroid, SHBG, FAI, and E2/T across the various BMI categories with age and ancestry as covariates: underweight ($<18.5 \text{ kg/m}^2$), normal weight (≥ 18.5 to $<25 \text{ kg/m}^2$), overweight (≥ 25 to $<30 \text{ kg/m}^2$), and obesity ($\geq 30 \text{ kg/m}^2$) in men and women.

Power analysis of sample size

Partial correlation analysis was used to assess the relationship between plasma steroid hormones and body composition/fat distribution at baseline and training response, with adjustment for age, ancestry, menopause status (women), OC/HRT status (women), and baseline value (training response). Based on previously published studies, we posit that the true correlations will be in the range from 0.25 to 0.50, with $\alpha = 0.0001$ and two-sided tests. If the true correlations are about 0.50, a sample size of about 60 is required to achieve a statistical power of about 85%. For coefficients of the order of 0.25, only about 140 subjects are needed if the alpha threshold is set at 0.05 but about 375 subjects are required for a threshold of 0.0001. Thus, when the multiple testing stringent alpha level is used, an adequate statistical power is achieved when correlations are based on the whole sample of men or women with correlations >0.25 . Lower statistical power is attained when subgroups are compared and when correlations reflect smaller effect sizes.

All analyses were performed using the SAS statistical package (SAS Institute, Inc., Cary, NC). Given the number of statistical tests performed, a Bonferroni-adjusted level of

significance was calculated and set at a P -value threshold of <0.0001 .

Results

Table 1 shows the distributions of the sedentary subjects before and in response to endurance training stratified by age, gender, and ancestry. At baseline, there were 270 men and 304 women with complete data (29% of males and 34% of females were Blacks). For the training response traits, there were 242 men and 238 women (25% of men and 24% of women were Blacks). At baseline, there are 261 premenopausal and 43 postmenopausal women. Among them, 83 premenopausal women were on OC, and 21 postmenopausal women reported using HRT. There were 198 premenopausal and 41 postmenopausal women who completed the exercise program. Among them, 71 were on OC and 19 were postmenopausal women on HRT.

Table 2 summarizes the associations of steroid hormone concentration levels with body composition/fat distribution in men. Shown are partial correlations (controlling for age and ancestry) between baseline plasma steroid hormone levels and baseline body composition/fat distribution. DHT, 17 hydroxy PROG (OHPROG), SHBG, and TESTO were significantly negatively correlated with nearly all body composition/fat distribution variables. SHBG and TESTO were not correlated with absolute fat-free mass but inversely associated with fat-free mass/ H^2 (Table 2) and positively associated with percentage fat-free mass (Table S9). Additionally, DHT, OHPROG, and SHBG were positively correlated with percentage fat-free mass (Table S9).

Table 3 depicts the partial correlations (controlling for age, ancestry, menopause status, OC/HRT status) in women. SHBG was significantly and negatively correlated with all fat mass/distribution variables, with the exception of the ASF/AVF ratio. The plasma level of SHBG was also significantly negatively correlated with fat-free mass (fat-free mass/ H^2 , and fat-free mass) but positively associated with percentage fat-free mass (Table S10). TESTO was not correlated with fat-free mass, fat-free mass/ H^2 and percentage of fat-free mass. DHT was significantly and positively correlated with fat-free mass and fat-free mass/ H^2 (Table S10). FAI was positively associated with all body composition/fat distribution variables except ASF/AVF ratio.

Hormonal levels exhibited substantial differences across the four quartiles of body composition and fat distribution in men (Table S11-S21). Plasma levels of SHBG (Figs. 1a, b) and TESTO (Figs. 1c-f) decreased significantly from Q1 to Q4 for nearly all body composition and fat distribution variables. The hormonal levels across quartiles of adiposity variables in women are detailed in Tables S22-S32. After

Table 1 Basic characteristics and changes in response to the exercise training program of sedentary males and females

	Training response (%) ^a										
	Baseline					Training response (%) ^a					
	N	Age (yr)	BMI (kg/m ²)	Percent body fat	Waist girth (cm)	AVF(cm ²)	N	ΔBMI	ΔPercent body fat	ΔWaist girth	ΔAVF
Men											
All	270	36 ± 14	26.6 ± 4.7	22.6 ± 8.5	93.2 ± 13.4	98.1 ± 61.8	242	-0.5 ± 2.6	-4.4 ± 10.8***	-1.0 ± 2.7***	-6.2 ± 18.5***
Blacks	77	33 ± 11	27.2 ± 4.9	23.2 ± 7.4	91.9 ± 14.3	74.0 ± 47.8	61	-0.3 ± 3.2	-3.4 ± 8.7	-1.1 ± 3.3	-5.1 ± 22.1
Whites	193	37 ± 15	26.4 ± 4.6	22.3 ± 9.0	93.8 ± 13.1	107.8 ± 64.2	181	-0.5 ± 2.4	-4.8 ± 11.5***	-1.0 ± 2.4***	-6.6 ± 17.2***
17–29 yrs	125	23 ± 4	24.6 ± 4.3	17.8 ± 8.6	85.9 ± 12.2	60.4 ± 36.2	113	-0.1 ± 2.9	-5.4 ± 13.3***	-0.9 ± 3.0	-4.9 ± 21.1
30–49 yrs	86	40 ± 7	28.9 ± 4.2	26.4 ± 5.8	99.3 ± 11.2	112.9 ± 54.1	74	-1.0 ± 2.6	-3.4 ± 9.2***	-1.3 ± 2.4***	-7.5 ± 15.9***
50–65 yrs	39	56 ± 4	27.7 ± 4.2	27.1 ± 6.2	100.0 ± 11.0	156.7 ± 61.3	55	-0.6 ± 1.9	-3.8 ± 5.9***	-0.8 ± 2.2	-7.4 ± 15.8
Women											
All	304	34 ± 14	25.5 ± 5.4	31.4 ± 9.9	86.4 ± 14.3	70.2 ± 48.5	238	-0.1 ± 3.5	-2.2 ± 7.6***	-1.2 ± 3.9***	-1.1 ± 18.2***
Blacks	102	32 ± 12	28.0 ± 6.3	35.9 ± 8.9	89.9 ± 15.0	67.8 ± 41.3	57	-0.3 ± 4.1	-2.4 ± 5.9	-1.1 ± 4.4	0.6 ± 19.7
Whites	202	35 ± 14	24.3 ± 4.5	29.1 ± 9.6	84.6 ± 13.6	71.4 ± 51.8	181	-0.1 ± 3.3	-2.2 ± 8.1***	-1.3 ± 3.8***	-1.7 ± 19.9
17–29 yrs	156	22 ± 4	24.2 ± 5.5	27.5 ± 9.7	80.4 ± 12.2	45.4 ± 23.8	118	0.1 ± 3.7	-2.5 ± 9.6	-0.8 ± 4.3	0.3 ± 19.6
30–49 yrs	95	40 ± 6	26.4 ± 4.8	33.6 ± 8.3	90.0 ± 11.9	81.3 ± 38.5	71	-0.2 ± 3.6	-1.8 ± 5.4	-1.8 ± 3.5***	-1.2 ± 17.0
50–65 yrs	53	55 ± 4	28.1 ± 5.0	39.1 ± 6.7	97.3 ± 15.4	123.3 ± 66.3	49	-0.4 ± 3.1	-2.2 ± 4.5	-1.5 ± 3.4	-4.8 ± 17.1

Values are shown as mean ± SD

AVF abdominal visceral fat

****P* < 0.0001, paired *t*-test comparing before and after training.^a The training response is reported in percent from baseline (%)

Table 2 Partial correlations between baseline plasma steroid hormone levels and baseline body composition and fat distribution in men (*N* = 270)

	Body composition				Fat distribution							
	BMI	Percent body fat	Fat mass/H ²	Fat-free mass/H ²	Fat-free mass	Waist girth	Hip girth	WHR	ATF	AVF	ASF	ASF/AVF
ADTG	0.02	0.10	0.08	-0.07	-0.06	0.05	0.02	0.07	0.06	0.04	0.06	0.04
ALDO	-0.04	-0.14	-0.09	0.04	0.08	-0.07	-0.04	-0.09	-0.07	0.01	-0.09	-0.08
CORT	-0.10	-0.10	-0.10	-0.07	-0.07	-0.13	-0.10	-0.14	-0.11	-0.04	-0.12	-0.05
DELTA	-0.11	-0.12	-0.11	-0.07	-0.05	-0.12	-0.09	-0.13	-0.11	-0.05	-0.12	-0.10
DHEA	-0.02	-0.02	-0.02	-0.01	-0.00	-0.04	-0.02	-0.06	-0.03	-0.01	-0.03	0.02
DHEAE	0.10	0.11	0.09	0.09	0.05	0.09	0.06	0.13	0.12	0.09	0.12	0.07
DHEAS	0.08	0.02	0.04	0.12	0.09	0.06	0.03	0.09	0.05	0.03	0.06	-0.00
DHT	-0.26***	-0.28***	-0.29***	-0.13	-0.04	-0.26***	-0.21	-0.27***	-0.28***	-0.20	-0.28***	-0.14
DIOLG	0.02	0.08	0.08	-0.07	-0.09	0.02	0.00	0.04	0.05	0.04	0.05	0.02
Ln DIOLG	-0.02	0.04	0.01	-0.06	-0.08	-0.02	-0.05	0.04	0.01	0.04	0.00	-0.01
E2	-0.01	-0.02	0.01	-0.01	0.00	-0.02	-0.03	-0.02	-0.01	0.04	-0.02	-0.11
OHPROG	-0.23***	-0.31***	-0.28***	-0.09	-0.08	-0.26***	-0.22**	-0.25***	-0.27***	-0.18*	-0.27***	-0.17
PREG	-0.05	-0.04	-0.06	-0.02	-0.04	-0.07	-0.07	-0.04	-0.05	-0.06	-0.04	0.02
PROG	-0.10	-0.14	-0.13	-0.04	-0.00	-0.13	-0.14	-0.08	-0.10	-0.04	-0.11	-0.09
SHBG	-0.38***	-0.38***	-0.37***	-0.27***	-0.18	-0.35***	-0.32***	-0.32***	-0.38***	-0.33***	-0.36***	-0.15
TESTO	-0.37***	-0.37***	-0.38***	-0.23***	-0.19	-0.38***	-0.34***	-0.35***	-0.38***	-0.25***	-0.39***	-0.21
FAI	0.07	0.14	0.10	0.01	-0.04	0.06	0.04	0.10	0.11	0.14	0.09	0.05
E2/T	0.20	0.19	0.22	0.12	0.10	0.19	0.18	0.14	0.21	0.13	0.22	0.06

Age and ancestry were controlled for in the partial correlations between baseline plasma steroid hormone levels and body composition and fat distribution

ATF abdominal total fat, ASF abdominal subcutaneous fat, AVF abdominal visceral fat, ASF/AVF abdominal subcutaneous fat/abdominal visceral fat, WHR waist girth/hip girth ratio

****P* < 0.0001

Table 3 Partial correlations between baseline plasma steroid hormone levels and baseline body composition and fat distribution in women ($N = 304$)

	Body composition					Fat distribution						
	BMI	Percent body fat	Fat mass/ H^2	Fat-free mass/ H^2	Fat-free mass	Waist girth	Hip girth	WHR	ATF	AVF	ASF	ASF/AVF
ADTG	0.09	0.07	-0.06	0.13	0.15	0.15	0.09	0.17	0.09	0.14	0.07	-0.03
ALDO	-0.02	-0.11	-0.06	0.08	0.02	-0.02	-0.07	0.03	-0.07	-0.02	-0.08	-0.06
CORT	-0.17	-0.18	-0.16	-0.12	-0.09	-0.17	-0.15	-0.14	-0.18	-0.20	-0.16	0.00
DELTA	0.00	-0.09	-0.06	0.13	0.19	-0.01	0.02	-0.04	-0.04	-0.06	-0.02	0.08
DHEA	0.01	-0.06	-0.03	0.11	0.07	-0.01	0.00	-0.03	-0.03	-0.06	-0.02	0.10
DHEAE	0.12	0.06	0.10	0.13	0.10	0.09	0.08	0.07	0.10	0.12	0.09	-0.08
DHEAS	0.17	0.06	0.12	0.22	0.19	0.18	0.14	0.15	0.13	0.11	0.13	-0.00
DHT	0.10	-0.04	0.03	0.23***	0.23***	0.04	0.07	-0.00	0.05	0.04	0.05	0.09
DIOLG	0.12	0.09	-0.09	0.12	0.12	0.11	0.08	0.10	0.09	0.12	0.08	-0.03
E2	0.01	-0.04	-0.02	0.06	0.02	-0.02	-0.03	0.01	-0.01	-0.04	-0.01	0.05
Ln E2	-0.02	-0.06	-0.05	0.05	0.01	-0.04	-0.04	-0.02	-0.05	-0.04	-0.04	0.00
OHPROG	-0.04	-0.12	-0.08	0.07	0.07	-0.05	-0.03	-0.07	-0.08	-0.07	-0.07	0.01
PREG	0.10	0.01	0.07	0.14	0.11	0.04	0.07	-0.01	0.05	0.00	0.06	0.02
PROG	-0.02	-0.12	-0.07	0.07	0.05	-0.06	-0.07	-0.02	-0.08	-0.09	-0.06	0.01
SHBG	-0.31***	-0.25***	-0.29***	-0.27***	-0.25***	-0.36**	-0.28***	-0.32***	-0.32***	-0.35***	-0.29***	0.06
TESTO	0.07	0.01	0.05	0.11	0.10	0.01	0.08	-0.08	0.05	-0.00	0.06	-0.10
FAI	0.39***	0.26***	0.34***	0.38***	0.34***	0.35***	0.34***	0.20**	0.35***	0.35***	0.32***	-0.02
E2/T	0.03	-0.05	-0.02	0.13	0.05	0.03	-0.01	0.08	0.01	-0.05	0.02	0.07

Age, ethnicity, menopause status, and oral contraceptives/hormonal replacement therapy were controlled for in the partial correlations between baseline plasma steroid hormone levels and body composition and fat distribution in women

ATF abdominal total fat, ASF abdominal subcutaneous fat, AVF abdominal subcutaneous fat, WHR waist girth/hip girth ratio

*** $P < 0.0001$

Hormone Levels by Quartiles of Baseline Body Composition and Fat Distribution Variables in Men

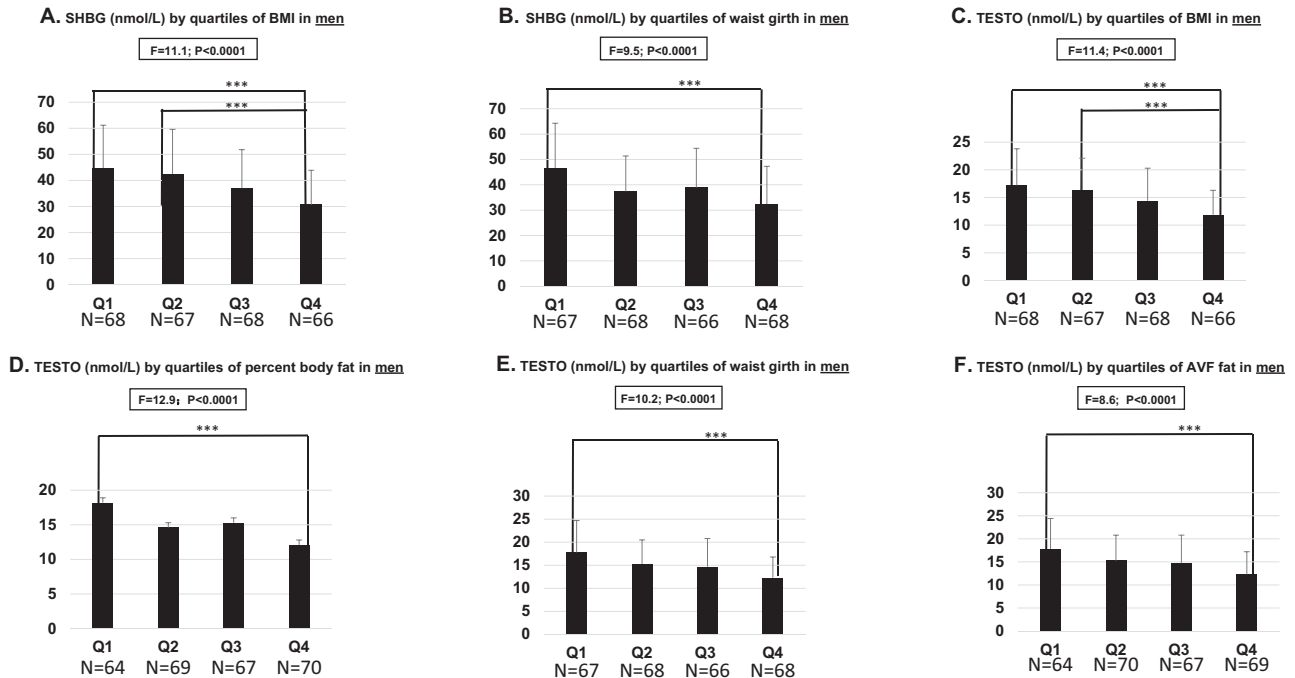


Fig. 1 Hormone levels by quartiles of baseline body composition and fat distribution variables in men. **a** Plasma level of SHBG in Q4 of BMI was lower than Q1 and Q2. **b** Plasma level of SHBG in Q4 of waist girth was lower than Q1. **c** Plasma level of TESTO in Q4 of BMI was lower than Q1 and Q2. **d** Plasma level of TESTO in Q4 of percent body fat was lower than Q1. **e** Plasma level of TESTO in Q4 of waist

girth was lower than Q1. **f** Plasma level of TESTO in Q4 of AVF was lower than Q1. SHBG sex hormone-binding globulin, TESTO Testosterone, AVF abdominal visceral fat. Values are shown as mean ± SD. One-way ANOVA was used to compare each plasma steroid across quartiles of baseline body composition or fat distribution variables. ****P* < 0.0001

adjustment for age, ancestry and OC/HRT status, the plasma levels of SHBG decreased progressively from Q1 to Q4 (Figs. 2a–c), and that of FAI (Figs. 2d–f) increased significantly from Q1 to Q4 for all variables.

Figure 3 depicts hormonal levels that differed significantly among normal weight, overweight, and obese subgroups in men and women, as defined by standard BMI thresholds. In men, with age and ancestry as covariance, the plasma levels of SHBG and TESTO decreased gradually from the normal weight to the obese group. In women, when age, ancestry, menopause status, and OC/HRT status were used as covariates, the levels of SHBG decreased progressively across normal weight, overweight, and obese categories, whereas FAI increased across the same weight gradient. Other hormone levels across BMI categories are detailed in Table S33.

In response to the exercise program, percent body fat, waist girth, and AVF decreased significantly, but individual differences in response (Δ) were large (see Table 1). Table S34 summarizes the other body composition and fat distribution exercise training responses (Δ). We verified the degree of skewness of response distributions and did not find any strong evidence that skewness was an issue (all < 3.0). Body composition and fat distribution variables changed significantly in response to the exercise training

program (*P* < 0.0001), except for BMI. In brief, there were no significant correlations between baseline hormonal levels and the exercise training response of body composition and fat distribution traits in men or women. Moreover, hormonal levels were not different across quartiles of body composition and fat distribution responses. These results are shown in Tables S35–S56.

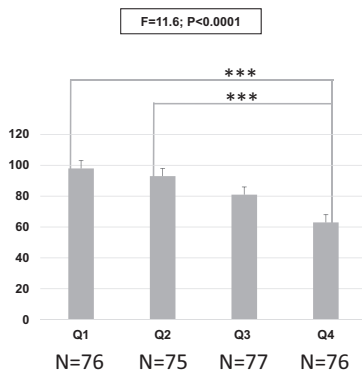
Discussion

The present study is the most comprehensive report published to date on the relationships between a large panel of plasma steroid hormones and body composition or indicators of fat distribution in sedentary men and women at baseline and in response to a standardized and fully monitored 20-week exercise program.

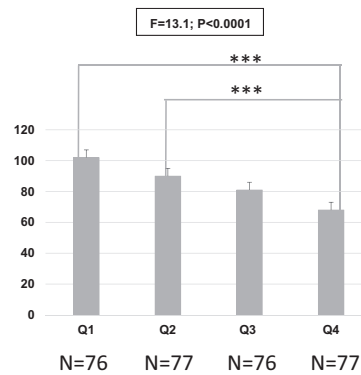
Negative associations were found between fat mass and adipose tissue distribution variables (BMI, percent of body fat, fat mass/H², waist circumference, hip circumference, WHR, ATF, AVF, and ASF) with plasma levels of DHT, OHPROG, SHBG, and TESTO in men. Surprisingly, the plasma concentration of TESTO was inversely correlated with fat mass/H² but not with total fat-free mass, whereas it was positively correlated with percentage fat-free mass. In

Hormone Levels by Quartiles of Baseline Body Composition and Fat Distribution in Women

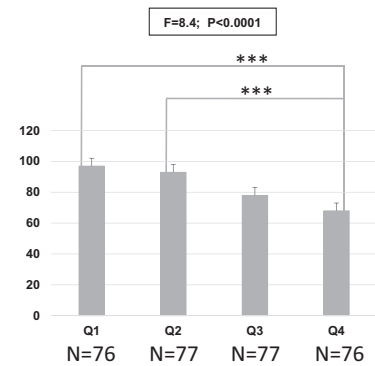
A. SHBG (nmol/L) by quartiles of BMI in women



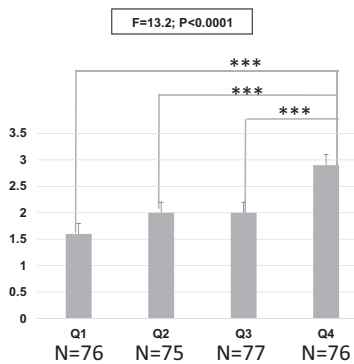
B. SHBG (nmol/L) by quartiles of waist girth in women



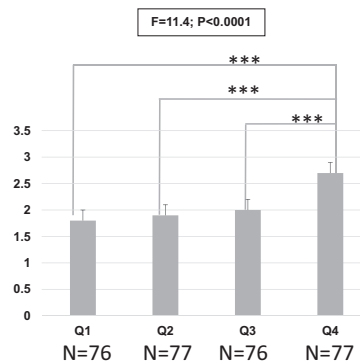
C. SHBG (nmol/L) by quartiles of AVF in women



D. FAI by quartiles of BMI in women



E. FAI by quartiles of waist girth in women



F. FAI by quartiles of AVF in women

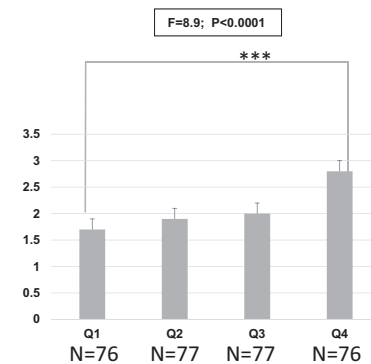


Fig. 2 Hormone levels by quartiles of baseline body composition and fat distribution in women. **a** Plasma level of SHBG in Q4 of BMI was lower than Q1 and Q2. **b** Plasma level of SHBG in Q4 of waist girth was lower than Q1 and Q2. **c** Plasma level of SHBG in Q4 of AVF was lower than Q1 and Q2. **d** Plasma level of TESTO in Q4 of BMI was higher significantly than that of Q1, Q2 and Q3. **e** Plasma level of TESTO in Q4 of waist girth was higher Q1, Q2 and Q3. **f** Plasma level

of TESTO in Q4 of AVF was higher than Q1. Free androgen index (FAI), calculated as $\text{TESTO}/\text{SHBG} \times 100$. AVF abdominal visceral fat. Values are shown as mean \pm SEM. The general linear model (GLM) procedure was used to compare each plasma steroid across quartiles of baseline body composition or fat distribution variables. *** $P < 0.0001$

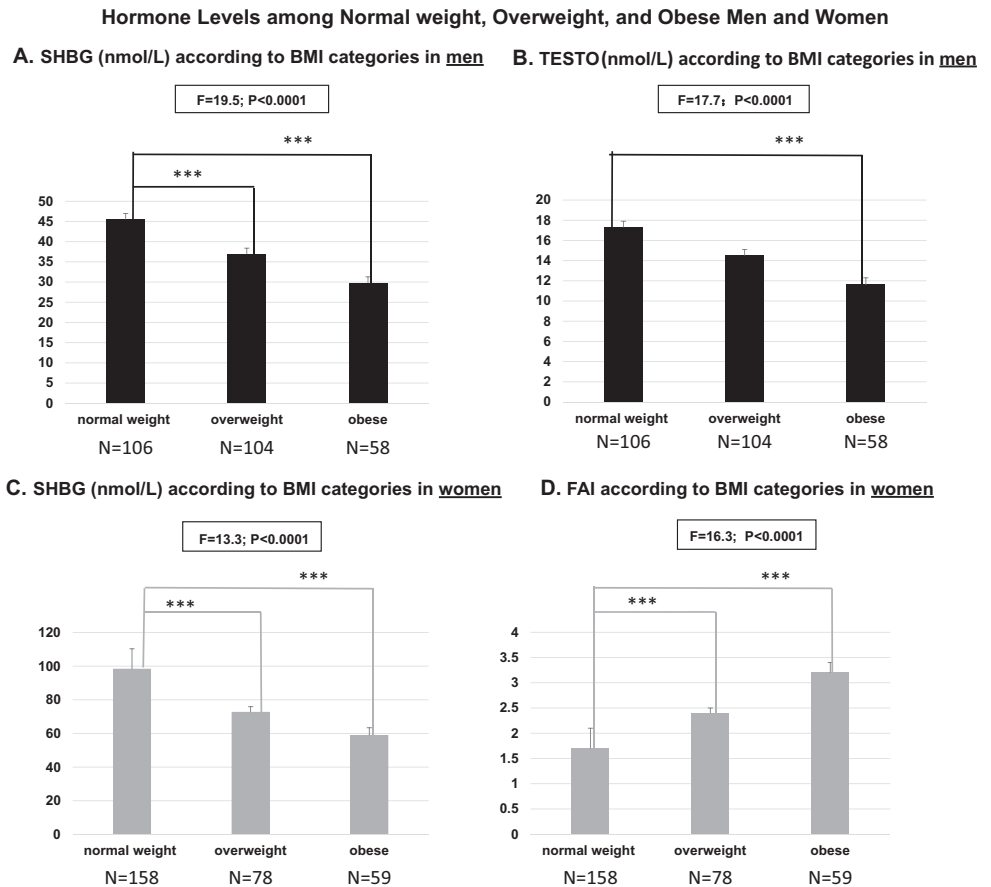
contrast, in women, SHBG was negatively correlated with all fat mass and distribution variables, whereas FAI was positively associated with all of them. Baseline plasma steroid levels, SHBG concentrations, FAI, or E2/T were not associated with the changes in body composition and fat distribution traits resulting from exposure to the 20-week endurance exercise program after adjustment for baseline values, age, ancestry, menopause status, and OC/HRT (women).

The present results reveal that body composition and fat distribution are negatively correlated with SHBG in men and women and with TESTO in men. This sex dimorphism might be partly attributed to the marked sex-related difference of SHBG binding to TESTO [40]. Non-SHBG-bound TESTO seems to be the best parameter for serum levels of bioactive T^{20} . Androgens exert their effects on adipocyte differentiation in a depot-specific manner, via the androgen receptor, leading to modulation of adipocyte size and specific fat depot expansion [41]. Androgens also impact key

adipocyte functions including insulin signaling, lipid metabolism, fatty acid uptake, and adipokine productions, with frequently observed sex-specific effects [42]. Furthermore, sex differences in body fat distribution are commonly recognized, with males usually characterized by a predominant accumulation of fat in the abdominal region, whereas women generally display a greater proportion of their body fat in the gluteal–femoral region [43].

An excessive amount of body fat, such as in obesity, can lead to impaired testicular T biosynthesis. Furthermore, depressed levels of plasma TESTO increases fat deposition, particularly AVF. As abdominal fat is a risk factor for the development of metabolic syndrome, type 2 diabetes mellitus and cardiovascular disease [44], abdominal adiposity and the ensuing hyperinsulinemia [45] can synergistically reduce SHBG production [46]. Thus, low physiological levels of TESTO and SHBG could represent components of a hormonal milieu that is favorable to fat storage with potential adverse metabolic impact.

Fig. 3 Hormone levels among normal weight, overweight, and obese men and women. **a** Plasma level of SHBG was higher in normal weight group than overweight and obese. **b** Plasma level of TESTO was higher in normal weight group than obese. **c** Plasma level of SHBG was higher in normal weight group than overweight and obese. **d** FAI was lower in normal weight group than overweight and obese. SHBG sex hormone-binding globulin, TESTO testosterone. Values are shown as mean ± SEM. The GLM procedure was used to compare each plasma steroid across the various BMI categories with age and ancestry as covariates. *** $P < 0.0001$



In men, the major source of E2 is through the conversion of TESTO via aromatase, which is significantly expressed in adipose tissue, suggesting that higher levels of adiposity favor synthesis of E2 [1]. We did not find that E2 was associated with body composition and fat distribution traits in men and in women in contrast to positive associations reported in 30- to 90-year-old men from the United States [1], 69- to 80-year-old Swedish men [3], and 40- to 80-year-old Dutch men [17], as well as in premenopausal African–American women [15]. Importantly, in the present study, age, ancestry, menopause, OC, and HRT were controlled for.

In women with androgen excess, higher TESTO level is a fairly consistent predictor of abdominal adipose tissue accumulation, but it is not the case in non-hyperandrogenic women [47]. Our results confirm that androgens are not related to fat mass and visceral fat accumulation in women. Hence, in women without clinical manifestations of hyperandrogenism, there is no clear association between androgens and adiposity, particularly abdominal and visceral fat levels.

We found that baseline steroid hormonal levels were not associated with the adiposity changes observed in response to the exercise training program. This is concordant with other reports even though prior studies were based on rather

small sample sizes. For instance, Hawkins et al. [25] also did not find that TESTO, DHT, and SHBG were associated with exercise-induced changes in body fat in men after 12 months of a home-based exercise intervention.

Interestingly, the World Anti-Doping Agency (WADA) introduced a ‘hyperandrogenism’ rule in 2011 that aims at excluding women with a serum TESTO > 10 nmol/L from participating in elite sport. It is based on the premise that the greater lean body mass in men is a consequence of their higher serum TESTO [48]. The Court of Arbitration for Sport suspended the ‘hyperandrogenism regulation’ in 2015. Unless sufficient scientific evidence is provided in support of the ‘hyperandrogenism’ rule within 2 years, it will be withdrawn permanently. Even though our findings are based on sedentary women, as opposed to athletes, they strongly suggest that endogenous plasma TESTO levels in the normal range are not associated with absolute or relative fat-free mass in women once age, ancestry, menopause status, OC, and HRT have been taken into account. However, in the present study, none of the women reached the 10 nmol/L threshold (the highest plasma level of TESTO was 5.905 nmol/L).

The present study is characterized by several strong points. It is based on a relatively large sample with

substantial subgroups defined by age, ancestry, and sex. Two blood samples obtained on consecutive days were used to assay the steroid hormones. Of particular significance, all blood samples were drawn in the early follicular phase of the menstrual cycle for eumenorrheic women. Also of significance, information on use of OC and HRT was available and could be adjusted for in the present analyses. Furthermore, as a large number of tests of significance was performed, an alpha threshold of $P < 0.0001$ was used to reduce the risk of false-positive results. Additionally, body composition and fat distribution phenotypes were measured directly in the laboratory on all participants and a large panel of steroid hormones was assayed. Finally, adiposity traits changed marginally but significantly ($P < 0.0001$) in response to the standardized exercise training program, with considerable individual difference in the changes observed.

There are some limitations to the data reported herein. As fat-free mass was assessed by underwater weighing, we could not distinguish between truncal vs. limb lean masses, a distinction that was shown to be useful in some studies [6, 49]. As our sample of postmenopausal women was not large enough, we could not compare early and late menopausal periods [13]. Additionally, despite the fact that the study is based on a relatively large sample size, it was not sufficiently large to compare various age groups within sex and ethnic groups. Finally, the data of the HERITAGE study used herein, with the exception of the response to the exercise intervention, are observational and cross-sectional in nature, which imposes limits on the assessment of causality.

We conclude that baseline levels of DHT, OGPROG, SHBG, and TESTO in men and SHBG and FAI in women are associated with individual differences in fat mass and fat distribution phenotypes. TESTO was not correlated with absolute fat-free mass in men and women, but DHT was positively correlated with fat-free mass in women, and TESTO was positively corrected with % fat-free mass in men. Additionally, no association could be detected between baseline hormonal levels and adiposity changes following a standardized and monitored 20-week exercise program. Low physiological concentrations of DHT, OHPROG, SHBG, and TESTO are potential risk factors for high adiposity and undesirable patterns of fat distribution in men. The same appears to be true for SHBG in women.

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Compliance with ethical standards

Conflict of interest AT receives research funding from Johnson & Johnson Medical Companies for studies unrelated to the present paper. The remaining authors declare that they have no conflict of interest.

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