Sex steroids in relation to sexual and skeletal maturation in obese male adolescents

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Background: Childhood obesity is associated with an accelerated skeletal maturation. However data concerning pubertal development and sex steroids levels in obese adolescents are scarce and contrasting.

Objectives: To study sex steroids in relation to sexual and skeletal maturation and to serum PSA, as a marker of androgen activity, in obese boys from early to late adolescence.

Methods: 90 obese boys (10–19 y) at the start of a residential obesity treatment program and 90 age-matched controls were studied cross-sectionally. Pubertal status was assessed according to the Tanner method. Skeletal age was determined by an X-ray of the left hand. Morning concentration of total testosterone (TT) and estradiol (E2) by LC-MS/MS, free T (FT) by equilibrium dialysis, and LH, FSH, SHBG and PSA by immunoassays, were measured.

Results: Genital staging was comparable between the obese and non-obese group, whereas skeletal bone advancement (mean 1 year) was present in early and mid- adolescence in the obese males. While both median SHBG and TT concentrations were significantly ($p<0.001$) lower in obese subjects during mid- and late- puberty, median FT, LH, FSH and PSA levels were comparable to those of controls. In contrast, serum E2 concentrations were significantly ($p<0.001$) higher in the obese group at all pubertal stages.

Conclusion: Obese boys have lower circulating SHBG and TT, but similar FT concentrations during mid- and late-puberty in parallel with a normal pubertal progression and serum PSA levels. Our data indicate that in obese boys, serum FT concentration is a better marker of androgen activity than TT. On the other hand, skeletal maturation and E2 were increased from the beginning of puberty, suggesting a significant contribution of hyperestrogenemia in the advancement of skeletal maturation in obese boys.

It is well-known that obesity in childhood is associated with an accelerated growth and skeletal maturation (1, 2, 3, 4), but it remains unclear which hormonal changes are most important for stimulating the skeletal maturation in this particular condition (5, 6, 7). Moreover, only very limited and contrasting data have been published concerning pubertal development and sex steroids (testosterone (T) as well as estradiol (E2) concentrations), especially in male obese adolescents. Whereas some authors found an advanced sexual maturation in obese boys (8, 9, 10), others reported a normal (1, 2) or even a delayed genital development (1, 3, 11).

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Abbreviations:

Furthermore, there is controversy about T concentrations in obese children and adolescents. In prepubertal obese boys increased total T (TT) concentrations (12, 13), as well as normal (14) and low TT levels have been described (2, 15), whereas in pubertal boys normal (12), as well as decreased TT concentrations have been reported (2, 16, 17, 18). Poor assessment of pubertal staging, small study groups (14, 15), lack of age- matched control group (2) and the use of direct immunoassays for TT determination (12, 16, 17) might explain these contrasting hormonal findings between studies. Moreover, the well-known lower SHBG production in obesity, especially during puberty, might be responsible for the finding of lower TT concentration in obese adolescents (2, 12, 15, 16). Little experience exits with free T (FT) in adolescent obesity (16, 18). Therefore, in the present study TT, SHBG and FT were assessed as markers of androgen secretion and both clinical (genital development) and biological (prostatic specific antigen (PSA) concentrations) markers of androgen activity were assessed in a well described group of obese adolescents. We hypothesized a normal genital development in association with normal FT concentrations and a more rapid skeletal maturation in relation to increased E2 levels.

Materials and Methods

Subjects

Ninety male obese adolescents (BMI SDS $> +2$), aged 10–19 years, were investigated at the entry of a residential weight-loss program at the Zeepreventorium in De Haan, Belgium. Ninety age-matched healthy normal-weighted controls were randomly selected from an ongoing longitudinal study evaluating changes in bone geometry, bone maturation and muscle strength in relation to sex steroids in childhood and adolescence. These healthy children were recruited by letters distributed in several schools within the Ghent area. Subjects with a history of hypogonadism, panhypopituitarism, diabetes, previous or ongoing treatment with T or oral steroids were excluded. Both study protocols were approved by the Ethical Committee of the Ghent University Hospital. Informed consent was obtained from the parents and all participants gave their assent.

Methods

Anthropometry and sexual maturation

Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd, Crymuch, UK). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg. Waist circumference, defined as the smallest abdominal circumference if present or otherwise measured halfway between the iliac crest and the rib cage, was determined to the nearest 0.1 cm. All anthropometric measurements were performed by the same trained physician (SV). The standard deviation score (SDS) for body height, weight, waist circumference and BMI was computed using the reference data of the 2004 Flemish growth study (19). Pubertal status of the subjects was assessed by trained pediatricians according to the method established by Tanner (Tanner Genital Staging: stage 1: prepuberty; stage 5: postpuberty). Testicular volume was determined with a Prader orchidometer in a subgroup of 40 consenting obese boys and their respective controls.

Bone age determination

Bone age reading of an X- ray of the left hand and wrist was done by two independent experienced pediatric radiologists, blinded for the chronological age, using the Greulich and Pyle method (20). The mean of both readings was taken, but if the difference was more than one year a third independent reading (by a trained pediatrician) was performed and the two closest estimates were retained for final calculations. Skeletal age differences (SAD) were calculated by subtracting the chronological age (CA) from the skeletal age (BA) (SAD = BA –CA): positive differences reflecting an accelerated skeletal maturation and negative differences a delayed bone maturation.

Hormonal measurements

Venous blood samples in the obese group were obtained between 0800 and 1000 hours after overnight fasting. Blood samples in the age-matched control group were collected within the same time interval, but allowing a small breakfast. All samples were stored at – 80°C until batch analysis. Commercial automated immunoassays were used to measure SHBG, LH, FSH, DHEAS, PSA (Roche Diagnostics, Mannheim, Germany). The intra- and interassay coefficients of variation (CV's) for all assays were less than 10%. The lower detection limit for PSA was 0.003 ng/ml and the intra-assay and interassay CV's were respectively 1.2% and 3.5%. Serum E2, TT and androstenedione (A) were determined by liquid chromatography tandem mass spectrometry (AB Sciex 5500 triple-quadrupole mass spectrometer; AB 173 Sciex, Toronto Canada). Serum limit of quantification (LOQ) was < 0.5 pg/mL (1.9 pmol/L) for E2 and the interassay CV was 4.0% at 21 pg/mL (77 pmol/L) (21). Serum LOQ was 1.2 ng/dl for TT and the interassay CV was 8.3% at 36.7 ng/dl and 3.1% at 307.8 ng/dl. Serum LOQ was 4.25 ng/dl for A and the interassay CV was 2.9% at 59.8 ng/dl. Serum FT was determined by equilibrium dialysis (FT dialysis) (22), CV of the method calculated from duplicate measurements is 11.7%. FT was also calculated (cFT) in all subjects from the concentrations of TT, SHBG and albumin according to Vermeulen et al (22). The results for FT dialysis and cFT are not substantially different as can be seen from a comparison by Passing-Bablok and Bland-Altman analysis (see supplemental data).

Statistics

Normality was checked using QQ-plots and Shapiro-Wilk tests. The anthropometric data showed at normal distribution, hormonal data were however not normally distributed. Data are presented as mean ± standard deviation or as medians (25th – 75th percentile) in case of a non-normal distribution. Comparison between obese and control groups were performed using parametric independent T-tests or ANOVA, when criteria for normality were met. In other cases, Mann-Whitney U tests were used. Between-group differences of categorical variables were calculated with χ^2 tests. The difference was considered statistically significant at $P < .05$. To study hormonal parameters and anthropometric parameters in the obese boys compared to the

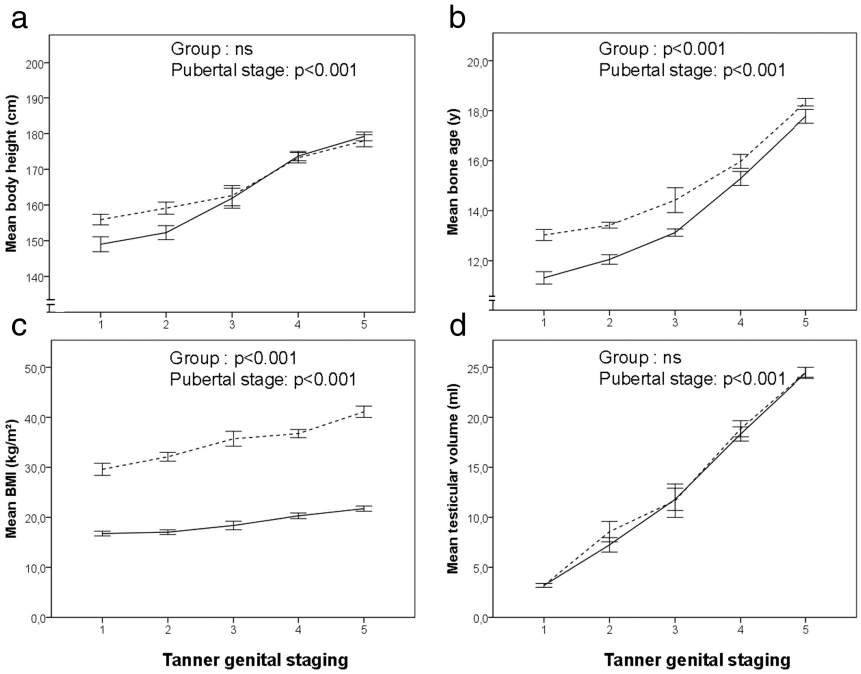

Figure 1. Height (a), bone age (b), BMI (c) and testicular volume (d) at different pubertal stages in obese adolescents compared to their age-matched controls. The line plots present mean height, BMI, bone age and testicular volume for each pubertal stage (prepuberty: 1; postpuberty: 5) for the two study groups. The error bars represent 1 SEM. The obese group is presented by the dotted line and the control group (age-matched controls) by the full line. The interconnecting lines do not present longitudinal data.

controls taking pubertal stage into account as presented in figure 1 and figure 2, linear regression analysis was used. Hormonal parameters TT, FT, E2, LH, SHBG, A, DHEAS and PSA underwent a Box-Cox transformation to enhance normality (transformation factors were TT:λ=0.49; FT: λ=0.42; E2: λ=0.32; LH: λ= 0.63; SHBG: λ=-0.29; PSA: λ=0.23, A: λ=0.40; DHEAS: λ=0.26). Box-Cox transformations were performed using MedCalc for Windows, version 12.5 (MedCalc Software, Ostend, Belgium). Based on the available literature (13, 18) on sex steroid (TT, FT) levels determined by LC-MS/MS and SHBG levels in obese prepubertal and late pubertal boys, sample size calculations were performed using Medcalc for Windows, version 12.4.00 (MedCalc Software, Ostend, Belgium) (α: 0.05; ß:0.20). We calculated a necessary sample size of 7 to 14 children in each group at the different pubertal stages to discern the published differences in TT, FT and SHBG between both groups. Post hoc power calculations on our E2 analyses using G*power (version 3.1.5) demonstrated a power between 70 and 99% at the different pubertal stages. Data were analyzed using SPSS software version 19.0.

Results

Comparison of anthropometric data and sexual and skeletal development between obese boys and age-matched controls

Table 1 summarizes the anthropometric characteristics and Tanner stages of both groups. Mean body weight (SDS), BMI (SDS) and waist circumference (SDS) of the obese group were almost double of those of normal-weighted peers. Mean BMI SDS did not differ between the different pubertal stages in the control group, while BMI SDS at Tanner stage G5 was found to be highest in the obese group (BMI SDS G4: 2.6 ± 0.25; BMI SDS G5:3.0 ± 0.35; $P < .001$) (figure 1). Moreover, waist circumference SDS at Tanner genital stage 5 was significantly higher than waist circumference SDS at Tanner genital stage 4 in the obese group (waist circumference SDS G4: 2.5 ± 0.28; waist circumference SDS G5:2.7 ± 0.24; $P < .05$). Height and height SDS were not significantly different between both groups by ANOVA analysis (figure 1, Table 1). Height and height SDS of prepubertal and early-pubertal (G1 and G2) obese children were significantly higher compared to their healthy peers (height : G1-G2: obese: 158 ± 6 vs. controls: 151 ± 7 cm, $P < .001$; height SDS:G1-G2: obese: 0.6 ± 1.1 vs. controls:-0.06 ± 1.1, $P < .05$). Although skeletal maturation is advanced in obese children from Tanner stage 1 to 4 (<0.001), there is no significant difference in pubertal development between both groups (figure 1, Table 1). Moreover, studying a subgroup of 40 obese adolescents and their matched controls no difference in testicular volume was observed (figure 1). Anthropometric characteristics of this subpopulation were similar to the whole population (data not shown).

Comparison of hormonal and biological parameters between obese adolescents and age-matched controls

Before pubertal onset, TT was similar, while SHBG concentrations were lower and FT, DHEAS, A and E2 higher in obese boys (Table 2). There was a significant positive correlation between FT and DHEAS and between FT and A (spearman rank correlation coefficient (r) r(DHEAS-FT)=0.79 $P < .001$; r(A-FT)= 0.71 $P < .001$). As shown in figure 2, significantly higher sex steroids (TT, FT and E2) and PSA concentrations were found with advancing pubertal stage in both groups ($P < .001$) (figure 2, Table 2). The subanalysis in the different Tanner genital stages showed that obese adolescents (at least stage G2) have lower SHBG levels at every pubertal stage, lower TT concentrations from stage G3 onward, but similar FT con-

centrations, except for adolescents in pubertal stage 5. There was no significant difference between values obtained from FT dialysis and cFT (cFT = 0.0109 + 0.9762 FT dialysis). No significant difference in circulating LH, FSH and PSA concentrations was found between the obese boys and their controls. Serum E2 concentrations and E2/TT ratio were significantly higher in the obese adolescents ($P < .01$).

Associations between the degree of obesity and hormonal parameters in the obese boys

In order to study the importance of the degree of obesity on hormonal parameters, associations between the degree of obesity, expressed as BMI SDS and waist circumference SDS, and TT, FT, SHBG, E2 and PSA were studied using linear regression (including pubertal stage) in the obese group only. Waist circumference SDS was a negative pre-

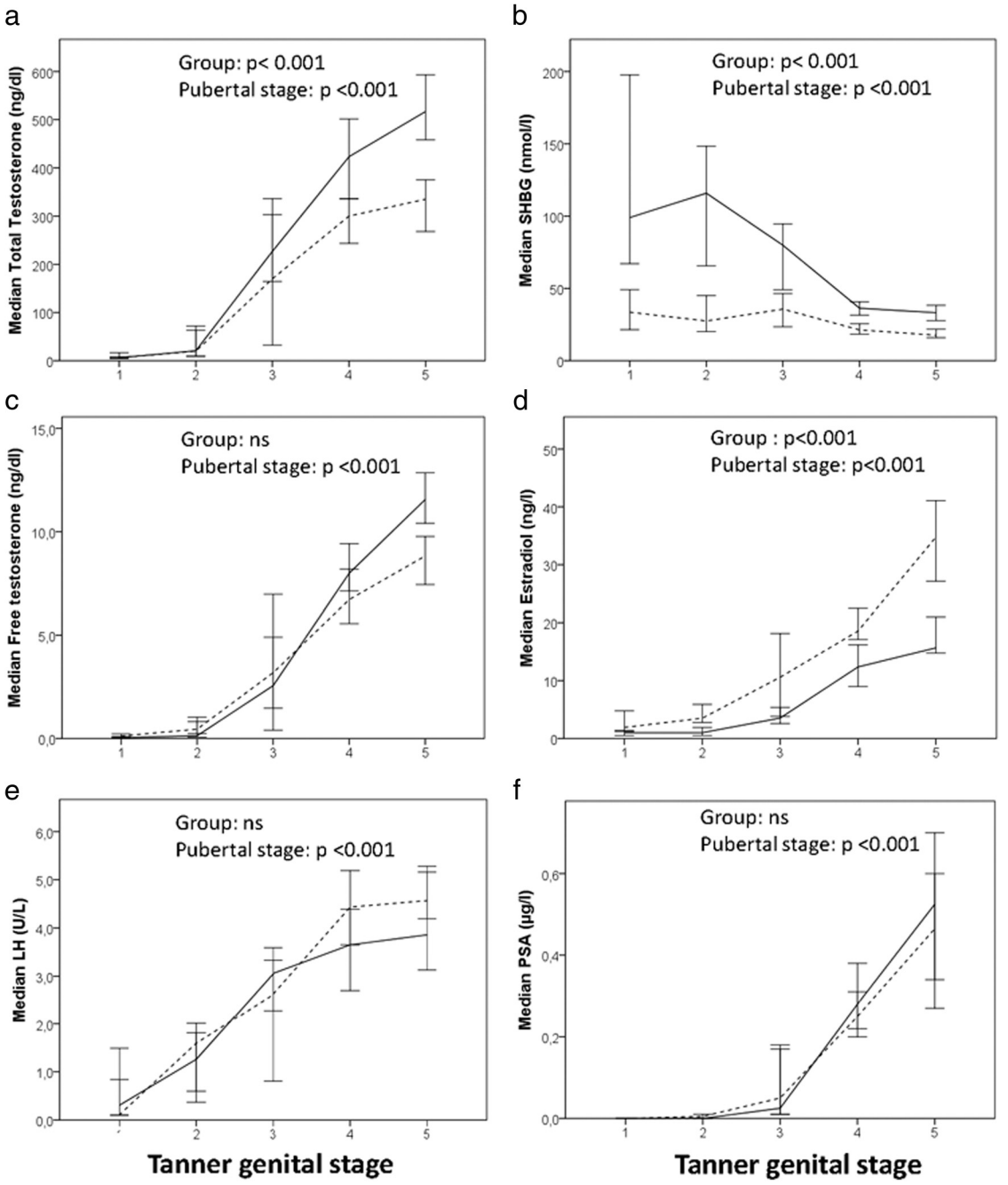

Figure 2. Total testosterone (a), SHBG (b), Free testosterone (c), Estradiol (d), LH (e) and PSA (f) in obese boys and their age-matched controls at different pubertal stages. The line plots present median TT, SHBG, FT, E2, LH and PSA levels for each pubertal stage (prepuberty: 1; postpuberty: 5) for the two study groups. Since the hormonal data do not meet the criteria for a normal distribution, data are presented as medians and the error bars represent 95th confidence interval (CI). The obese group is presented by the dotted line and the control group (age-matched controls) by the full line. The interconnecting lines do not present longitudinal data.

Table 1. Comparison of anthropometric data and skeletal and pubertal development between obese and age-matched control boys.

	n	Obese boys (mean ± SD)	n	Age-matched controls (mean ± SD)	Significance level
Anthropometry					
Age (y)	90	14.6 ± 2.2	90	14.5 ± 2.2	ns
Height (cm)	90	169.0 ± 11	90	167 ± 13	ns
Height (SDS)	90	0.36 ± 1.1	90	0.11 ± 1.0	ns
Weight (kg)	90	104.8 ± 26.0	90	55.6 ± 15.0	<0.001
Weight (SDS)	90	2.9 ± 0.6	90	0.03 ± 0.9	<0.001
BMI (kg/m^2)	90	36.2 ± 5.8	90	19.5 ± 3.2	<0.001
BMI (SDS)	90	2.6 ± 0.37	90	−0.04 ± 0.97	<0.001
Waist circumference (cm)	85	109 ± 15	85	69 ± 8	<0.001
Waist circumference (SDS)	85	2.5 ± 0.28	85	−0.06 ± 0.88	<0.001
Skeletal maturation					
Bone age (y)	90	15.6 ± 2.2	90	14.6 ± 2.6	<0.01
Difference age-bone age (y)	90	1.1 ± 0.9	90	0.2 ± 1.1	<0.001
Tanner genital stage		**Obese boys (frequency)**		**Age-matched controls (frequency)**	
G1	8	8.8%	11	12.1%	ns (χ^2)
G2	17	18.7%	14	15.4%	
G3	13	14.3%	12	13.2%	
G4	30	33.0%	31	34.1%	
G5	23	25.3%	23	25.3%	

Comparison between obese boys and age-matched controls were performed using parametric independent t-tests. Between-group differences of categorical variables were calculated using χ-square tests.

dictor of TT (waist circumference SDS: ß= - 0.18 $P < .01$) and FT testosterone (waist circumference SDS :ß= - 0.13 $P < .05$) levels. However, no association between BMI SDS and TT or FT was found. There was a trend to a negative association between BMI SDS and waist circumference SDS and SHBG (BMI SDS: ß= - 0.20 $P = .08$; waist circumference SDS :ß= - 0.20 $P = .06$). BMI SDS was a positive predictor of E2 levels (BMI SDS: ß=0.20 $P < .01$). No association was found between waist circumference SDS and E2. All results remained unchanged when FT dialysis was substituted by cFT in the analyses.

Discussion

The present study investigates both androgen secretion (assessed by serum TT and FT concentrations using sensitive assays) and androgen activity using detailed clinical and biological assessment of sexual (Tanner genital staging, PSA) as well as skeletal (bone age readings of the left hand and wrist) maturation in a group of obese prepubertal and pubertal boys. Our results demonstrate that pubertal obese boys have lower TT levels, higher E2 but normal FT levels, at least during mid- and late puberty These hormonal differences might be responsible for the observed dissociation between an advanced skeletal maturation (mean advancement around 1 year) and a normal sexual maturation (similar pubertal stage distribution and PSA concentrations). Our data indicate that FT is a better indicator of androgen exposure than TT, explaining the normal pubertal progression and PSA production in male obese adolescents and suggest that the increased estrogen production and aromatization might be linked to the advanced skeletal maturation during pubertal progression.

Firstly, we confirmed the presence of an accelerated growth and skeletal maturation during childhood and early stages of puberty in our group of obese children and adolescents, who had a longstanding and persisting obesity, necessitating a residential weight loss program (1, 2, 3, 4). This accelerated growth and bone maturation contrasts with the finding of a normal androgen secretion and activity during this period, but is in accordance with the increasing estrogen production and aromatization.

Table 2. Comparison of hormonal parameters and biochemical parameters between obese boys and age-matched controls.

	Tanner genital stage	n	Obese boys (median) (P25-P75)	n	Controls (median)(P25-P75)	Significance level (p)
TT (ng/dl)	G1	8	6.3 (5.8–10.3)	11	6.2 (4.8–7.3)	Ns
	G2	16	19.9 (10.1–67.2)	14	20.7 (9.0–44.8)	Ns
	G3	13	170 (32.6–317)	12	227 (170–293)	Ns
	G4	30	300 (201–365)	30	423 (326–504)	<0.001
	G5	23	335 (265–469)	23	517 (445–616)	<0.001
FT dialysis (ng/dl)	G1	8	0.12 (0.1–0.15)	11	0.04 (0.03–0.06)	<0.001
	G2	16	0.45 (0.24–1.0)	14	0.13 (0.08–0.40)	<0.05
	G3	13	3.2 (0.5–6.2)	12	2.6 (1.5–4.7)	Ns
	G4	30	6.7 (5.1–9.3)	30	8.0 (5.9–10.6)	Ns
	G5	23	8.8 (6.7–10.3)	23	11.6 (9.1–13.3)	<0.01
cFT (ng/dl)	G1	8	0.13 (0.11–0.16)	11	0.04 (0.03–0.06)	<0.001
	G2	16	0.44 (0.24–1.1)	14	0.14 (0.07–0.39)	<0.05
	G3	13	3.1 (0.5–6.5)	12	2.5 (1.4–4.7)	Ns
	G4	30	6.5 (5.2–8.8)	30	7.8 (5.4–9.9)	Ns
	G5	23	8.9 (7.1–9.8)	23	11,3 (9.8–12.8)	<0.01
SHBG (nmol/liter)	G1	8	33.6 (28.4–40.4)	11	99.0 (73.0–187)	<0.001
	G2	16	27.5 (20.2–44.9)	14	115.9 (73.6–148.2)	<0.001
	G3	13	35.7 (23.7–43.9)	12	79.9 (52.0–93.1)	<0.001
	G4	30	21.3 (17.2–29.7)	30	36.3 (28.7–44.5)	<0.001
	G5	23	17.6 (15.4–23.8)	23	33.2 (26.4–38.5)	<0.001
E2 (ng/liter)	G1	8	1.9 (1.4–3.0)	11	1.0 (0.5–1.0)	<0.001
	G2	16	3.5 (2.8–5.4)	14	1.0 (0.5–1.4)	<0.001
	G3	13	10.6 (4.0–16.9)	12	3.5 (2.7–5.3)	<0.05
	G4	30	18.6 (14.9–25.3)	30	12.4 (8.5–17.0)	<0.001
	G5	23	34.8 (25.6–41.1)	23	15.7 (13.2–21.0)	<0.001
Ratio E2/TT	G1	8	0.29 (0.20–0.44)	11	0.14 (0.08–0.19)	<0.01
	G2	16	0.16 (0.07–0.37)	14	0.04 (0.02–0.1)	<0.01
	G3	13	0.06 (0.05–0.12)	12	0.02 (0.013–0.021)	<0.001
	G4	30	0.07 (0.05–0.08)	30	0.03 (0.02–0.04)	<0.001
	G5	23	0.11 (0.06–0.13)	23	0.03 (0.02–0.05)	<0.001
LH (U/liter)	G1	8	0.1 (0.1–1.1)	11	0.3 (0.1–0.5)	Ns
	G2	16	1.6 (0.65–1.8)	14	1.3 (0.53–1.8)	Ns
	G3	13	2.6 (1.1–3.2)	12	3.1 (2.3–3.5)	Ns
	G4	30	4.4 (3.4–5.5)	30	3.7 (2.5–4.5)	<0.05
	G5	23	4.6 (3.8–6.0)	23	3.9 (3.0–5.3)	Ns
FSH (U/liter)	G1	8	1.8 (1.1–2.8)	11	1.6 (1.1–2.1)	Ns
	G2	16	2.1 (1.6–3.1)	14	2.1 (1.3–2.3)	Ns
	G3	13	3.0 (1.8–4.6)	12	2.0 (1.8–2.8)	Ns
	G4	30	3.7 (2.8–5.1)	30	3.1 (2.1–4.7)	Ns
	G5	23	2.6 (2.0–4.1)	23	2.7 (1.5–4.7)	Ns
DHEAS (μg/dl)	G1	8	138 (114–152)	11	89.6 (41.1–106)	<0.01
	G2	16	174 (114–244)	14	133 (81.5–180)	Ns
	G3	13	225 (144–288)	12	142 (87.1–181)	<0.05
	G4	30	204 (162–315)	30	221 (157–322)	Ns
	G5	23	370 (268–469)	23	348 (223–438)	Ns
A (ng/dl)	G1	8	26.1 (15.7–46.2)	11	17.5 (10.8–20.2)	0.06
	G2	16	46.7 (38.7–57.0)	14	23.1 (18.9–34.3)	<0.001
	G3	13	64.6 (49.6–102)	12	31.1 (25.5–47.5)	<0.001
	G4	30	79.4 (58.7–93.5)	30	47.1 (35.8–73.0)	<0.001
	G5	23	81.7 (63.0–117)	23	80.9 (66.3–85.3)	Ns
PSA (μg/liter)	G1	8	undetectable	11	undetectable	-
	G2	17	0.005 (0–0.01)	14	undetectable	-
	G3	13	0.05 (0.01–0.17)	12	0.025 (0.01–0.16)	Ns
	G4	30	0.25 (0.16–0.35)	31	0.28 (0.19–0.44)	Ns
	G5	23	0.46 (0.33–0.67)	23	0.52 (0.27–0.62)	Ns

Non-Gaussian distribution: data presented as median (25^{th} -75^{th} percentile (P25-P75)). Comparison between obese boys and age-matched controls were performed using non-parametric Mann-Whitney-U tests.

Conversion factor to SI-units for TT from ng/dl to nmol/liter is 0.0347, for FT from ng/dl to nmol/liter is 0.0347, for E2 from ng/liter to pmol/liter is 3.671, for DHEAS from μg/dl to nmol/liter is 2.714 , for A from ng/dl to nmol/liter is 0.0349.

Secondly, we found a normal genital development in obese adolescents. Only few other studies have examined the pubertal development in obese adolescents (1, 2, 3, 11, 23). Our results of a normal pubertal progression is in accordance with the findings of Denzer et al (2007), reporting a normal genital development in German boys in comparison with the historical Swiss standard of Largo and Prader (2). Laron et al (2004) also reported in a short communication no difference in pubertal timing among 136 obese boys and 48 nonobese boys (23). On the other hand, an increased prevalence of delayed pubertal development in obese males has been observed by some pediatric obesity clinics (1, 24). The reasons for this phenomenon, is not known, although some recruitment bias might be involved, given the well-known accelerated body fat accumulation in boys occurring before pubertal onset, promoting some overrepresentation of obese boys with a delayed sexual maturation in obesity clinics.

Thirdly, we found normal serum PSA concentrations in obese adolescents. During male development, PSA concentrations correlate with the rise in T levels, being high during mini-puberty, declining to undetectable values by six months, reappearing by about age 10 years and increasing in concentration thereafter until adulthood (25). Serum PSA concentration have been found to correlate with T concentrations during puberty, especially when T concentrations were adjusted for SHBG levels (26, 27). This study is the first report of PSA concentrations in obese boys and suggests that PSA seems to be a better marker for evaluating androgen activity at tissue level than skeletal maturation, given it is less influenced by estrogens during pubertal development

Our study design allowed us to assess the difference in circulating sex steroids between obese and nonobese adolescents at different pubertal stages. We found clearly lower TT in obese adolescents from stage 3 onwards. Most studies have reported low TT levels in obese subjects during pubertal progression (2, 16, 17, 18), although two studies did find normal TT concentrations at Tanner stage G2 (12, 14). As previously described by Denzer et al 2007, we found markedly lower SHBG levels at every pubertal stage possibly caused by the increased insulin levels (28). Since approximately half of TT is bound to SHBG, it is likely that the lower SHBG concentrations in obese adolescents can account at least in part for these lower TT concentrations. Moreover, FT concentrations -as assessed by the equilibrium dialysis method- were comparable with the concentrations in nonobese subjects at mid- and late -puberty (G3 and G4). The findings of a normal pubertal development and normal PSA values indicate that FT levels seem to be a more representative index of androgen activity during adolescence than TT levels in obese pubertal boys. Our finding of higher FT concentrations in prepubertal and early-pubertal obese boys is probably related to an increased adrenal activity (2, 13) in obese children and adolescents which seem to be supported by the increased DHEAS and A levels in our prepubertal and early-pubertal obese boys and the strong positive correlation between DHEAS and FT and between A and FT. Taneli et al(2010) reported lower FT in obese boys at Tanner stage 2, but not at Tanner stage 4 (16). However, in the latter study FT concentrations were measured by direct radioimmunoassay (RIA), an inaccurate method that underestimates FT concentrations by manifold and is dependent upon SHBG concentrations (29, 30). In accordance with Mogri et al(2013), also using a equilibrium dialysis method, we found that postpubertal obese males (G5) had significantly lower FT concentrations compared to their lean counterparts (18). In adult men FT concentrations have been reported to be preserved in moderately obese men and decreased in severely obese subjects due to a deficient gonadotropin secretion, as evidenced by a decreased amplitude of secretory LH pulses (31, 32). The lower FT concentrations at completion of puberty in obese boys might be related to increasing body fat accumulation since a higher degree of obesity (assessed as waist circumference SDS) was negatively associated with FT levels in the obese group. Although the obese adolescents with a G5 status studied by us had indeed the most severe degree of obesity, -as shown by their higher BMI SDS and waist SDS, and highest E2 concentrations, known to play a major role in negative feedback regulation of LH (33, 34), their E2/TT was not higher in comparison with earlier pubertal stages and a single point LH measurement was not different from lean controls.

The few studies reporting on estrogens in obese boys, did not find a significant difference in E2 levels between obese boys and lean controls, but these studies were hampered by a very small sample size (14, 16, 18, 35) as well as the use of inaccurate immunoassays (14, 16, 35).

The strength of the present study is the comprehensive and reliable evaluation of pubertal development (by trained pediatricians), skeletal maturation (by two experienced radiologists) and sex steroids (measured by highly sensitive and accurate mass spectrometry-based methodology as required when studying low androgen and estrogen serum levels in children and adolescents) in an large group of obese adolescents. Since there are no universally accepted reference ranges for TT and FT concentrations in pubertal boys, we used age-matched controls, recruited in

parallel with the obese study subjects to avoid secular trends. As far as we know this is the first study to present sex steroid data at different pubertal stages with an acceptable sample size in obese boys using state-of-the-art techniques and to have evaluated PSA levels as a marker for androgen responsiveness in an obese pediatric population in relation to FT, measured by equilibrium dialysis. Given the wide usage of free testosterone calculations, the cFT was also included in the analyses. All results remained unchanged when FT by dialysis was substituted by cFT in our analyses.

Our study has some limitations. Firstly, our sample size in the prepubertal and early pubertal group is rather small especially in comparison with our late-and postpubertal group. However, after sample size calculations based on the available literature we are confident that this is not a major drawback. Secondly, our study is limited by the fact that we only have assessed cross-sectional data. In order to confirm the underlying mechanisms in the dissociation between skeletal and sexual maturation, prospective longitudinal studies are required, ideally with a follow-up from early childhood at onset of obesity until adulthood.

In conclusion, obese male mid- and late-pubertal adolescents have lower TT concentration, but similar FT levels compared to age-matched lean controls. This normal androgen activity is reflected in a normal sexual development and similar PSA levels. On the other hand, skeletal maturation and E2 were increased from the onset of puberty, suggesting a significant contribution of hyperestrogenemia in the advancement of skeletal maturation in obese boys.

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