Changes in Visceral Adiposity, Subcutaneous Adiposity, and Sex Hormones in the Diabetes Prevention Program

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Context: The degree to which changes in visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) relate to corresponding changes in plasma sex steroids is not known.

Objective: We examined whether changes in VAT and SAT areas assessed by computed tomography were associated with changes in sex hormones [dehydroepiandrosterone sulfate (DHEAS), testosterone, estradiol, estrone, and sex hormone binding globulin (SHBG)] among Diabetes Prevention Program participants.

Design: Secondary analysis of a randomized trial.

Participants: Overweight and glucose-intolerant men (n = 246) and women (n = 309).

Interventions: Intensive lifestyle change with goals of weight reduction and 150 min/wk of moderate intensity exercise or metformin administered 850 mg twice a day or placebo.

Main Outcome Measures: Associations between changes in VAT, SAT, and sex hormone changes over 1 year.

Results: Among men, reductions in VAT and SAT were both independently associated with significant increases in total testosterone and SHBG in fully adjusted models. Among women, reductions in VAT and SAT were both independently associated with increases in SHBG and associations with estrone differed by menopausal status. Associations were similar by race/ethnicity and by randomization arm. No significant associations were observed between change in fat depot with change in estradiol or DHEAS.

Conclusions: Among overweight adults with impaired glucose intolerance, reductions in either VAT and SAT were associated with increased total testosterone in men and higher SHBG in men and women. Weight loss may affect sex hormone profiles via reductions in visceral and subcutaneous fat.
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Women's lower cardiovascular disease risk has been attributed to sex differences in sex hormone profiles (1), which are associated with the deposition of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) (2). Specifically, cross-sectional studies have reported that lower androgen levels in men and higher

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Abbreviations: BMI, body mass index; CT, computed tomography; DHEAS, dehydroepiandrosterone sulfate; DPP, Diabetes Prevention Program; ILS, intensive lifestyle modification; IQR, interquartile range; MESA, Multi-Ethnic Study of Atherosclerosis; SAT, subcutaneous adipose tissue; SD, standard deviation; SHBG, sex hormone binding globulin; SWAN, Study of Women's Health Across the Nation; VAT, visceral adipose tissue.

androgen levels in women are associated with greater visceral fat area measured by computed tomography (CT) (2, 3). The relationship between sex hormones and adiposity is likely bidirectional: weight gain is associated with declines in testosterone in men and predicts estradiol trajectories in perimenopausal women (4–6). However, the extent to which changes in specific fat depots affect sex steroid profiles are poorly understood, in part due to the lack of longitudinal studies assessing both sex steroids and body fat distribution with precise imaging methods.

The Diabetes Prevention Program (DPP) randomized racially and ethnically diverse overweight, nondiabetic glucose-intolerant participants to a program of intensive lifestyle modification (ILS), metformin, or placebo (7). Randomization to ILS led to large reductions in VAT in both men and women and smaller reductions in SAT (8, 9), as well as changes in sex hormone profiles in men and women (10, 11), but the relationship between VAT, SAT, and sex hormones was not examined. For the present report, we examined whether changes in VAT and SAT were associated with corresponding changes in sex hormone profiles in men and women. On the basis of previous cross-sectional studies (2, 3, 12–15), we hypothesized that reductions in VAT and SAT would be associated with increases in sex hormone binding globulin (SHBG), as well as with increases in androgens in men and decreases in androgens in women. We also hypothesized that reductions in VAT and SAT would be associated with reductions in estradiol and estrone in both men and women, but these associations would be less pronounced than those observed for testosterone and SHBG in the overweight/obese population enrolled in the DPP. Finally, we hypothesized that the strength of these associations would be greater for VAT than for SAT.

Materials and Methods

The design, methods, and baseline characteristics of the DPP have been previously described (7). Briefly, participants were recruited across 27 clinical centers located throughout the United States. Inclusion criteria were age ≥25 years, body mass index [(BMI) ≥24 kg/m^2 (≥22 kg/m^2 for Asian-Americans)], a fasting plasma glucose level of 5.3 to 7.0 mmol/L (95 to 125 mg/dL), and a 2-hour plasma glucose level of 7.8 to 11.1 mmol/L (140 to 199 mg/dL) following an oral 75-g glucose load. Eligible participants were randomly assigned to one of three interventions: 850 mg metformin twice daily, placebo twice daily, or ILS. The goals of ILS were to achieve and maintain a weight reduction of at least 7% through consumption of a low-calorie, low-fat diet, plus moderate physical activity for at least 150 minutes per week. Weight was measured semiannually and waist circumference was measured annually. Each participating institution was overseen by its respective ethics review board. For the purposes of this analysis, we included participants who had an acceptable quality CT-assessment of adiposity both at baseline and at year 1 (n = 718). From these, we excluded participants who did not have sex steroid measures either at baseline or at year 1 (n = 53); we further excluded participants who reported exogenous sex steroid use at baseline or at year 1 (n = 110) leaving a total of 555 participants (246 men and 309 women) for the analysis. Compared with those excluded, participants in this analysis were younger, had higher BMI, glucose, adiposity, DHEAS, testosterone, estradiol, and lower SHBG (see Supplemental Table 1).

CT assessment of adiposity was performed at 18 of 27 participating sites, and imaging procedures have been previously described (8, 9). An anterior-posterior scout and two 10-mm thick axial images at the L2-L3 and L4-L5 disc spaces were read at a central reading facility by readers blinded to randomization assignment. Blood samples for sex hormone measurements were collected fasting, before 1000 hours. Sex hormones were measured by Endoceutics (Quebec City, Canada). SHBG was measured using an enzyme-linked immunosorbent assay (Bioline, Taunton, MA) with interassay coefficients of variation of 7.8% and 5.0% at 18.2 and 63.1 nmol/L, respectively. Sex steroids were measured using gas chromatography/mass spectrometry (16). The lower limits of detection for dehydroepiandrosterone sulfate (DHEAS), testosterone, estradiol, and estrone were 20 ng/dL, 10, 0.2, 0.8 pg/mL, respectively. The lower limits of quantification for DHEAS, testosterone, estradiol, estrone, and estrone sulfate were 100, 50,1, 4, pg/mL and 4 nmol/L for SHBG. Interassay variation (coefficient of variation) was 5.2%, 10.7%, 7.0%, 12.5% for DHEAS, testosterone, estradiol, and estrone, respectively at the lower limits of quantification level. Bioavailable testosterone and bioavailable estradiol were calculated according to the method described by Södergård *et al.* (17) (courtesy of Frank Stanczyk, University of Southern California, Los Angeles, CA) taking the concentrations of total testosterone, total estradiol, and SHBG into account and assuming a fixed albumin concentration of 4.0 g/dL.

Statistical analysis

Due to the dimorphic associations between sex steroids and fat depots, men and women were examined separately. Baseline characteristics were described using n (percentages) for categorical variables and means [standard deviation (SD)] or median [interquartile range (IQR)] for quantitative variables with normal and skewed distributions, respectively. Differences between men and women were tested using the χ^2 test for independence for categorical variables and the t test or the nonparametric Wilcoxon test, as appropriate, for continuous variables. Cross-sectional linear regression models were created to examine the associations between baseline measures of VAT at L2-3 and 3-4 and SAT area at L2-3 and L3-4 area in cm^2 with baseline measures of sex hormones. Sex hormone measures were log-transformed to satisfy assumptions of normality. To explore the relationship of baseline fat depot with sex hormones, linear regression models adjusted for age, race/ethnicity, and treatment arm were created. Additional evaluations for interactions with randomization arm and race/ethnicity were performed by adding interaction terms in the models to determine whether the fat depot associations varied by treatment assignment or race/ethnicity. Interaction terms by randomization arm were not significant and thus models combined data across randomization arms, although results stratified by randomization arm are presented in the Appendix (Supplemental Table 2). Similar linear regression models were created to

examine changes in VAT with changes in sex hormones, as well as changes in SAT with changes in sex hormones. Changes were defined as the difference of the year 1 values minus the baseline values. These models included additional adjustment for baseline fat depot measure.

To examine whether changes were more associated with visceral or subcutaneous adiposity, we also examined the change in the ratio of VAT/SAT at each cross-sectional slice between baseline and year 1. Additional models also included changes in VAT and SAT entered as separate independent variables in the models. Adjustment for BMI did not change the pattern of results (not shown), so BMI was not included as an adjuster in the final models. Analyses were performed using the SAS version 9.2 (SAS Institute, Cary, NC), and all tests were two sided with statistical significance set at $P < 0.05$.

Results

Table 1 shows participant characteristics at baseline by sex. Men and women were randomized in equal proportions to placebo, metformin, or ILS. Slightly less than a third of participants were less than 45 years of age with a mean age of 51 years; men averaged 54 (±11) years of age, whereas women averaged 49 (±10) years of age. Among the women, 178 were premenopausal and 131 were postmenopausal. Slightly more than half of the participants were non-Hispanic white, approximately one-quarter of the participants were black, and the remainder were Hispanic or of Asian race/ethnicity. Men had significantly more VAT but less SAT than women, and thus men had higher VAT/SAT than women. Men also had higher DHEAS and testosterone levels but lower estrone levels than women and similar estradiol and SHBG levels compared with women.

Table 2 shows the cross-sectional associations between baseline measures in VAT and SAT with baseline measures of sex hormone concentrations after adjustment for age, race/ethnicity, and randomization assignment. Among men, baseline measures of VAT and SAT were inversely associated with baseline measures of testosterone and positively associated with baseline measures of estrone. Baseline VAT (β-coefficient −0.16, P = 0.022) and SAT (β-coefficient −0.25, $P < 0.001$) were

Table 1. Baseline Participant Characteristics

	All N = 555	Men N = 246	Women N = 309	*P* Value
Treatment group, %				0.330
Placebo	181 (33)	75 (30)	106 (34)	
Metformin	187 (34)	91 (37)	96 (31)	
ILS	187 (34)	80 (33)	107 (35)	
Age group, %				<0.001
25–44 y	170 (31)	50 (20)	120 (39)	
45–59 y	262 (47)	120 (49)	142 (46)	
≥60 y	123 (22)	76 (31)	47 (15)	
Race/ethnicity, %				<0.001
Non-Hispanic white	309 (56)	155 (63)	154 (50)	
African-American	124 (22)	33 (13)	91 (29)	
Hispanic	99 (18)	44 (18)	55 (18)	
Asian	23 (4)	14 (6)	9 (3)	
BMI, kg/m^2	32.9 (5.3)	32.2 (5.3)	33.6 (5.3)	0.002
VAT L2-L3, cm^2	207.8 (88.5)	264.8 (86.5)	162.3 (58.9)	<0.001
VAT L3-L4, cm^2	160.9 (65.8)	182.3 (73.7)	143.8 (53.1)	<0.001
SAT, L2-L3, cm^2	293.6 (117.4)	246.7 (105.4)	330.9 (113.2)	<0.001
SAT, L3-L4, cm^2	424.3 (147.1)	346.9 (126.0)	485.8 (133.2)	<0.001
VAT/SAT L2-L3	0.83 (0.51)	1.21 (0.52)	0.52 (0.20)	<0.001
VAT/SAT L3-L4	0.43 (0.23)	0.57 (0.24)	0.31 (0.13)	<0.001
DHEAS, ng/mL	823.4 (498.8–1276.5)	946.9 (580.7–1589.0)	713.2 (441.1–1120.7)	<0.001
DHEAS, μmol/L	2.22 (13.47–34.47)	2.56 (1.57–4.29)	1.93 (1.19–30.26)	<0.001
Testosterone, ng/dL	31.72 (15.27–289.18)	304.70 (239.77–388.76)	16.00 (12.00–22.00)	<0.001
Testosterone, nmol/L	1.10 (0.53–10.03)	10.57 (8.32–13.49)	0.56 (0.42–0.76)	<0.001
Bioavailable T, ng/dL	16.83 (7.52–158.61)	162.47 (127.00–207.63)	7.75 (5.49–11.36)	<0.001
Bioavailable T, nmol/L	0.58 (0.26–5.50)	5.64 (4.41–7.20)	0.27 (0.19–0.39)	<0.001
Estradiol, pg/mL	22.3 (14.1–38.4)	21.4 (17.9–26.5)	27.9 (8.3–81.1)	0.064
Estradiol, pmol/L	81.86 (51.76–140.97)	78.56 (65.71–97.28)	102.42 (30.47–297.72)	0.064
Bioavailable E2, pg/mL	14.63 (9.78–26.61)	13.70 (11.11–17.86)	19.46 (6.08–49.30)	0.011
Bioavailable E2, pmol/L	53.70 (35.89–97.68)	50.61 (40.80–65.55)	71.45 (22.31–180.98)	0.011
Estrone, pg/mL	43.8 (33.4–73.7)	36.4 (29.5–44.5)	88.5 (55.8–120.8)	<0.001
Estrone, pmol/L	161.97 (123.51–272.54)	134.61 (109.09–164.56)	327.27 (206.35–446.72)	<0.001
SHBG, nmol/L	38.9 (28.1–57.0)	39.7 (26.3–56.0)	38.8 (29.0–57.3)	0.452

Mean (SD) or n (percentage) shown; sex hormone medians and interquartile ranges shown.

Table 2. Cross-Sectional Associations Between Baseline Measures of SAT and VAT With Baseline Measures of Log-Transformed Sex Hormones in Linear Regression Models (Standardized β-Coefficient and *P* Value)

	Log DHEAS		Log Testosterone		Log Estradiol		Log Estrone		Log SHBG	
Model	**β (ng/dL per cm²)**	***P* Value**	**β (nmol/L per cm²)**	***P* Value**	**β (pg/mL per cm²)**	***P* Value**	**β (pg/mL per cm²)**	***P* Value**	**β (nmol/L per cm²)**	***P* Value**
Men										
VAT (L2-L3)	0.01216	0.833	**−0.20420**	**0.003**	0.12885	0.060	**0.16873**	**0.013**	−0.10241	0.092
VAT (L3-L4)	−0.04556	0.429	**−0.18506**	**0.007**	0.06056	0.380	0.10257	0.133	−0.05910	0.333
SAT (L2-L3)	**−0.17842**	**0.002**	**−0.22074**	**<0.001**	**0.14473**	**0.033**	**0.13585**	**0.043**	0.02644	0.661
SAT (L3-L4)	**−0.15476**	**0.006**	**−0.21749**	**0.001**	**0.18839**	**0.005**	**0.15881**	**0.018**	−0.00665	0.912
Women										
VAT (L2-L3)	−0.00917	0.866	−0.08950	0.133	−0.00275	0.957	0.02394	0.773	**−0.22734**	**<0.001**
VAT (L3-L4)	−0.00944	0.860	−0.04653	0.431	−0.02342	0.644	−0.04261	0.595	**−0.18471**	**0.002**
SAT (L2-L3)	−0.02169	0.676	0.04048	0.481	−0.04091	0.404	−0.00547	0.945	**−0.14697**	**0.013**
SAT (L3-L4)	0.02846	0.588	0.05054	0.387	−0.04159	0.400	−0.01651	0.838	−0.06186	0.305

β-coefficients are SD changes in the log-transformed sex hormones per SD increase in cm^2 of adipose tissue depot. Models adjust for age, race/ethnicity, and randomization assignment. Boldface indicates $P < 0.05$.

also associated with lower levels of log bioavailable testosterone. SAT, but not VAT, was associated with total estradiol and inversely associated with DHEAS. Levels of VAT (β-coefficient 0.16, $P = 0.015$) were also associated with lower levels of log bioavailable estradiol, whereas levels of SAT had borderline associations with lower levels of log bioavailable estradiol (β-coefficient 0.12, $P = 0.07$). Among women, measures of VAT and SAT were not associated with sex steroids or bioavailable testosterone and estradiol. Both SAT and VAT were inversely associated with baseline SHBG.

Table 3 shows the associations between changes in VAT and SAT with changes in sex hormone concentrations after adjustment for age, race/ethnicity, randomization assignment, and size of the adipose tissue measure at baseline. Among men, changes in both VAT and SAT were inversely related to changes in SHBG and changes in testosterone. For example, at the L2-L3 level, for each cm^2 decline in VAT, there was a 0.021 nmol/L increase in total testosterone ($P < 0.001$) and a 0.073 increase in SHBG ($P = 0.008$). In contrast to models which examined only baseline measures of fat and sex hormones, no statistically significant associations were observed between changes in fat depot and changes in estradiol, estrone, and DHEAS. Declines in VAT between baseline and year 1 were also associated with increases in bioavailable testosterone (β-coefficient −0.20, *P* value 0.011) but not with changes in bioavailable estradiol (β-coefficient 0.06, $P = 0.51$). These patterns were similar across randomization arms, although the statistical significance of the associations was reduced (Supplemental Table 2). Among women, changes in both VAT and SAT were inversely related to changes in SHBG. For example, at the L2-L3 level, for each cm^2 decline in VAT, there was a 0.17-nmol/L increase in SHBG ($P = 0.010$) in SHBG. Declines in VAT at the L2-L3 level were associated with declines in estrone, although the association did not reach statistical significance at the L3-L4 level. Changes in VAT and SAT were not associated with changes in testosterone, DHEAS, or estradiol. As in men, these patterns were similar across

Table 3. Association Between Change in VAT and SAT With Changes in Sex Hormone in Linear Regression Models (Standardized β-Coefficient and *P* Value)

	ΔDHEAS		ΔTestosterone		ΔEstradiol		ΔEstrone		ΔSHBG	
Model	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**
Men										
ΔVAT (L2-L3)	0.04415	0.569	**−0.41134**	**<0.001**	−0.03506	0.685	0.02990	0.708	**−0.20557**	**0.008**
ΔVAT (L3-L4)	0.00693	0.932	**−0.29744**	**<0.001**	−0.01145	0.898	0.07068	0.389	**−0.18587**	**0.022**
ΔSAT (L2-L3)	0.08165	0.262	**−0.30809**	**<0.001**	−0.01236	0.880	−0.09880	0.177	**−0.18563**	**0.012**
ΔSAT (L3-L4)	0.04266	0.561	**−0.35370**	**<0.001**	−0.02041	0.808	0.01098	0.886	**−0.20499**	**0.006**
Women										
ΔVAT (L2-L3)	−0.09837	0.130	−0.12410	0.075	0.05825	0.397	**0.19065**	**0.036**	**−0.16411**	**0.010**
ΔVAT (L3-L4)	−0.09112	0.175	0.01060	0.882	0.04481	0.529	0.16172	0.095	**−0.13485**	**0.040**
ΔSAT (L2-L3)	−0.08060	0.216	−0.11613	0.093	−0.00306	0.965	0.08715	0.355	**−0.22157**	**<0.001**
ΔSAT (L3-L4)	−0.04453	0.485	−0.11611	0.086	0.03797	0.575	0.08725	0.325	**−0.13853**	**0.025**

β-coefficients are in SD of changes (Δ) in sex hormones per SD increase in the change of adipose tissue depot, in cm^2 (Δcm^2). Models adjust for age, race/ethnicity, randomization assignment, and size of adipose tissue measure at baseline. Boldface indicates $P < 0.05$.

randomization arms, but statistical significance was reduced. Reductions in VAT and SAT were associated with SHBG in both pre- and postmenopausal women (Table 4), although statistical significance was reduced. Reductions in VAT and SAT were not associated with changes in bioavailable testosterone or bioavailable estradiol in women. Among premenopausal women but not postmenopausal women, decreases in VAT were associated with decreases in estrone. Among postmenopausal women but not premenopausal women, decreases in SAT were associated with increases in estrone.

To determine their relative influences on sex hormones, we examined models including both VAT and SAT (Table 5). Among men, greater declines in the ratio of VAT/SAT at the L2-L3 level were also associated with increases in total testosterone, suggesting that alterations in VAT were more associated with total testosterone. Similarly, in models that included VAT and SAT as independent variable in the same model, declines in VAT but not SAT at the L2-L3 level were associated with increases in testosterone. However, models at the L3-L4 level did not show a significant association between the ratio of VAT/SAT to testosterone and also showed that declines in VAT and SAT were both independently associated with testosterone. Among women, the ratio of VAT/SAT was not significantly associated with SHBG. The lack of association between VAT/SAT with SHBG also suggests that declines in VAT and SAT were similarly associated with increases in SHBG, rather than with VAT alone. This was supported by models that included both VAT and SAT as independent variables. In these models, neither VAT nor SAT was consistently associated with SHBG, suggesting that the high correlation between VAT and SAT and the similarity of their relationship with SHBG reduced the significance of associations in models that included both variables.

Discussion

In the unique setting of a randomized trial of weight loss among overweight men and women, we found that reductions in adiposity were significantly associated with sex hormone changes that differed by gender and fat depot. In men, reductions in VAT were associated with increases in total testosterone and SHBG. Reductions in SAT were associated with a similar pattern. In women, reductions in VAT and SAT were associated with increases in SHBG. Although changes in adiposity and sex hormones occurred concurrently and thus may be bidirectional, the fact that adipose tissue changes occurred in the context of a weight reduction trial suggests that significant decreases in adipose tissue depots can impact sex hormone profile. Whether these changes in sex profile are clinically significant is less certain; a previous report noted that DPP men randomized to ILS lost approximately a median (IQR) of 55 cm^2 (76.4 cm^2) VAT at the L2-L3 area (9). Thus, a 55-cm^2 VAT loss would translate into 55 cm^2 × 0.021 nmol/L per cm^2 or a median (IQR) increase of approximately 1.15 nmol/L (1.60 nmol/L) in testosterone. The same report noted that women randomized to ILS lost approximately 23.9 cm^2 (45.1 cm^2) VAT at the L2-L3 area (9). Thus, a 24-cm^2 VAT loss would translate into a 3.36 nmol/L (6.31 nmol/L) increase in SHBG.

Previous reports that assessed adiposity by radiographic imaging have suggested similar relationships between adiposity and androgens in men (2, 12–14). Using data from the Multi-Ethnic Study of Atherosclerosis (MESA), Mongraw-Chaffin *et al.* (2) reported that higher amounts of visceral fat were associated with lower calculated bioavailable testosterone and SHBG in men. Similar patterns were observed with subcutaneous fat although strength of association was less pronounced

Table 4. Association Between Changes in VAT and SAT With Changes in Sex Hormone in Linear Regression Models (Standardized β-Coefficient and *P* Value), Stratified by Menopausal Status

	ΔDHEAS		ΔTestosterone		ΔEstradiol		ΔEstrone		ΔSHBG	
Model	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**
Women premenopausal at baseline (n = 178)										
ΔVAT (L2-L3)	−0.05136	0.571	0.04573	0.616	0.12832	0.170	**0.23419**	**0.017**	**−0.21550**	**0.016**
ΔSAT (L2-L3)	−0.14989	0.105	−0.00748	0.936	0.05347	0.585	0.11046	0.280	**−0.24544**	**0.007**
Women postmenopausal at baseline (n = 131)										
ΔVAT (L2-L3)	−0.13070	0.119	**−0.27727**	**0.015**	−0.07827	0.452	−0.16603	0.441	−0.10542	0.252
ΔSAT (L2-L3)	−0.06767	0.421	−0.19859	0.077	−0.17761	0.092	**−0.48883**	**0.021**	**−0.22479**	**0.014**

β-coefficients are in SD of changes in sex hormones per SD increase in the change of adipose tissue depot in cm^2 (Δcm^2). Models adjust for age, race/ethnicity, randomization arm, and size of adipose tissue measure at baseline. Boldface indicates $P < 0.05$.

Table 5. Association Between Change in VAT and SAT With Changes in Sex Hormone in Linear Regression Models (Standardized β-Coefficient and *P* Value)

	ΔDHEAS		ΔTestosterone		ΔEstradiol		ΔEstrone		ΔSHBG	
Model	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**	**β**	***P* Value**
Men										
ΔVAT/SAT (L2-L3)	−0.02163	0.753	**−0.14717**	**0.034**	0.01657	0.825	0.13482	0.056	0.03523	0.610
ΔVAT/SAT (L3-L4)	−0.08639	0.226	−0.07009	0.334	0.03595	0.639	0.03554	0.625	−0.01969	0.787
ΔVAT+ΔSAT										
ΔVAT (L2-L3)	−0.02142	0.811	**−0.35215**	**<0.001**	−0.04845	0.628	0.08643	0.339	−0.13947	0.120
ΔSAT (L2-L3)	0.09122	0.278	−0.14255	0.075	0.01069	0.909	−0.13659	0.098	−0.12242	0.146
ΔVAT+ΔSAT										
ΔVAT (L3-L4)	−0.05313	0.549	**−0.18248**	**0.035**	−0.02181	0.826	0.04406	0.627	−0.11545	0.194
ΔSAT (L3-L4)	0.06705	0.407	**−0.28501**	**<0.001**	−0.00641	0.944	−0.00280	0.973	**−0.16024**	**0.049**
Women										
ΔVAT/SAT (L2-L3)	−0.05731	0.358	−0.03425	0.609	0.01335	0.841	0.07792	0.362	0.02295	0.708
ΔVAT/SAT (L3-L4)	−0.09030	0.153	0.05729	0.400	−0.04630	0.499	0.02323	0.795	−0.04707	0.451
ΔVAT+ΔSAT										
ΔVAT (L2-L3)	−0.09284	0.240	−0.08180	0.338	0.09420	0.262	**0.21859**	**0.045**	−0.05128	0.504
ΔSAT (L2-L3)	−0.01829	0.817	−0.07418	0.382	−0.06505	0.441	−0.05249	0.636	**−0.19472**	**0.012**
ΔVAT+ΔSAT										
ΔVAT (L3-L4)	−0.08976	0.233	0.07866	0.324	0.02963	0.710	0.14627	0.189	−0.07556	0.298
ΔSAT (L3-L4)	−0.00756	0.915	**−0.14886**	**0.049**	0.02798	0.708	0.02856	0.775	−0.10776	0.116

Models enter both VAT and SAT simultaneously as a ratio or as separate independent variables. β-coefficients are in SD of changes (Δ) in sex hormones per SD increase in the change of adipose tissue depot, in cm^2 (Δcm^2). Models adjust for age, race/ethnicity, randomization assignment, and size of adipose tissue measure at baseline. Boldface indicates $P < 0.05$.

than with visceral fat. Among Japanese men (12) and Danish men (14), lower visceral fat was also correlated with higher total and calculated bioavailable testosterone and SHBG concentrations. Although the majority of total adipose tissue mass is SAT, VAT has the highest risk for metabolic dysregulation, including insulin resistance, presumably due in part to increased release of fatty acids and other metabolites into the portal vein, as well as increased secretion of harmful adipocytokines relative to subcutaneous fat (18). In animal models, increased VAT is associated with hypothalamic inflammation and impaired release of gonadotropin releasing hormone, which impacts testosterone release (19). The associations between both fat depot areas and SHBG may be explained by the fact that both VAT and SAT, but particularly VAT, are associated with hepatic adiposity, which in turn is negatively correlated with hepatic SHBG production (20). Similarly, the DPP has previously reported that randomization to lifestyle intervention led to significant reductions in VAT and SAT, and thus reductions in these fat depots are highly correlated with each other (8, 9).

We found that reductions in the ratio of VAT/SAT were significantly associated with increases in total testosterone at the L2-L3 level, suggesting that the reductions in VAT were more significant for testosterone levels than SAT. This was supported by results showing that L2-L3 VAT, but not L2-L3 SAT, was associated with testosterone in models that included both fat depots. However, these findings were not corroborated at the L3-L4 level, where we found no association between VAT/SAT ratio with testosterone, and in models that included both VAT and SAT, both VAT and SAT were significantly associated with testosterone. In contrast to findings at the L2-L3 level, the models from the L3-L4 level suggest that both fat depots could be important for determinants of testosterone concentration. These conflicting patterns suggest that reductions in VAT may be slightly more impactful for testosterone, but the concurrent reduction in both VAT and SAT in the DPP may have minimized this impact.

Previous cross-sectional studies have also suggested that visceral adiposity is associated with greater androgenicity in women, *i.e.* higher calculated bioavailable testosterone and lower SHBG concentrations (2, 3, 15). Because SHBG preferentially binds to testosterone over estradiol (17, 21), and due to the fact that bioavailable testosterone is usually not measured directly but calculated (22), low SHBG levels may indicate greater relatively androgenicity in women, even in the presence of normal total testosterone and estradiol levels (2, 3, 15). Both MESA and the Study of Women's Health Across the Nation (SWAN) reported that visceral adiposity was associated with higher bioavailable testosterone and lower SHBG in women (2, 3, 15). Similarly, we found that changes in both VAT and SAT were inversely related to changes in SHBG, suggesting that decreases in fat were associated with decreases in androgenicity in women. We did not find associations in between reductions in the

ratio of changes in VAT to changes in SAT, nor we did we find that VAT was more associated with SHBG than SAT in models containing both fat depots. This suggests that both VAT and SAT were important for SHBG levels or that the correlation between VAT and SAT was so high that we could not detect differences in their relative importance for sex hormone levels. It is possible that we observed a greater number of associations in cross-sectional as opposed to longitudinal analyses for several reasons, including lack of impact of changes in fat depot upon specific sex hormone profiles, as well as assay imprecision that reduced statistical power for change analyses.

We did not find that changes in adiposity were associated with changes in estradiol or DHEAS in men or women. Although models examining baseline measures only found significant associations between fat depot with DHEAS, estradiol, and estrone, these associations were generally not present in models examining changes in fat and changes in sex hormones. This suggests that the discrepant results were due the longitudinal nature of the analysis of changes. Previous reports have conflicted regarding the association between estradiol and fat depots, with no association noted in Japanese men between baseline hormone measurement and change in VAT over 10 years (13) and in a cross-sectional study of women in SWAN (3, 15) whereas MESA has previously reported a direct cross-sectional association between visceral adiposity and estradiol, as well as DHEAS concentrations among men and women (2).

Among women, we did note an association between VAT and estrone, the predominant estrogen in postmenopausal women (23), which has not been examined in conjunction with adiposity depot in other studies. Estrone is predominantly manufactured by adipose tissue rather than the ovaries (23), and thus reductions in estrone could reasonably be expected with reductions in adiposity. In addition, estrone concentrations were higher than estradiol concentrations in our study, and it is possible that changes in this sex steroid were easier to detect. Explanations as to why reductions in VAT were associated with reductions in estrone among premenopausal women, whereas reductions in SAT were associated with increases in estrone in postmenopausal women, are speculative. It is possible that in postmenopausal women, aromatization from estradiol to estrone is altered or that the relationship between adiposity and estrogen production is subject to other factors that change with the menopause; in SWAN, the relationship between waist circumference and estradiol concentrations also differed by menopausal status (6). To our knowledge, no other studies have reported associations between weight loss and changes in DHEAS concentrations, although cross-sectional associations and trials examining the impact of DHEAS supplementation upon weight loss have been conducted with the latter showing equivocal results (24).

It is likely that the relationship between sex hormones and adiposity is bidirectional. Although the present report was conducted in the setting of a weight-loss intervention, we excluded users of exogenous sex steroids, and the pattern of results was similar after adjustment for menopausal status, our report does not preclude significant effects of sex steroid changes upon fat mass. The majority of persons excluded were excluded due to exogenous sex hormone use and had lower BMI, weight, and fasting glucose than included persons, suggesting that these factors and exogenous sex steroid use might be associated. Low testosterone concentrations independently predict future increases in intra-abdominal fat, even after adjustment for baseline intra-abdominal fat (25). VAT has a higher concentration of androgen receptors than SAT, and these androgen receptors may facilitate local sex steroid effects (26, 27). Similarly, adipose tissue also contains α and β estradiol receptors that may influence the deposition and activity of adipose tissue (28). Men who are randomized to sex steroid suppression with goserelin and therapy with testosterone and anastrozole to suppress conversion to estradiol demonstrate significant increases in intraabdominal fat area (29). Similarly, among women undergoing the menopausal transition, estradiol declines predict increases in waist circumference in the early transition, although later postmenopausal changes in waist circumference are stronger predictors of estradiol changes than vice-versa (6).

The strengths of this report include the use of serial CT imaging to assess adiposity, as opposed to reliance on anthropometric measures only. Although waist circumference correlates with visceral fat, correlation coefficients range between 0.3 and 0.5 for women and 0.5 and 0.6 for men due to the inclusion of subcutaneous fat, as well as abdominal muscle in waist circumference measurements (30). We also used sensitive mass spectrometric methods to assess sex steroid measures, which is particularly pertinent for measures of sex steroids in postmenopausal women. CT and sex steroids were assessed contemporaneously and in the setting of a randomized trial of weight loss, suggesting that reductions in weight resulted in changes in sex hormone profile. However, there are several limitations. This is a secondary analysis of a randomized trial, and thus it is possible that the associations between adiposity and sex steroids resulted from confounders that affected both sex steroids and weight rather than from direct effects of weight changes upon sex steroids. We used single measures of sex hormones at baseline and at follow-up rather than multiple measures, which may have reduced statistical power, although other cohorts have

noted that single measures of sex hormones reflect sex hormone levels over several years (31). The use of frozen rather than fresh sera may have affected steroid concentrations. The examination of estradiol changes in premenopausal women were not timed to the menstrual cycle, thus reducing our ability to assess significant estradiol changes with weight reduction. Finally, our statistical power to detect differences by race/ethnicity was limited. Although other cohorts have not found significant differences in the relationship between sex steroids and adiposity by race/ethnicity (2), racial/ethnic subgroups have been reported to have different patterns of adipose tissue deposition, as well as sex steroid profiles (32).

We conclude that reductions in fat mass, particularly VAT, are associated with increases in total testosterone in men and greater production of SHBG in men and women. These results suggest that lifestyle modification should be investigated further as a means of addressing androgen deficiency and excess in men and women, respectively. These findings also suggest that reductions in VAT may contribute to declines in estrone in women. Further investigation is needed to determine to what extent sex hormones interact with adipokines and whether modification of fat mass can lead to improvement of symptoms attributed to androgen excess or deficiency.

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