Published in final edited form as:

Clin Endocrinol (Oxf). 2010 December ; 73(6): 744–751. doi:10.1111/j.1365-2265.2010.03866.x.

Luteinizing hormone, testosterone and inhibin B levels in the peripubertal period and racial/ethnic differences among boys aged 6–11 years: analyses from NHANES III, 1988–1994

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Abstract

Objective—To determine whether the initial physical findings of puberty are accompanied by hormonal evidence of pubertal activation of the hypothalamic–pituitary–gonadal (HPG) axis and whether racial/ethnic differences exist, we have analysed hormone levels in relation to age, onset of puberty and race/ethnicity.

Design—Cross-sectional analysis of luteinizing hormone (LH), testosterone (T) and inhibin B from banked sera from a representative sample of US boys aged 6·0–11·99 years who participated in the National Health and Nutrition Examination Survey (NHANES) III.

Patients—Eight hundred and twenty-eight boys having sera including 228 non-Hispanic white (NHW), 266 non-Hispanic black (NHB), 288 Mexican-American (MA) and 46 ‘other’.

Measurements—Using analysis of variance and linear regression techniques, concentrations of LH, T and inhibin B were compared by race/ethnicity for all boys and pubertal status (Tanner's Staging 1, 2 and 3+) for boys aged 8 years and older. Receiver operating curves were utilized to identify cut-points predictive of pubertal HPG status.

Results—Mean hormones levels progressively increased with age. Receiver operating characteristic (ROC) curves indicate hormones are consistent with pubertal onset as indicated by Tanner stage 2, except for T and genital stage 2. Inhibin B and LH levels increased significantly by genital stage after adjusting for age and race/ethnicity, while LH and T concentrations increased significantly across pubic hair stages. Levels of inhibin B were significantly higher for NHB boys compared with other racial/ethnic groups.

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No authors have anything to declare.

Conclusions—In these cross-sectional findings, hormone levels rise gradually as boys approach the peripubertal age, whereas an abrupt rise was not associated with the onset of physical changes of puberty.

Introduction

In boys, concentrations of luteinizing hormone (LH), T and inhibin B are positively associated with age and pubertal progression, albeit with considerable variation in concentrations within categories of pubertal staging.^{1,2} While such biological variation is expected, it would be useful to determine whether or not biomarkers can be used to determine whether the initial physical changes of puberty are indicative of upregulation of the hypothalamic–pituitary–gonadal (HPG) axis. There are convincing data to indicate that physical changes are occurring earlier than in previous decades,^{3–5} but the cause of these physical changes is unclear and such changes may not progress as expected during puberty. Thus, it is unclear whether the earlier age of physical changes is a consequence of earlier onset of pubertal HPG activity. While GnRH stimulation testing may be necessary to verify the HPG axis activity, hormone profiles from a large representative population such as studied here should provide useful information for the assessment of secular trends⁶ of pubertal onset. Further, these data will also clarify whether racial/ethnic differences in age of pubertal onset are reflected by different hormonal profiles.

The aim of this study was to determine whether serum concentrations of three hormonal biomarkers, LH, T and inhibin B, in a large representative sample of US boys aged 6·00–11·99 years, suggest that the onset of physical pubertal status is consistent with hormonal evidence of a pubertal HPG axis.

Materials and methods

Study sample

The referent study population comprised 1767 boys aged 6·0–11·99 years who participated in the National Health and Nutrition Examination Survey (NHANES) III. The study sample was restricted to the 839 (47%) boys who had stored residual serum samples. LH was determined in 839 stored sera samples, testosterone in 825 and inhibin B in 817. Definitions of race/ethnicity were those utilized by the NHANES III. Our final sample included 228 non-Hispanic white (NHW) boys, 266 non-Hispanic black (NHB) boys, 288 Mexican-American (MA) boys and 46 ‘other’ race boys. Signed informed consent was obtained before participation in NHANES III.

Data collection

Existing data from the NHANES III were obtained from the public user data set (<http://www.cdc.gov/nchs/nhanes.htm>) and included anthropometric measurements, physical examination data as conducted by 23 paediatricians participating with the study, socio-demographic data from the standardized interview and pubertal staging for boys ≥8 years of age. Blood samples, which were obtained between 1988 and 1994, have a label indicating the date and time obtained. Samples were stored at –70 °C and are expected to yield valid results. Sex hormones have been reported to be stable when exposed to multiple freeze–thaw cycles.⁷ Also, LH levels have been successfully measured in stored sera for women aged 35–60 years who participated in the NHANES III.⁸ Inhibin B stability information is not available although, because it is a peptide hormone like LH, stability characteristics are likely similar.

Laboratory measurements

Sera were made available by the National Center for Health Statistics/Centers for Disease Control and Prevention and were subsequently assayed for LH, T and inhibin B by an immunoassay laboratory (Rules-Based Medicine, Inc., Austin, TX, USA, <http://www.rulesbasedmedicine.com>).

The LH ELISA kit (Bio-Quant BQ049F; Bio-Quant, Inc., San Diego, CA, USA, <http://www.bio-quant.com>) is a solid-phase direct sandwich method that uses two anti-LH antibodies. The intra-assay variation is 10.58%, 7.60% and 6.18% for samples with mean values of 1.7, 16.3 and 47.2 IU/l, respectively. Interassay precision is 11.57%, 10.83% and 8.13% for samples with means of 1.9, 15.6 and 46.1 IU/l, respectively. The functional sensitivity of the assay is 0.12 IU/l; the minimal detectable level is 0.035 IU/l.

Total testosterone (T) was assayed using 20 µl of serum and an ELISA (Bio-Quant BQ080S). This competitive binding assay has an intra-assay variation of 8.4%, 4.4% and 3.7% for samples with a mean value of 0.12, 0.17 and 0.47 nmol/l, respectively. Interassay precision CV of 10%, 8.3% and 5.0% for samples with a mean value of 0.13, 0.18 and 0.44 nmol/l, respectively, and a sensitivity of 0.17 nmol/l. LH/T ratios have been calculated for age and pubertal stage.

Inhibin B was measured using 10 µl of serum and the Gen I DSL-10-841000 ACTIVE® Inhibin B ELISA (Diagnostic Systems Laboratories, Inc., Webster, TX, USA, <http://www.dslabs.com>). The sensitivity of this assay is 7.0 ng/l, and the coefficient of variation (CV) for intra-assay variation is 3.5%, 4.6% and 5.6% for samples with a mean of 69, 274 and 472 ng/l, respectively. The Gen I assay has excellent internal validity; however, since the inhibin B analyses were run, a Gen II assay for inhibin B has been introduced⁹ that yields levels more comparable with other commercially available assays.¹⁰ Given the reported correlation between the Gen I and Gen II assays, it has been suggested that the Gen I results can be compared across studies using the following equation: $\text{GenII} = 1 \cdot 57(\text{GenI}) + 11 \cdot 29 \text{ ng/l}$.¹⁰

Statistical analysis

Descriptive analysis of the hormones were undertaken to assess their distributions and interrelatedness with age, race/ethnicity and pubertal stage for children with recorded stage (over age 8.0 years). Race/ethnicity comparisons were restricted to three groups and excluded the small 'other race' boys ($n = 46$). Pubertal onset was defined as having reached Tanner stage 2, while overall pubertal comparisons involved Tanner stage 1 compared with both 2 and 3–5 or stages 2–5. For analysis, sampling times were grouped: morning (8 AM–<1 PM; 41%), afternoon (1 PM–5 PM; 32%) and evening (after 5 PM; 27%).

Analysis of variance (ANOVA) and linear regression were used to compare hormonal concentrations by age, race/ethnicity and Tanner stages (1,2 and 3+) using weighted least-square means for each hormone by year and Tanner stage for the three racial/ethnic groups; least-square means and 95% confidence intervals (CIs) were calculated. Socio-demographic characteristics and anthropo-metric measurements for the three racial/ethnic groups were evaluated by calculating weighted means and percentages consistent with the population-based sampling format of the NHANES III. Statistical significance was assessed using the chi-squared test for categorical variables, and 95% confidence intervals (CIs) were calculated from linear regression for continuous variables. CIs exclusive of zero for linear regression coefficients were considered significant.

The NHANES' weights were incorporated into both anova and linear regression techniques to account for the complex multistage sampling procedures utilized in designing the

NHANES III and to facilitate the external validity of our findings. Weighted and unweighted results for this paper were not substantially different. One testosterone value and one inhibin B value were excluded, because they were biologically implausible. Racial/ethnic differences in inhibin B were assessed by anthropometric measurements and other socio-demographic factors using weighted linear regression. The following variables were singly entered into the model with race/ethnicity (three categories) and age (months) to determine whether the racial/ethnic differences remained statistically significant: height (cm), waist circumference (cm), body mass index (BMI, kg/m²), subscapular and triceps skinfolds (mm), geographical region (north, south, midwest, west), poverty/income ratio (computed as the total income divided by the poverty threshold for the year of the interview), metropolitan residence (yes/no), time of blood draw (morning, afternoon, evening) and passive smoke exposure in the home as reported by parents (yes/no).

Hormone concentrations were log-transformed to approximate a normal distribution prior to inclusion in ANOVA or regression models. The normality test (Kolmogorov–Smirnov) was applied to the residuals, and inspection of the transformations affirmed that the data were approximately normally distributed. Exact logistic regression models were fitted with pubertal status (stage 1 or stage 2+) as the outcome and the hormone level (LH, T or inhibin B) as the regressor for each assay.

Receiver operating characteristic (ROC) curves were developed to assess the utility of the hormone levels (LH, T and inhibin B) as predictors of pubertal onset (Tanner stage 2+). An ROC curve illustrates the trade-off between sensitivity and specificity as the cut-off varies for a given marker, and the area under the curve (AUC) provides an index for the overall predictive performance of the marker. We constructed ROC curves for the individual hormones as well as a hybrid marker that combined information from the three hormones and race/ethnicity. The hybrid marker was based on a logistic regression model for predicting pubertal onset, which included all predictors as regressors.¹¹ To avoid overfitting the model, we adopted a cross-validation approach where two-thirds of the sample was used to fit the logistic regression model and the other one-third was used to estimate the ROC curve.¹² This procedure was repeated 200 times, and the results were averaged to obtain the final estimate of the ROC curve for the hybrid marker. SAS 9.1 statistical software (SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513-2414, USA) was used for these analyses.

Results

Assessment for potential selection bias

The sample of NHANES III boys who had hormone measurements ($n = 782$; 44%) was compared with the boys (NHW, NHB, MA) who had insufficient stored sera ($n = 902$) to assess potential selection bias. Socio-demographic, anthropometric and haematologic characteristics did not significantly vary by the availability of sera with the exception of age (boys with sera were significantly ($P < 0.0001$) older than boys without stored sera; 109.5 vs 105.4 months, respectively), genital stage (genital stage 2 or higher: 51% vs 47%, respectively), race/ethnicity (analytic sample had fewer NHB boys: 14% vs 20%) and passive smoke exposure (analytic sample less often reported to be living with a cigarette smoker: 34% vs 40%). The mean (95% CI) BMI for boys in our sample was 17.6 (17.2, 18.0) kg/m² with a mean standing height of 135.1 cm.

Comparison of racial/ethnic groups

Socio-demographic and anthropometric differences by race/ethnicity are noted in Table 1. Boys were comparable in age, height, weight and subscapular skinfold across the race/

ethnicity groups; however, NHB boys had the lowest skinfold indices, while MA boys had the highest. NHB boys were more likely to be classified as Tanner stage 3 than the other racial/ethnic groups ($P = 0.02$ genital stage; $P = 0.0001$ pubic hair stage).

Hormone profiles

Mean levels of LH, T and inhibin B (Fig. 1) gradually increased with age, while LH/T ratios decreased as the age of puberty was approached and then increased again. No significant association was found between timing of blood collection (morning, afternoon or evening) in either of the log-transformed hormone concentrations even after adjustment for age and race/ethnicity (data not shown). In weighted ANOVA, levels of LH and T increased significantly with age ($P \leq 0.001$ and $P = 0.01$), but did not vary by race/ethnicity ($P = 0.34$ and $P = 0.77$) (data not shown). LH levels, when analysed by Tanner stage (1, 2 and 3+), significantly ($P \leq 0.03$) increased across both the three pubic hair and genital stage categories before and after adjustment for race/ethnicity and age (Table 2). T levels significantly ($P < 0.0001$) increased across the three pubic hair stage categories before and after adjustment for race/ethnicity and age ($P = 0.004$). In contrast, T was not significantly related to genital stage before or after adjustment for race/ethnicity and age. In a subanalysis in which T was restricted to morning samples only, the morning sample profiles appeared similar to the overall T values. The relationship of T with both pubic hair and age was significant, while the relationship with genital stage and race was not.

The progressive increases in inhibin B concentrations for boys in pubertal stages 1 to 2 and from 2 to 3+ are shown in Table 2 for genital stages and in Fig. 2 for pubic hair stages. The positive association using weighted linear regression models was significant ($P < 0.0001$) between inhibin B concentrations and age (Fig. 1) and both genital and pubic hair stages (Table 2). After adjustment for age and race/ethnicity, inhibin B remained significantly associated with genital stage ($P < 0.0001$) but not with pubic hair stage. When compared across all ages for all race/ethnic groups, levels of inhibin B were significantly ($P < 0.01$) higher in NHB boys than the remaining two racial/ethnic groups (Fig. 3). Among all boys at genital stage 1, there was no significant effect for age, but a significant effect for race/ethnicity was found. Specifically, NHB had the highest levels of inhibin B in comparison with the other groups of boys. Conversely, among boys with Tanner stage 2 or greater, there was no significant effect of race/ethnicity, but values increased significantly ($P = 0.01$) with age.

Hormone levels and BMI

Body mass index was not significantly associated with inhibin B, either unadjusted ($P = 0.57$) or adjusted for age, race or Tanner stage ($P = 0.3$). For T, when adjusted for age, race and pubic hair Tanner stage, there was no significant association with BMI ($P = 0.36$).

The risk of exceeding pubertal levels of LH (i.e. greater than the prepubertal ranges of G1 and PH1) was not associated with BMI in logistic regression models even after adjusting for age, race or Tanner stage.

Evidence of HPG pubertal axis activity

While hormone levels rise as pubertal stages progress, individual hormone levels overlap extensively for those in Tanner stage 1 and thereafter. As physical changes are not necessarily associated with significantly higher levels of hormones, it may not be possible to determine whether the physical changes are a consequence of pubertal activation of the HPG axis or a consequence of a steroid or steroid-like effect independent of this axis. The ROC curves fail to denote a hormone level above which puberty onset and progression is present. Using ROC curves, all three hormones predict puberty as measured by attainment of Tanner

stage 2 in the sense that the AUC is significantly greater than one half ($P < 0.0001$) with the sole exception for T and genital staging. The misclassification rate (MCR), defined as the percentage of individuals (pubertal or not) who are misclassified, was determined for each hormone and for the combination (hybrid marker). A minimal MCR of 14.4% was found for an LH value of 2.05 mIU/ml, suggesting that this percentage of boys would be misclassified as having attained puberty when in fact they are prepubertal. The minimal MCR for testosterone of 13.2% was found for a value of 0.98 ng/dl and a value of 16% for an inhibin B value of 198 pg/ml. The minimal MCR for the hybrid marker (combination of the hormone for the construction of a ROC curve) was 15.2%. Hence, these MCR levels are too high to be useful for the clinical prediction of pubertal status on an individual basis to assess whether a specific hormone level cut-off would affirm the physical changes of Tanner stage 2. Table 3 shows mean and percentile data for unweighted hormone levels for stages 1 and 2 or greater. There is considerable overlap for all hormones levels between the prepubertal and pubertal (stage 2 or greater) range.

Discussion

This study involving a large cross-sectional study of 828 boys aged 6.0–11.99 years reflects significant increases in LH, T and inhibin B with age and when comparing pubic hair stage categories 1, 2 and 3+. LH and inhibin B concentrations increased over genital stage categories, although T concentrations did not differ between genital stages 1 and 2. The rise in LH and T was most noticeable from ages 9 through 11 years, while the LH/T ratio decrease as puberty was approached consistent with an earlier increase in LH, then followed by an increase in this ratio consistent with a subsequent rise in T. Progressive rise in inhibin B was seen from age 6 years onwards. Inhibin B concentration was higher in NHB than NHW or MA boys irrespective of age or genital stage. Hormone concentrations were not associated with BMI.

While measured hormone levels predicted pubertal status at the group level, levels for any given individual may be inadequate to verify pubertal stage 2 as indicated based upon the AUC for pubertal stage 2. Hence, a single hormone measurement such as that evaluated in clinical practice may not always predict or validate pubertal onset of the functioning of the HPG axis among individual boys. Application of the single hormone measurement in this study would lead to misclassification of pubertal status in about 15% of the study subjects. Thus, in situations in which this occurs and verification of pubertal onset is required, LH measurements following stimulation with gonadotrophin-releasing hormone (GnRH) or a GnRH analogue may be required.¹³ Further, cautious interpretation is required in the event we have not fully adjusted for diurnal variation¹⁴ and our reliance on Tanner genital staging and not measured testicular growth as the so-called best marker of puberty.¹⁵ In attempting to explain the racial/ethnic difference in inhibin B using multivariable models, adjusting for timing of biospecimen collection, anthropometric and socio-demographic factors did not account for our observed differences.¹⁶

The changes in all hormone concentrations for age and pubertal stages are consistent with previous reports.^{1,2,17} The levels of inhibin B among the boys reported here were all collected after the 5–7 AM nadir reported for peripubertal boys. Further, our findings are consistent with the relation reported between LH nocturnal secretion during the peripubertal period and the inverse correlation with the time before onset of puberty.¹⁸ Further, the variation in testosterone levels is consistent with the lack of indication of overall secretion previously reported at early puberty.^{18–20} Thus, while these results are consistent with previous reports, a single level of any of these three hormones may not correlate with physical development. The largest portion of the sampling in this study is for those during G1, including those who have begun puberty hormonally but not physically who may have

early elevation of testosterone levels. Hence, profiles would be expected to be very different than a comparison of equal numbers of ages and stages across puberty. This may particularly apply to the lack of correlation with progressive G stages because the patient population is young and those in G2 and G3+ stages are relatively few and hence relatively early within each stage. Racial differences in other hormone levels and binding globulins have been previously reported for pubertal children; higher estradiol concentrations in black boys compared to white boys, higher androstenedione concentrations in white than black girls and²¹ higher sex hormone binding globulin in African-American than white prepubertal boys.²²

This study did not find significant associations of hormones with BMI. It is of interest that recently published reports regarding BMI and the onset of puberty among boys are inconclusive. BMI trajectories (highest, intermediate and lowest) were created from measured heights at weights over 8 ages ranging from 2 to 11.5 years in one report.²³ The highest trajectory was associated with the greater relative risk of being prepubertal compared with the lowest trajectory. Another study comparing cross-sectional data from two recent time periods found an earlier onset of puberty and higher LH levels among the most recent group from 2006 to 2008 but after adjustment for BMI, neither the earlier onset nor LH levels were significantly different.²⁴

Nevertheless, hormone levels are consistent with their expected properties: LH as an initial stimulant of puberty and inhibin B rising coincident with the testicular growth characteristic of genital stage 2 and testosterone elevation at the onset of pubic hair growth. As the main action of T during early puberty is paracrine, an intratesticular process,²⁵ it is not surprising that a significant rise in plasma was not detected at the onset of genital stage 2. Relatively high hormone levels among some boys during stage 1 are consistent with the increases preceding the outward physical changes of puberty.^{18,26} Higher luteinizing hormone (LH) to testosterone cross-correlation coefficients have been observed in peripubertal boys than in adult men, with the highest testosterone levels occurring during early morning hours¹⁰ well before the morning sampling was carried out in the NHANES.

No single or combination cut-point was found to be predictive of physical pubertal status, genital or pubic hair stage 2, nor the onset of up-regulation of the HPG axis at the time of Tanner stage designation. This may be a consequence of intra- and interindividual, including diurnal, variation of levels, plus the evidence that elevations of these hormones may occur prior to the onset of physical changes.

In this study, a significant effect was observed for race/ethnicity among boys in genital stage 1 with NHB boys having the highest levels of inhibin B irrespective of age. While there was no significant race/ethnicity effect in Tanner stage 2+ boys, there was a significant age effect. The latter finding may suggest that once boys reach a critical level of maturation, the racial/ethnic differences in inhibin B are not substantial, the difference being related to the onset of puberty.

Previous work with NHANES III data has suggested the median age at entry to Tanner genital stage 2 was younger in NHB boys (age = 9.2 years) when compared with non-Hispanic white (NHW) (age = 10.0 years) and Mexican-American (MA) (age = 10.3 years) boys³. It is interesting to note that for all boys in the year following the median age at entry to genital stage 2, inhibin B is significantly higher. The observed difference in inhibin B levels in NHB may be a reflection of earlier onset and progression of the physical changes of puberty. It appears not to be related to testicular volume because prepubertal and pubertal non-Hispanic black (NHB) boys have similar testicular volumes as comparably developed boys of other races, although there are limited data.²⁷

These findings need to be cautiously interpreted, largely given the cross-sectional nature of the National Health and Nutrition Examination Survey (NHANES) III. Nevertheless, this study comprises the largest sample of boys representative of the United States and is the first to investigate the relation between these three hormonal biomarkers and pubertal staging. These data underscore the wide interindividual variation in hormone profiles between and within Tanner stages and fail to provide support that the purported earlier onset of puberty observed among more recent birth cohorts of boys is a consequence of earlier pubertal activation of the hypothalamic–pituitary–gonadal (HPG) axis. Thus, critical data gaps remain in understanding the notable differences in inhibin B concentrations by race/ethnicity.

Acknowledgments

Support with intramural funding from the *Eunice Kennedy Shriver* National Institute of Child Health & Human Development.

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Fig. 1. Mean luteinizing hormone (IU/L), testosterone (nmol/L) and inhibin B (ng/L concentrations by age for 828 boys aged 6.0–11.99 years.

Fig. 2.

Weighted means (unadjusted least-square means) of luteinizing hormone (LH), testosterone and inhibin B levels according to pubic hair stage for racial/ethnic groups. The *P*-value derived from weighted ANOVA comparing hormone concentrations by Tanner pubic hair stage across all race/ethnicities was <0.0001 for all three hormones. The *P*-value derived from weighted ANOVA comparing hormone concentrations by Tanner pubic hair stage after race/ethnicity and age adjustment was 0.03 for LH, <0.001 for testosterone and 0.91 for inhibin B.

Fig. 3.
Mean inhibin B levels for age from 6 through 11 years according to racial/ethnic designation.

Table 1

Distribution of anthropometric, pubertal and socio-demographic characteristics by race/ethnicity for boys, aged 6–11 years

Characteristics	Non-hispanic white			Non-hispanic black			Mexican-American		
	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	n	Mean (95% CI)	P-value
Age (mo)	228	109.6 (108.0, 111.2)	266	108.5 (104.7, 112.3)	288	108.3 (103.7, 112.9)			0.79
Height (cm)	229	135.0 (134.4, 135.6)	266	135.7 (134.3, 137.0)	288	133.6 (132.0, 135.2)			0.15
Weight (kg)	229	32.6 (32.0, 33.3)	265	33.0 (31.5, 34.5)	282	34.0 (32.1, 35.8)			0.40
Body mass index (kg/m ²)	229	17.6 (17.3, 17.8)	264	17.6 (17.0, 18.1)	282	18.6 (17.9, 19.3)			0.02
Waist circumference (cm)	223	62.1 (61.4, 62.8)	265	60.1 (58.5, 61.8)	285	64.9 (62.9, 66.9)			0.002
Waist/height ratio	223	0.46 (0.46, 0.47)	265	0.44 (0.43, 0.45)	285	0.49 (0.47, 0.50)			<0.0001
Subscapular skin fold (mm)	223	8.3 (7.8, 8.8)	265	7.8 (6.7, 8.9)	283	9.7 (8.4, 11.0)			0.08
Triceps Skin fold (mm)	223	11.7 (11.2, 12.1)	264	9.9 (8.8, 11.0)	285	12.4 (11.0, 13.7)			0.006
	n	Percentage (95% CI)	n	Percentage (95% CI)	n	Percentage (95% CI)	n	Percentage (95% CI)	P-value*
Pubic hair stage									
1	122	87.2 (80.9, 93.6)	120	70.4 (63.5, 77.3)	172	92.8 (88.5, 97.0)			0.0001
2	12	7.7 (2.8, 12.7)	37	21.7 (15.9, 27.5)	9	6.0 (2.4, 9.6)			
3+	5	5.0 (0.2, 9.8)	13	7.9 (3.7, 12.0)	3	1.2 (0.0, 2.9)			
Genital stage									
1	59	50.6 (40.4, 60.8)	64	33.2 (25.7, 40.7)	98	57.0 (41.2, 72.7)			0.02
2	61	38.5 (28.9, 48.0)	78	46.1 (36.8, 55.3)	74	37.8 (24.8, 50.8)			
3+	19	10.9 (5.8, 16.0)	28	20.7 (9.4, 32.0)	13	5.2 (0.5, 9.9)			
Poverty/income ratio									
<1	28	12.3 (6.7, 17.9)	123	42.3 (32.1, 52.5)	135	51.4 (43.8, 59.0)			<0.0001
1–2	52	21.9 (16.4, 27.4)	71	27.9 (21.2, 34.7)	70	26.4 (19.5, 33.3)			
>2	142	65.8 (59.2, 72.4)	62	29.8 (21.7, 37.8)	47	22.2 (14.9, 29.5)			
Residence									
Metropolitan	82	42.2 (28.9, 55.5)	152	58.1 (44.3, 71.9)	152	62.0 (46.9, 77.1)			0.02
Non-metropolitan	147	57.8 (44.5, 71.1)	115	41.9 (28.1, 55.7)	137	38.0 (22.9, 53.1)			
Region									
Northeast	41	22.6 (17.0, 28.3)	36	12.2 (7.9, 16.6)	1	1.6 (1.3, 1.9)			<0.0001
Midwest	72	30.8 (22.1, 39.5)	77	29.5 (22.9, 36.0)	36	11.6 (1.8, 21.3)			

	<u><i>n</i></u>	<u>Percentage (95% CI)</u>	<u><i>n</i></u>	<u>Percentage (95% CI)</u>	<u><i>N</i></u>	<u>Percentage (95% CI)</u>	<u><i>P</i>-value[*]</u>
South	82	24.6 (17.0, 32.2)	131	48.6 (41.1, 56.0)	81	22.7 (13.3, 32.2)	
West	34	22.0 (10.2, 33.8)	23	9.7 (4.4, 15.0)	171	64.1 (51.7, 76.5)	

Means, percentages and 95% CIs are weighted.

Anthropometric characteristics were age-adjusted.

CI, confidence interval; cm, centimetre; kg, kilogram.

^{*} *P*-values were calculated with weighted linear regressions for continuous variables and weighted chi-square tests for categorical variables.

Table 2

Weighted least-square means and 95% confidence intervals by Tanner genital stage and race

Non-hispanic white				Non-hispanic black			Mexican-American			P-value*	P-value†
n	Weighted means	95% CI	n	Weighted means	95% CI	n	Weighted means	95% CI			
Luteinizing hormone (IU/l)											
1	60	0.21	(0.15, 0.29)	64	0.10	(0.07, 0.16)	98	0.30	(0.23, 0.39)	<0.0001	<0.0001
2	61	0.53	(0.36, 0.76)	78	0.31	(0.22, 0.44)	75	0.29	(0.21, 0.41)		
3+	19	0.85	(0.43, 1.70)	28	0.41	(0.24, 0.68)	13	0.71	(0.29, 1.72)		
Testosterone (nmol/l)											
1	58	0.87	(0.67, 1.07)	64	1.11	(0.87, 1.42)	98	0.97	(0.83, 1.14)	0.13	0.79
2	57	0.97	(0.76, 1.25)	76	0.97	(0.79, 1.18)	72	0.94	(0.76, 1.14)		
3+	19	1.12	(0.76, 1.87)	27	0.80	(0.69, 1.28)	13	1.98	(1.14, 3.40)		
Inhibin B (ng/l)											
1	58	27.8	(23.2, 33.5)	63	38.3	(30.7, 45.4)	95	38.5	(34.0, 43.6)	<0.0001	<0.0001
2	60	52.1	(42.3, 64.2)	76	55.1	(46.6, 65.2)	71	46.0	(39.5, 53.7)		
3+	19	62.0	(42.1, 91.2)	26	82.7	(64.1, 106.9)	12	81.2	(52.6, 125.4)		

* P-value derived from weighted ANOVA comparing hormone concentrations by Tanner stage across all race/ethnicities.

† P-value derived from weighted ANOVA comparing hormone concentrations by Tanner stage after race/ethnicity and age adjustment

Table 3

Unweighted empirical percentiles for prepubertal and pubertal boys

Tanner Stage	Mean Age(y)	Mean	N	% below LOD	10th	25th	50th	75th	90th
Luteinizing hormone (IU/ml)									
PH 1	9.94	0.62	442	30.3	*	*	0.32	0.78	1.55
PH 2+	10.88	1.24	83	19.3	*	0.22	0.87	2.05	2.81
G 1	9.75	0.50	231	35.1	*	*	0.23	0.59	1.03
G2+	10.35	0.89	295	23.4	*	0.10	0.55	1.30	2.22
Testosterone (nmol/l)									
PH 1	9.94	1.31	434	9.0	0.38	0.62	1.01	1.63	2.11
PH 2+	10.84	2.50	79	6.3	0.45	1.01	1.77	3.33	5.48
G 1	9.75	1.46	229	5.7	0.45	0.69	1.07	1.77	2.67
G2+	10.34	1.56	285	10.9	*	0.59	1.07	1.84	3.40
Inhibin B (ng/l) Non-hispanic black									
PH 1	9.91	57.82	115	4.3	17.6	28.2	54.8	78.3	103.3
PH 2+	10.70	86.72	50	0.0	26.2	46.2	73.4	106.5	176.3
G 1	9.72	47.10	63	3.2	18.3	25.2	42.6	67.5	80.6
G2+	10.42	78.60	102	2.9	20.8	42.3	67.3	103.0	149.5
Inhibin B (ng/l) Other racial/ethnic groups									
PH 1	9.94	54.06	313	3.2	17.0	28.4	42.3	69.8	107.0
PH 2+	11.11	77.33	31	3.2	28.2	32.2	61.7	120.0	155.0
G 1	9.76	43.40	162	3.7	13.9	26.1	38.3	54.5	74.3
G2+	10.29	67.50	183	2.7	21.2	32.0	54.8	95.0	135.0

* Below LOD.