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Sex steroids and adiposity in a prospective observational cohort of youth

Running title: Sex steroids and adiposity

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Abstract

Objective Adiposity, particularly visceral adipose tissue (VAT), predicts adverse cardiovascular risk factor profiles in children as well as adults. Although endogenous sex steroids likely influence VAT in adults, such an association has not been established in youth. The association between childhood and adolescent sex steroids with adiposity, specifically VAT, was examined before and after adjustment for other hormone changes.

Methods These analyses examined longitudinal associations between sex steroids (testosterone, estradiol, dehydroepiandrosterone [DHEA]) and magnetic resonance imaging assessments of VAT in 418 children, 49% of whom were non-white, at approximately 10 years old at visit 1 (V1) and 17 years old at visit 2 (V2). Linear mixed effects models adjusted for maternal education, household income, child caloric intake, physical activity, fasting insulin and leptin, and hepatic fat fraction. Differences in associations by race and pubertal stage were also assessed.

Results At V1, mean BMI for boys was 19.1 (4.7) kg/m² and for girls was 18.5 (4.1) kg/m². At visit 2, mean BMI for boys was 23.7 (5.5) kg/m² and for girls was 23.6 (5.7) kg/m². For each ng/dl (0.035 nmol/l) increase in testosterone at V1, there was a 0.25 cm² increase in concurrent and future VAT in non-white (p=0.04) but not white girls (p=0.78). Higher levels of testosterone and DHEA at V1 were associated with greater concurrent and future VAT at V2. These associations were consistent regardless of pubertal stage. In boys, higher testosterone predicted higher future VAT but lower concurrent VAT. Estradiol and DHEA did not predict future VAT in boys. In girls, DHEA predicted future SAT, and no sex steroids predicted future SAT in boys.

Conclusions Testosterone levels predict VAT in boys and girls, and DHEA predicts VAT in girls, even after adjustment for other hormone changes.

Introduction

Visceral adiposity predicts adverse cardiovascular risk factor profiles in children (1, 2) as well as adults (3). Therefore, understanding the determinants of visceral adipose tissue (VAT) in youth is important to improving metabolic health. Although endogenous sex steroids predict visceral adipose tissue (VAT) area in adults (4), such an association has not been established in youth for several reasons. First, precise measurement of VAT can be difficult due to varying correlations between anthropometric and radiographic measures by race and sex (5, 6). Second, children experience changes in other hormones that likely influence VAT deposition, and it is unclear whether sex steroids are influential after adjustment for these other hormones, particularly leptin and insulin. Third, undetectable sex steroid levels are common in children (7), and no consensus exists regarding the optimal statistical approach for handling such values. Finally, longitudinal studies are optimal during this period of rapid development, but are difficult to perform.

The Exploring Perinatal Outcomes in Children (EPOCH) study is a longitudinal cohort study of youth in Colorado that measured VAT and subcutaneous adipose tissue (SAT) by MRI, and sex steroids at approximately 10 years of age and again six years later.(7) EPOCH also assessed other hormones that change during childhood, including leptin. Approximately half of the population was Hispanic or African-American, reflecting the racial/ethnic distribution of Colorado. Thus, EPOCH is one of the few studies which assesses repeated measures of adiposity and sex steroids in a diverse ethnic population of youth. Using these data, along with statistical techniques developed for addressing non-detectable levels of sex steroids, the association between testosterone, dehydroepiandrosterone (DHEA), and estradiol with concurrent and future VAT and SAT was examined. A previous reports in adults has suggested that higher androgen

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levels were associated with greater amounts of VAT in women and lesser amounts of VAT in men.(8) Therefore, hypotheses for these analyses were that higher androgen levels would predict greater VAT and SAT in girls and lesser VAT and SAT in boys, and greater estradiol levels would predict greater VAT in both girls and boys, after adjustment for diet, physical activity, changes in other hormones including leptin, and that such associations would be consistent across pubertal stage.

Methods

Participants

EPOCH is a historical prospective cohort of youth with the original aim of characterizing long-term consequences of in-utero exposure to maternal diabetes. Children exposed to maternal diabetes in-utero and a random sample of children not exposed to maternal diabetes were also enrolled. EPOCH recruited healthy 6- to 13-yr old children who were offspring of singleton pregnancies, born at a single hospital in Denver between 1992 and 2002, whose biological mothers were members of Kaiser Permanente of Colorado (KPCO) (9). The study population was sampled to reflect similar racial and ethnic distributions of Colorado. Children and their mothers were invited to participate in 2 research visits at average ages of 10.5 (SD=1.5) and 16.7 (SD=1.2). This analysis focused on the 418 children who attended both visits. All participants provided informed consent, and youths provided written assent. The study was approved by the Colorado Multiple Institutional Review Board.

Main Outcome Measures

At both visits, MRI of the abdominal region was used to quantify VAT and SAT with a 3 T HDx Imager (General Electric, Waukegan, WI, USA); the same MRI machine was used. Each study participant was placed supine and a series of T1-weighted coronal images were taken to locate the L4/L5 plane. One axial, 10 mm, T1-weighted image, at the umbilicus or L4/L5 vertebrae, was analyzed to determine VAT and SAT content. Images were analyzed by a single reader, blinded to exposure status. With measures of VAT and SAT, VAT/SAT ratios were generated. Hepatic imaging was performed at the 2nd research visit using a magnitude based, 6-echo, spoiled gradient-recalled echo sequence. Hepatic fat fraction (HFF) was calculated from the mean pixel signal intensity data, for each echo acquisition using an open source Osirix algorithm. This fraction was then multiplied by 100 such that a value of 1 is equivalent to 1% HFF.

DHEA, testosterone, estradiol measurements

A fasting blood draw between 7 – 10 a.m. occurred for all consenting children at both the 1st and 2nd visits. Sera from the 1st research visit were frozen and stored at -80°C for an average of 6.2 years, and then analyzed when the participant completed the 2nd research visit. Sera from the 2nd research visit were refrigerated and analyzed within 24 hours of collection. All laboratory measurements were performed at the Colorado Clinical Translational Science Institute Core Laboratories. Serum DHEA was measured by using a Beckman Coulter chemiluminescent assay with a limit of detection of 0.05 micromol/l. Serum total testosterone was measured by using a Beckman Coulter 1-step competitive with a limit of detection of 0.59 nmol/l. Serum estradiol was measured by using a Beckman Coulter chemiluminescent with a limit of detection of 36.7 pmol/l.

Covariate measurements

Race/ethnicity was self-reported using 2000 U.S. Census-based questions and categorized as Hispanic (any race), non-Hispanic white, non-Hispanic African-American, and non-Hispanic other. Maternal level of education (high school or less versus more than high school) and total household income (less than \$50,000 versus more than \$50,000) were self-reported at the study visit. Pubertal development was self-assessed using a diagrammatic representation of Tanner staging (10); this method was recently shown to be in excellent agreement with physician-assessed Tanner stage (11, 12).

Childhood height and weight were measured in light clothing and without shoes. Weight was measured to the nearest 0.1 kg using an electronic scale. Height was measured to the nearest 0.1 cm using a portable stadiometer. BMI was calculated as kg/m^2 , and Z-score and percentile calculated based on Centers for Disease control growth charts. Caloric intake was assessed at both research visits with the Block Kids Food Frequency Questionnaire, which has been developed and validated for children >8 years.(13) Physical activity was assessed at both research visits with the 3-day Physical Activity Recall. Participants recalled prior day activities in 30-minute blocks, along with intensity level (light, moderate, hard, and very hard) as appropriate. Per guidelines, responses were used to calculate the average daily number of 30-minute blocks of physical activities with moderate intensity (>6 metabolic equivalents).(14) Fasting insulin was measured by a radioimmunoassay method (Millipore, Billerica MA). Plasma leptin concentration was measured by using a Millipore radio-immunoassay with a sensitivity of 0.5 ng/ml.

Statistical analysis

Girls and boys were examined separately. Baseline characteristics were described using numbers (percentages) for categorical variables and means (standard deviations) and medians (interquartile ranges) for quantitative variables with normal and skewed distributions, respectively (Table 1). To determine the association between sex steroids and adipose tissue depot, separate general linear mixed models were fit, one for each sex hormone (DHEA, testosterone, estradiol) and fat depot (VAT and SAT and VAT/SAT) combination. Models fit a random intercept for each participant and random slope for age with unstructured covariance, with degrees of freedom adjusted using methods from Kenward and Roger.(15)

First, the association between sex hormones (predictors) and fat depots (outcomes) was examined using the “at or before” approach.(16) In this analytic approach, interaction terms are used to assess whether associations between predictors and outcomes differed depending upon they were measured at the same visit or different visits. If associations differed depending upon whether the sex steroid and fat depot were assessed concurrently vs. at different visits, the concurrent associations were reported separately and significance tested separately. If associations did not differ depending upon whether the sex steroid and fat depot were assessed concurrently, the association was reduced to a single parameter and tested as such. Cross-sectional associations between sex hormones and fat depot both assessed at visit 2 were assessed separately.

Sex steroid levels below detectable range are common in children, and common approaches to handling such missing values are to conduct multiple imputation or to omit individual data, but such strategies may introduce bias (17, 18). In order to avoid these biases, indicator variables were created to distinguish measurements below the limits of detection from detectable hormones, thus allowing for individuals with undetectable levels to be included in the analysis while avoiding inclusion of potentially biased estimates based upon imputation. While the continuous association between adiposity and sex hormone below the limit of detection could not be estimated, the indicator variable allowed for an estimate of the mean level of adiposity over time. Interaction terms allowed the assessment of differences in magnitude between associations between variables measured at the same time vs. different times, both for those with hormones below the limit of detection, and for those above; interaction terms for the indicator variables for detectable hormone levels were not significant, suggesting that the pattern of results was not altered regardless of whether sex steroid levels were in detectable range.

Models were further adjusted for several variables: indicators of maternal education and household income at visit 1, physical activity and diet at the same visit as the predictor; and fasting insulin and leptin and hepatic fat fraction at visit 2; these variables were selected to reduce possible confounding between sex steroids and adipose tissue deposition. To determine whether the pattern of associations differed by race/ethnicity or pubertal stage, interactions with race/ethnicity or pubertal stage at the initial visit were assessed and removed if non-significant. For each best-fitting model, modeling assumptions were evaluated with jackknife residuals, and reported parameter estimates with 95% confidence intervals, model generated Wald tests at an

alpha level of 0.05, and graphics. Analyses were performed using the Statistical Analysis Software (SAS) version 9.4 (SAS Institute).

Results

Table 1 shows participant characteristics by sex. At the first visit, both boys and girls were on average 10.5 ± 1.5 years of age. At the second visit, both boys and girls were on average 16.7 ± 1.2 years of age. At the first visit, approximately half of the boys were pre-pubertal compared to about 1/3 of the girls; by visit 2, all of the children had reached puberty. About half were non-Hispanic white. Although most mothers had more than a high school education, almost half of the population had an annual income less than \$50,000 per year. On average, caloric intake and physical activity were similar between visit 1 and visit 2. Table 1 also shows average adipose tissue areas and sex steroid levels at each visit, all of which increased during this period of rapid development.

Table 2 shows the association between sex steroids and VAT in girls. The associations between sex steroid levels and VAT both measured at visit 1 were similar to the associations between sex steroid levels at visit 1 and VAT at visit 2, so these parameters were combined. Higher androgen levels at visit 1, i.e. higher DHEA and testosterone, were associated with greater VAT depots. Of note, higher androgen levels at visit 2 and VAT at visit 2 were not significantly associated, suggesting that androgen levels predicted current and future VAT in girls at younger ages, but this association weakened as girls aged. Associations were similar regardless of pubertal stage, since there was no effect modification by pubertal stage. However, race modified the association between testosterone at visit 1 and VAT at visits 1 and 2 ($p=0.04$): non-white girls had a 0.25

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cm² increase in VAT for each unit increase in testosterone, but the association was not significant in white girls (p=0.78). Similar associations were observed when VAT/SAT was the outcome.

Table 2 also shows associations between sex steroid levels and SAT in girls. If the association between sex steroid levels at visit 1 and adipose tissue area at visit 1 were statistically similar to associations between sex steroid levels at visit 1 and adipose tissue area at visit 2, as well as those between sex steroid levels at visit 2 and adipose tissue area at visit 2, the parameters were combined; otherwise, separate coefficients are shown. In general, the strongest associations were when the sex steroid (DHEA, testosterone, and estradiol) as well as SAT were both measured at visit 1. Higher androgen levels at visit 1 predicted higher SAT at visit 1; although DHEA at visit 1 also predicted SAT at visit 2, testosterone at visit 1 did not predict SAT at visit 2. In contrast to associations between sex steroids and VAT which waned as girls aged, cross-sectional associations between androgen levels at visit 2 and SAT levels at visit 2 remained significant.

Table 3 shows the association between sex steroids and VAT in boys. The relationship between endogenous testosterone and VAT changed direction over time, so that higher androgen levels at visit 1 predicted higher VAT at visit 2, but higher androgen levels at visit 2 predicted lower VAT at visit 2. Specifically, a visit 1 testosterone level of 1.66 nmol/l predicts a visit 2 VAT of 0.144 cm², whereas a visit 2 testosterone level of 16.8 nmol/l predicts a visit 2 VAT of -4.1 cm². Associations did not differ by pubertal stage. However, race modified the association between testosterone at visit 2 and VAT at visit 2 (p<0.01), so that associations were stronger among non-

white boys. Among non-white boys, for each unit increase in testosterone, there was a 0.02 decrease in VAT ($p<0.001$), but this association was not significant among white boys ($p=0.36$). Similar associations were observed when VAT/SAT was the outcome.

Table 3 also shows associations between sex steroid levels and SAT in boys. Sex steroid levels at visit 1 did not predict SAT at visit 2. However, as with associations between testosterone and VAT, higher levels of testosterone at visit 2 were associated with lower levels of SAT at visit 2, again suggesting that the relationship between endogenous testosterone and SAT changes over time in boys. DHEA levels were associated with higher SAT at both visits; however, the association was modified by pubertal status (interaction $p=0.041$). At visit 1, among pre-pubescent boys, each unit increase in DHEA was associated with a 0.49 cm² increase in SAT, whereas pubertal boys had a 0.01 cm² decrease in SAT. At visit 2, among pre-pubescent boys, each unit increase in DHEA was associated with a 0.26 cm² increase in SAT, but this association was much weaker in pubertal boys, among whom each unit increase in DHEA was associated with only a 0.02 cm² increase in SAT.

Discussion

In a large, racially and ethnically diverse sample of youth, sex steroid levels were significantly associated with VAT area, even after adjustment for caloric intake and levels of other hormones such as insulin and leptin. The nature of the associations was dynamic and changed as children aged. Although associations were generally similar with those observed in adults, there were also several key differences depending upon whether children were white or non-white. Among non-white girls, higher androgen levels predicted greater VAT, particularly at younger ages. Among

both white and non-white boys, higher testosterone in younger boys predicted greater future VAT. However, this association changed as boys aged, so that among non-white boys, higher testosterone was associated with lower concurrent VAT when children were approximately 17 years of age, similar to the association observed in adults. Among girls, higher androgen levels predicted greater SAT at younger ages, and the strength of these associations waned as children aged. In contrast, among boys, higher testosterone in older boys was associated with lower concurrent SAT at older but not at younger ages. This relationship was stronger among non-white boys. Although these associations do not demonstrate causation, they suggest that sex steroids may contribute to fat deposition or serve as a marker for other unmeasured factors that affect fat deposition during adolescence.

These findings align with another report in healthy children noting significant associations between sex steroids and adiposity during childhood. In the EarlyBird cohort, a British study of white children, higher DHEA-sulfate levels were associated with greater body fat assessed by dual-energy x-ray absorptiometry (DEXA) and greater android/gynecoid fat distribution as assessed by DEXA in girls but not in boys,(19, 20) similar to the findings that higher DHEA predicted higher VAT and SAT in girls while weaker associations were observed in boys. In that study, higher testosterone was associated with lower body fat in boys and greater android/gynecoid fat distribution in girls (19, 20) similar to the observations that testosterone had inverse associations with VAT in older boys and direct associations with VAT in girls. The results of present analysis build upon this prior research in that the pattern of associations in EPOCH differed with stronger associations observed in non-white children, suggesting that sex steroid profiles are particularly important for fat deposition in minority children. Added strengths

of the present study are that the use of MRI rather than DEXA for assessment of VAT, and that the examination of longitudinal as well as cross-sectional associations, leading to the finding that testosterone may have different implications for VAT as boys aged. Models also adjusted for other hormones, particularly leptin and insulin, suggesting that sex steroids may have independent associations with VAT and SAT, apart from the other hormonal changes that occur during childhood.

Associations were stronger in non-white children. No other studies have examined relationships between adipose tissue depot and sex steroids in non-white children. The differences in timing and magnitude of associations between sex steroids and adipose tissue depot between white and non-white children may reflect different locations of adipose tissue deposition by race/ethnicity. As in adults,(8) non-white children appear to have different patterns of adipose tissue deposition than white children.(5, 21) Non-white children, particularly African-American children, undergo pubertal maturation earlier than white children,(22) and it is possible that associations between sex steroids and adiposity were more easily detectable in these children. It is also possible that effect modification by race was due to other factors that change during childhood including leptin, insulin-like growth factor-1, and insulin levels. However, the associations between sex steroids and adipose tissue deposition persisted after adjustment for fasting insulin and leptin levels, which would reduce confounding by these factors. Additional study in multiethnic cohorts is needed to determine how each of these factors, in addition to sex steroid levels, varies by race/ethnicity.

The extent to which obesity leads to earlier and higher sex steroid production in children or vice-versa is not established, although studies in both children (23, 24) and adults suggest that the relationship is likely bi-directional.(4, 25) Of note, overweight and obesity in children are often represented by BMI Z-scores, which may not correlate with body fat percentage.(26) In cross-sectional (23, 24, 27, 28) as well as longitudinal studies including the Danish National Birth Cohort and its subgroup Puberty Cohort, childhood overweight and obesity were associated with earlier age at puberty in both boys and girls.(29) A previous report in EPOCH noted that more rapid accumulation of VAT between visits 1 and 2 was associated with lower testosterone at visit 2 in boys.(7)

It is unclear why the association between androgens and adiposity may change over puberty in boys. In pre-pubertal boys, others have reported a positive association between circulating androgens and obesity indices.(30) Studies have focused upon testosterone, a relatively stronger androgen than the precursor DHEA. In pre-pubertal boys (Tanner stage I), testosterone therapy upregulates 11- β -HSD1 mRNA expression and 11- β -HSD1 activity in omental adipose tissue, which in turn converts inactive cortisone to cortisol.(31) In transgenic mice, expression and increased activity of 11-beta-HSD1 in adipose tissue leads to visceral obesity and insulin resistance.(32) This purported mechanism corresponds with our finding that pre-pubertal testosterone concentrations in boys were directly associated with greater VAT. This positive association in boys is lost during puberty. Testosterone therapy leads to decreases in visceral fat mass in hypogonadal adolescent males.(33) In a hypogonadal (castrated) mouse model, treatment with testosterone significantly reduces visceral fat mass, largely as a result of reductions in adipocyte size via increased androgen receptor activity.(34) Compared to other cell types,

adipocytes were highly sensitive to androgens.(34) However, it is not known why the impact of testosterone would differ in pre- vs. postpubertal boys, and what signaling triggers these differences in effects. Further studies are needed to elucidate the mechanisms for these changes.

In contrast, the relationship between androgens and visceral fat remains consistent in girls through the lifespan, although women become relatively hyperandrogenic after menopause. In women as in men, androgen levels correspond with expression of particular genes in adipose tissue. In a monozygotic twin study,(35) expressions of steroid sulfatase and expression of AKR1C3 and AKR1C2, which regulate conversion between different androgens, as well as HSD11B1 (responsible for cortisone conversion to cortisol, as has been found in men) in adipose tissue are significantly higher in the heavier twins, suggesting that there might be local differences in androgen uptake and regulation, even as serum androgen levels were similar. Along similar lines, in women with PCOS, androgen exposure increases adipocyte de novo lipid synthesis.(36)”

We did not find longitudinal associations between sex hormones and SAT in boys, and higher DHEA levels were longitudinally associated with SAT in girls. The clinical significance of this is not clear. Although both SAT and VAT are correlated with metabolic risk factors, VAT has been more consistently associated an adverse metabolic risk profile.(37, 38, 39, 40) This may be because visceral fat has different metabolic activity than subcutaneous fat, particularly in production of harmful adipokines that lead to elevations in cardiovascular risk factors.(41) Whether the size of the SAT depot is clinically significant apart from its association with greater

VAT is not clear. Removal of SAT has no impact on CVD risk profile in adults,(42) but similar studies have not been performed in children.

The strengths of this report include a relatively large, diverse longitudinal cohort which characterized adiposity using MRI at 2 points in time along with sex hormone measures and possible mediators. Associations between adiposity and sex steroids have been difficult to study for several reasons. First, the relationship between VAT and sex steroids is likely bidirectional, thus requiring longitudinal studies with repeated measures over extended periods of time.(4, 8, 43, 44, 45) Second, anthropometry is limited for assessing area of VAT and SAT, thus requiring technologies which are typically not available in clinical practice. Finally, sex steroids are frequently below the limit of detection in pre-pubescent children, making modeling of associations challenging.,(17, 18)

Although these concerns were addressed in the present study, the analyses have several limitations. As an observational study, it cannot demonstrate that sex steroids directly affect fat deposition. Multiple sex steroids were examined, and multiple comparisons were performed. Thus, some of the observed associations may have been due to chance and need to be replicated. Free fractions of sex steroids using mass spectrometry and sex hormone binding globulin were not assessed, and it is possible that use of these measures might yield a different pattern of results. It is likely that the use of less-sensitive immunoassays biased results to the null. However, hepatic fat fraction which is an indicator of fatty liver and correlated with sex hormone binding globulin, was a covariate and adjustment for hepatic fat fraction did not alter the pattern of results. Dietary assessment of caloric intake is less precise compared with energy assessments

of individuals in standardized settings,(46) although this also would have likely biased associations to the null. Similarly, physical activity assessment with questionnaires may be less accurate compared with devices such as accelerometry or direct observation in standardized settings, although use of more precise measures would have also likely biased associations to the null. Although the Block Kids Food Frequency Questionnaire was validated for children > 8 years, such instruments tend to underestimate energy consumption from fats and sugars compared to 24-hour questionnaires, particularly in younger children.(13) Finally, the possibly changing pattern of associations during this rapid period of development are complex, and replication in longitudinal studies should be performed.

Conclusion

These analyses suggest that sex steroid levels in childhood are associated with regional adipose tissue deposition. Additional studies are needed to determine whether regional adipose deposition persists into adulthood. In particular, studies are needed as to how exogenous sex steroid use, including oral contraceptive pill use in adolescent girls and testosterone therapy in boys with delayed puberty, may influence adipose tissue deposition. Since therapies for androgen excess can lead to lower VAT deposition in girls,(47) studies in broader populations are particularly important due to the potential for additive negative effects of pregnancy and childbearing upon adipose tissue profile. Such studies should examine both non-white and white populations.

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Data availability: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Table 1. Participant characteristics in EPOCH. Means (standard deviations), median (interquartile range), or n (percent) shown.

	Boys (N=209)		Girls (N=209)	
	Visit 1	Visit 2	Visit 1	Visit 2
Age (years)	10.5 (1.5)	16.6 (1.2)	10.4 (1.5)	16.7 (1.2)
Stage of puberty (n, %)				
Tanner 1	113 (54%)	0	74 (35%)	0
Tanner 2	65 (31%)	3 (1%)	77 (37%)	0
Tanner 3	24 (12%)	5 (2%)	38 (18%)	16 (8%)
Tanner 4	6 (3%)	73 (35%)	19 (9%)	93 (45%)
Tanner 5	0	126 (61%)	1 (0.5%)	100 (48%)
Race/ethnicity (n, %)				
Non-Hispanic White	101 (48%)		112 (54%)	
Hispanic	81 (39%)		68 (33%)	
African-American	15 (7%)		18 (9%)	
Other	12 (6%)		11 (5%)	
Maternal education, high school or less (n, %)	35 (17%)		39 (19%)	
Household income < \$50,000 (n, %)	98 (47%)		89 (43%)	
Total caloric intake (kcal/day)	1878 (579)	1836 (785)	1710 (508)	1482 (635)
Moderate physical activity blocks	4.49 (3.02)	4.77 (4.10)	4.12 (2.70)	4.63 (3.70)
Height (cm)	143.8 (11.1)	175.3 (7.3)	143.5 (11.4)	163.7 (7.5)
Weight (kg)	40.6 (14.8)	73.1 (18.9)	38.8 (13.0)	63.2 (15.9)
BMI (kg/m ²)	19.1 (4.7)	23.7 (5.5)	18.5 (4.1)	23.6 (5.7)
BMI ≥95 th percentile	48 (23%)	45 (22%)	28 (13%)	36 (17%)
BMI Z-score	0.31 (1.27)	0.43 (1.20)	0.15 (1.56)	0.40 (1.04)
Fasting insulin (pmol/L)		113.2 (78.5)		121.5 (79.2)
Leptin (ng/ml)		6.6 (8.2)		19.6 (15.4)
Hepatic fat fraction (%)		2.7 (3.7)		2.2 (2.2)
Visceral adiposity (VAT) (cm ²)	22 (15)	32 (22)	22 (15)	34 (22)
Subcutaneous adiposity (SAT) (cm ²)	116 (113)	172 (154)	119 (98)	232 (144)
Estradiol below the level of detection (n, %)	141 (67%)	12 (6%)	78 (37%)	15 (7%)
Estradiol (pmol/L)	67.6 (33.8)	99.1 (45.9)	132.5 (121.1)	316.8 (317.9)
T below the level of detection (n, %)	125 (60.0%)	2 (1%)	155 (74%)	25 (12%)

T (nmol/l)	4.3 (3.6)	17.0 (5.1)	1.1 (0.5)	1.4 (0.56)
DHEA (nmol/l)	247.8 (187.4)	787.0 (373.7)	203.7 (139.5)	605.2 (288.4)

Table 2. Among girls, the association between sex steroid levels (predictor) and adipose tissue (outcome). A positive association indicates that higher levels of sex steroid predict greater adipose tissue area (cm²), or that detectable levels of sex hormone predicted greater adipose tissue area (yes/no).

	DHEA	T	E2
	β (95% CI)	β (95% CI)	β (95% CI)
Association between sex steroid and VAT*			
Sex steroid at visit 1 and VAT at time 1 and 2	0.10 (0.05, 0.15) p<0.0001	0.16 (0.04, 0.28)† p<0.01	0.06 (-0.005, 0.12) p=0.071
Sex steroid at visit 1 and VAT at visit 1	-	-	-
Sex steroid at visit 1 and VAT at visit 2	-	-	-
Sex steroid at visit 2 and VAT at visit 2	-0.01 (-0.02, 0.01) p = 0.43	-0.04 (-0.11, 0.03) p = 0.28	-0.01 (-0.03, 0.01) p = 0.19
Association between sex steroid and SAT*			
Sex steroid and SAT at time 1 and 2	-	-	-
Sex steroid at visit 1 and SAT at visit 1	0.92 (0.61, 1.24) p<0.0001	0.93 (0.33, 1.52) p=0.003	0.56 (0.29, 0.83) p<0.0001
Sex steroid at visit 1 and SAT at visit 2	0.40 (0.05, 0.75) p=0.024	1.10 (-0.89, 3.09) p = 0.28	0.32 (-0.20, 0.84) p = 0.23
Sex steroid at visit 2 and SAT at visit 2	0.20 (0.11, 0.30) p<0.0001	0.56 (0.29, 0.83) p=0.03	0.03 (-0.06, 0.12) p = 0.48

* Models adjust for maternal education and income, caloric intake, physical activity, hepatic fat fraction, leptin, and insulin levels.

†Interaction with race (p=0.04)

Table 3. Among boys, the association between sex steroid levels (predictor) and adipose tissue (outcome). A positive association indicates that higher levels of sex steroid predict greater adipose tissue area (cm²), or that detectable levels of sex hormone predicted greater adipose tissue area (yes/no).

	DHEA	T	E2
	β (95% CI)	β (95% CI)	β (95% CI)
Association between sex steroid and VAT*			
Sex steroid at visit 1 and VAT at time 1 and 2	-	-	-
Sex steroid at visit 1 and VAT at visit 1	-0.004 (-0.04, 0.04) p = 0.86	0.001 (-0.025, 0.026) p = 0.97	-0.05 (-0.23, 0.13) p = 0.59
Sex steroid at visit 1 and VAT at visit 2	0.04 (-0.02, 0.10) p = 0.18	0.03 (0.007, 0.05) p=0.01	-0.30 (-0.78, 0.18) p = 0.21
Sex steroid at visit 2 and VAT at visit 2	-0.003 (-0.02, 0.01) p = 0.70	-0.009 (-0.01, -0.002) p=0.007	-0.04 (-0.15, 0.07) p = 0.45
Association between sex steroid and SAT*			
Sex steroid and SAT at time 1 and 2			
Sex steroid at visit 1 and SAT at visit 1	0.24 (0.03, 0.45)† p=0.03	0.03 (-0.10, 0.16) p = 0.64	0.35 (-0.61, 1.30) p=0.48
Sex steroid at visit 1 and SAT at visit 2	-0.09 (-0.43, 0.25) p = 0.61	0.02 (-0.16, 0.20) p = 0.81	-1.50 (-4.46, 1.47) p = 0.32
Sex steroid at visit 2 and SAT at visit 2	0.14 (0.03, 0.24)† p=0.01	-0.04 (-0.07, -0.01) p=0.003	0.25 (-0.47, 0.97) p=0.50

* Models adjust for maternal education and income, caloric intake, physical activity, hepatic fat fraction, leptin, and insulin levels.

† Interaction with race and pubertal status (p=0.04)