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An Investigation of the Association of Tanner Stage and Dehydroepiandrosterone during Pubertal Development

By

Lauren M. Daniels
Master of Public Health

Epidemiology

_____

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An Investigation of the Association of Tanner Stage and Dehydroepiandrosterone during Pubertal Development

By

Lauren M. Daniels

B.S., Emory University, 2009

Thesis Committee Chair: Michele Marcus, Ph.D., MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Abstract

An Investigation of the Association of Tanner Stage and Dehydroepiandrosterone during Pubertal Development

By Lauren M. Daniels

Over time, a significant decrease in age at entry into puberty has been demonstrated in the United States. Several negative outcomes associated with early entry into puberty for males and females, include increased risk of testicular cancer in men and breast cancer in women. Previously, many studies investigating pubertal development utilized Tanner staging to assess maturation. However, biological hormone markers, such as Dehydroepiandrosterone (DHEA), may provide a more objective method for measuring pubertal development. Several studies have indicated a significant rise in DHEA concentrations during early stages of maturation. In a longitudinal study of 77 children and adolescents, aged 7 to 16, the relationship between Tanner stage and DHEA was evaluated. Measures of pubertal development, including self-assessed Tanner Stage, anthropometric measurements, and salivary DHEA concentrations, were taken at three separate visits over a six-month period. Additionally, parental assessment of Tanner Stage was performed at each visit for children 7 to 10 years old. Medical conditions, medication history, and other lifestyle factors were assessed by questionnaire. After adjusting for age, Tanner stage 2 for female pubic hair development was associated with a significant increase in DHEA concentration of 2.7 pg/mL (95% CI: 1.3 – 5.6). Similarly, when adjusting for age, Tanner stage 2 for male pubic hair development was associated with a significant increase in DHEA concentration of 2.5 pg/mL (95% CI: 1.4 – 4.6). For female pubic hair development, there was overall agreement between self-assessed Tanner stage and parental Tanner stage for 90% of children with a kappa statistic for inter-rater agreement of 0.62. For female breast development, there was overall agreement in 91% of children with a kappa statistic of 0.76. For male pubic hair development, there was overall agreement in 67% of children with a kappa statistic of 0.37. For male genital development, there was overall agreement 71% of children with a kappa statistic of 0.10. High levels of inter-rater reliability were found between the child and parental assessment of female Tanner stages. Additionally, the results of this study confirm the association between an increase in androgen levels and the development of pubic hair during the early stages of maturation.

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BACKGROUND

Introduction

Studies have shown a decrease in the age at entry into puberty in the United States over time. Due to the negative outcomes associated with early maturation in males and females, an accurate measure of pubertal development is essential. Studies investigating puberty in children and adolescents have largely utilized Tanner staging, a method based on the observation of the physical markers of pubertal development. However, the use of Tanner staging can be unreliable and subjective, as well as invasive or even uncomfortable for participants. Biological hormone markers, such as Dehydroepiandrosterone (DHEA), may provide a more objective method for measuring pubertal development. Until recently, invasive and sometimes painful collection methods were necessary in order to accurately determine hormone levels in blood serum. New collection methods now allow hormone levels to be measured from alternate bodily fluids, such as saliva. This unobtrusive method of hormone level detection is useful in longitudinal studies requiring multiple instances of biologic collection. However, there is little information examining the relationship between salivary levels of biological hormone markers of puberty and the known physical signs of pubertal development.

Early Pubertal Development

Differences in pubertal development exist among various races and ethnicities. Studies have shown that while on average all children in the United States complete sexual maturation at the same age, certain ethnicities experience earlier onset of sexual development (1). Specifically, non-Hispanic African-American boys experience the

beginning stages of pubertal development at a much earlier age than either Hispanic or non-Hispanic Caucasian males (1). Also, secular trends towards earlier age at maturation have occurred in non-Hispanic Caucasian boys as well as in Mexican-American boys over time (2). For girls entering puberty, on average, African-American girls enter puberty earliest, followed by Mexican-American girls, and then Caucasian girls (3). Additionally, another study has confirmed that non-Hispanic African-American girls experience the earlier stages of puberty at a much younger age than either Hispanic or non-Hispanic Caucasian females (1). Additionally, while Mexican-American females may not enter puberty at the earliest age compared to other ethnicities, secular trends overtime suggest movement towards an earlier age at maturation among Mexican-American girls in the United States (2).

Research findings from multiple studies on the timing of puberty in the United States, suggest an earlier trend of breast development onset and menarche in females (4). Utilizing data from the National Health Examination Survey (NHES) Cycles II and III with the National Health and Nutrition Examination Survey (NHANES) III, researchers showed that the average age at menarche in the United States decreased over the span of twenty-five years (5). In addition to a significant decline in the age at menarche in the female population overall, age at menarche has also declined among individual ethnic groups (6).

In females, earlier pubertal development is associated with multiple negative outcomes. Girls who mature earlier are more likely to be heavier and shorter in early adulthood (7). Increased height and adiposity in preadolescents is associated with pubertal development (8). Additionally, a sedentary lifestyle or poor diet in pre-

adolescents may lead to an increased risk of earlier maturation (8). Female individuals who enter puberty through the thelarche pathway have a greater chance of higher BMI, greater body fat composition throughout puberty, and may be more at risk for adult obesity (9). Additional studies have confirmed that early sexual maturation in girls is associated with being overweight and an increased risk of obesity (10). Additionally, in both African-American and Caucasian girls, being overweight can lead to the development of cardiovascular disease and a higher clustering of cardiovascular disease risk factors (11). Early menarche can also lead to higher blood pressure and glucose intolerance, increasing the later risk of cardiovascular disease (12). Furthermore, early age at menarche increases an individual's chance of developing breast cancer later in life (13).

Consistent data regarding the earlier timing of pubertal development among males in the United States is less clear (4). However, utilizing data from the NHANES III, among multiple ethnic groups, earlier genital maturation and pubic hair development occurred in boys when compared to earlier NHANES assessments (14). Additionally, a separate study found an earlier age of onset among some stages of pubertal development in boys from the NHANES III when compared to boys from the NHES, Cycles II and III (15).

Early pubertal maturation is also associated with several adverse outcomes in males. In boys, early puberty is associated with an increase in the risk for testicular cancer (16). The early onset of adrenarche in boys is linked to the alteration of the insulin like growth factor system and decreased insulin sensitivity, suggesting that early maturation in boys may lead to a greater risk of the development of diabetes (17).

Dehydroepiandrosterone (DHEA)

DHEA is an adrenal precursor of steroid biosynthesis and a neuro-steroid acting on the central nervous system (18). Additionally, DHEA is the most abundantly secreted human adrenal steroid (19). High DHEA production by the human adrenal gland occurs during intrauterine development and lasts until the end of gestation, suggesting DHEA may have a protective effect during fetal development (20). As children mature, decreased levels of 3beta-hydroxysteroid dehydrogenase in the adrenal reticularis contribute to the increased production of DHEA seen during adrenarche (21).

Several studies have shown an increase in DHEA levels in males and females during puberty. The average level of salivary DHEA is higher in boys and girls undergoing the later stages of pubertal development when compared to individuals in the early stages of maturation (22). In a cross-sectional study examining serum concentrations of boys and girls during puberty, a significant increase in DHEA is shown to occur in boys from the age of 8 until the age of 13 when adult levels of DHEA are reached (23). Additionally, the study found that females experience an abrupt increase in the level of serum DHEA from 11 to 12 years of age and the attainment of adult levels of DHEA at the age of 12 years old (23). A partly longitudinal study, with collections of DHEA 1.5 years apart, explored serum steroid and pituitary hormone levels in females 7 to 17 years old (24). The results of this study suggest that DHEA increases with age, displaying an initial rise in females beginning at the age of 7.5 years (24). Similarly, an investigation of the serum levels of subjects under 20 years old, found that the rise of DHEA levels in both males and females occurs at approximately 8 years of age (25). Additionally, throughout puberty DHEA fluctuates diurnally in both boys and girls (26).

However, following puberty, the circulating levels of DHEA decrease significantly as both men and women age from 20-30 years old to 50-60 years old (27).

Alterations in the levels of DHEA during puberty may have important implications in adolescents for immediate and long-term health outcomes. A higher level of DHEA in both male and female adolescents is associated with a higher risk of depressive symptoms in both populations (28). Hyper-secretion of DHEA is also affiliated with increased negative mood and feelings and a greater chance of developing major depressive episodes in adolescents (29). Additionally, a high cortisol and DHEA ratio in adolescents is associated with disturbed interpersonal behavior and increased risk for developing major depression (30). However, higher DHEA levels during adolescence do not lead to greater chronicity of major depression (31).

DHEA plays an integral role in the central nervous system. Improved results in males, in the presence of normally circulating testosterone levels, suggest that DHEA acts directly on the central nervous system (18). Additionally, animal rat models have suggested that pre-treating hippocampal cells with DHEA protects against the effects of certain toxins and may be useful in the treatment of certain diseases affecting the central nervous system (32). In another study, DHEA stimulated neurogenesis in the hippocampus of the rat, indicating that DHEA has an important role in modulating the formation of new neurons and their survival (33). Furthermore, animal models have suggested that DHEA plays a protective role in preventing thymic involution with age (19).

In humans, treatment with DHEA has led to an improvement in the overall well-being and sexuality in women with adrenal insufficiency (34). Prolonged DHEA treatment has had promising results in individuals suffering from Addison's disease (35). Specifically, DHEA replacement therapy in patients with Addison's disease has shown marked improvement in mood and fatigue (18). Conversely, some data show that DHEA does not improve cognitive performance in healthy elderly individuals (36). Nor do DHEA supplements in the elderly appear to improve memory or attention capabilities (37).

Tanner Stages

In epidemiological studies, Tanner staging has been the approved method for determining the progression of pubertal development in pre-adolescent and adolescent males and females (38, 39). Many studies have validated the accuracy of Tanner self-assessment. A high correlation of Tanner self-assessments has been shown in females, ages 11-13, when compared to physician examiners (40). An additional study validated that adolescents are able to accurately assess their own pubertal development utilizing Tanner stage drawings (41).

However, several studies have suggested that Tanner self-assessments are subjective and unreliable, especially among certain populations. Data show that adolescents may tend to overestimate their development in the early stages and underestimate their development in the later stages, signifying that it may be difficult to use Tanner staging to determine exactly when puberty has begun or has finished (42). Tanner stage self-assessment in minority populations has been shown not to accurately

assess breast or pubic hair maturation in an ethnically diverse population (43). Among young African-American girls, the validity of maturation self-assessments is questionable as African-American girls are usually more mature but often underestimate their development (44). Additionally, a study utilizing Tanner stage self-assessment in children, ages 6-12 years, showed that this form of self-assessment cannot be reliably used to estimate the breast development of obese girls or the pubic hair development of obese or non-obese boys (45).

Various new methods have been recommended in order to increase the feasibility of self-assessment of pubertal development in large-scale epidemiological studies. A new Audio Computer Assisted Self-Interview Method has been proposed as a valid alternative to assessing pubertal development in large studies (46). This automated method for measuring pubertal development, utilizes new drawings based on Tanner stages combined with an audio component for participants on the computer. A preliminary study found good to excellent agreement between the child's self-assessment using the new method and examiner assessment for the male genital development, male pubic hair, and female pubic hair questions. The study achieved excellent agreement between child's self-assessment and the examiner for the female breast development questions. The newly proposed method is fast, private, easy to use and available in both English and Spanish. However, further testing is needed to confirm the results of the preliminary study, particularly among Spanish speaking youth. In large studies where individual self-assessment is not feasible, a novel pubertal self-assessment questionnaire was created as another alternative method to Tanner staging (47). The preliminary study utilized gender specific line drawings of Tanner pubertal stages. When comparing the

new self-assessment method to clinician examination, the study achieved good agreement for the male and female pubic hair questions. Additionally, the study found moderate agreement for the male genital development and female breast development questions. Overall, the study discovered that children utilizing this method underestimated the stage of their pubertal development when compared to clinician examination. Further validation of this new approach is necessary before it can be employed to measure pubertal development in an adolescent population.

The use of a biological marker, such as DHEA, may provide an alternative method to assess pubertal development. Unlike Tanner stage assessment, the use of a biological marker does not rely on externally observed characteristics, is less inconvenient to the participant, and does not require the expensive cost associated with clinician examination. Overall, utilizing levels of DHEA to assess pubertal development, may prove to be a more accurate, standardized, and efficient method to measure maturation in males and females.

METHODS

Study Design and Population

The Growth and Puberty (GAP) study was undertaken to examine the feasibility of performing a prospective study of puberty among the children of agricultural workers in rural Iowa. Utilizing enrollment questionnaires obtained from the Agricultural Health Study Phase I and Phase II, all female participants with children under the age of 15 were identified. This list of children was used to identify potential participants for the Growth and Puberty pilot study. Only children living within 100 miles of Des Moines, Iowa were eligible to participate in the study. A total of 174 Agricultural Health Study families were identified for possible enrollment into the study. From these families, 77 children enrolled to participate in the GAP study.

Written consent was obtained from parents and verbal assent was obtained from children participating in the study. Data collection for each family in the study occurred during a six-month period over three different visits with a field interviewer. The first visit occurred at enrollment into the study, the second visit occurred three months after the initial visit, and the third visit occurred six months after the initial visit. At each of the three visits, questionnaires were administered to both the parent and the child regarding environmental exposures and variables that might affect the growth and development of the child. Additionally, at each visit, Tanner stage was self-assessed by each child. For children ages 7 to 10, parental assessment of Tanner stage was also performed at each study visit. For children ages 11 to 15, only the child's Tanner stage assessment was collected. In addition, the field interviewer measured the body size and

body composition of the child at each visit. Saliva samples were obtained from the child at each visit in order to assess the sample for DHEA, a hormone signaling sexual maturation.

Questionnaires

An initial questionnaire was mailed to parents to be completed prior to the first visit. This initial questionnaire included questions about the mother's exposures during pregnancy, the child's history of exposure to agricultural chemicals, the child's health history and nutritional supplement intake, as well as other social and environmental factors. The same parent was asked to complete follow-up self-administered questionnaires prior to Visit 2 and Visit 3.

At each of the three visits, the field interviewer questioned children regarding puberty, growth, smoking, farm tasks, pesticide exposure, use of dietary supplements, physical development, and activities occurring in fields in which pesticides had been applied. Parents were allowed to review the child questionnaires prior to administration and could identify questions that they did not want their children to be asked by the interviewer.

Tanner Stage Assessments

Tanner staging is an established method for assessing pubertal development in both male and female children and adolescents (38,39). In this study, drawings used for Tanner stage assessment were adapted from those developed by D. Udry of the University of North Carolina Population Center. The text and illustrations included in the Tanner assessment forms have been adapted from a pubertal maturation self-assessment

interview component that is undergoing validation by the Centers for Disease Control and Prevention (CDC), the National Center for Health Statistics (NCHS), and the National Health and Nutrition Examination Survey (NHANES) Program.

At each of the three study visits, a parent, usually the mother, was asked to use the Tanner stage drawings to assess the development of each child aged 7-10 years. The parent was asked to complete the form from memory without physical examination of the child. There was an option for the alternative parent to complete the Tanner form on the child if they would be better able to accurately assess the child's developmental stage. Parents were not asked to complete the form for children older than 10 years at the time of enrollment into the study.

Permission was obtained from the mother at Visit 1 to allow the child to complete a self-assessed Tanner form. If the parent refused to give permission, the study interviewer offered the option of having the child complete an abbreviated version of the Tanner form. The abbreviated Tanner form only includes the first three or four of the five Tanner stages of development. In this study, none of the parents requested the abbreviated form for their child. At Visit 1, participating children were given the Tanner stage drawings enclosed in an envelope and asked to move to another room to use a mirror for self-examination. The child was instructed to mark below the drawings that most closely matched their stage of physical development. The forms were then returned to the envelope, sealed and given to the interviewer. Each child was advised that they could choose not to complete the forms and should check the box indicating same on the Tanner forms. For Visit 2 and Visit 3, children were asked to complete the Tanner form prior to the visit by the interviewer.

Anthropometric Measurements

The field interviewer conducted anthropometric measurements at each study visit. The interviewer measured height (cm), weight (kg), waist circumference (cm), and hip circumference (cm) of each child. All measurements were performed in accordance with techniques adapted from the training manual for NHANES IV. Every measurement was performed twice and the average of the measurements was utilized in the analysis. If there was a significant discrepancy between the initial two measurements, a third measurement was taken by the interviewer. Additionally, body weight and body composition were determined using foot-to-foot bioelectric impedance analysis (BIA) at each study visit. The same type of portable digital scale, the Tanita 300, was used by each study interviewer and was brought to each participant's home during every study visit.

Collection of DHEA Samples

Each child was asked to collect a saliva sample on the morning of each study visit. Parents were then asked to refrigerate each sample until the examiner arrived to pick up the sample at each visit. At the initial visit, girls were asked if they have had their first period. All biological samples from girls who had reached menarche were not initially analyzed for hormone concentrations.

The saliva samples were collected by participants using a protocol designed by Salimetrics, LLC (State College, Pennsylvania), associated with the Pennsylvania State University Behavioral Endocrinology Laboratory. Sample collection occurred in the morning prior to teeth brushing or the consumption of any food or drink. In the event

that the sample was not collected prior to any of these activities, the child rinsed their mouth with water and waited 10 minutes before collecting the saliva. Saliva collection had to occur in the morning, as the interest was in the peak levels associated with diurnal variations in hormones. Additionally, parents were reminded via telephone call that saliva samples should not be collected from children with a body temperature over 102° F or with bleeding oral lesions. In the event that the child suffered from either of these conditions, the visit with the study interviewer was rescheduled.

During sample collection, children were asked to move their jaws as if they were eating, smelling, and chewing their favorite food. Approximately 2 mL of saliva was expectorated through a short plastic straw into a vial suitable for storage at ultra-low temperatures.

For each saliva sample, the mother was asked to complete the Specimen Checklist and Parent Questionnaire Update Form. Using this form, the mother noted the date and time of saliva collection, all medications the child had taken within the 48 hours prior to the sample collection, if and what the child ate within 30 minutes of collection, if the child drank dairy or fruit juice within 30 minutes of collection, and if the child had brushed their teeth prior to the collection.

If a child forgot to obtain a saliva sample on the morning of the visit or if an insufficient amount of saliva was collected, the child was asked to collect a sample or replacement sample. An additional collection kit was given to the child and a new appointment was scheduled, preferably within 2 weeks of the original appointment.

The saliva collection vials were pre-labeled with the study ID bar code. Participants stored the samples in the refrigerator until picked up by the study interviewer. The interviewer transported the saliva specimens in a portable refrigerator, a Koolatron, or on ice packs to a study site freezer (cooled to -20° C). The frozen saliva samples were packaged with dry ice and shipped to the Social & Scientific Systems (SSS) laboratory in North Carolina. All samples were shipped to arrive at the laboratory by the next day. At the laboratory, samples were electronically tracked by a computer system using the assigned bar code number. All samples were stored at -80° C in locked freezer storage until assay. At the end of the data collection period, selected samples were shipped to the Salimetrics laboratory in State College, Pennsylvania to be assayed for DHEA concentrations. During transport, all samples were packaged according to the Centers for Disease Control and Prevention guidelines for the transport of biological specimens. All samples only underwent a single freeze-thaw cycle prior to laboratory testing.

Laboratory Analysis of DHEA Samples

All analysis of DHEA samples was performed by Salimetrics laboratory. Each sample was centrifuged at 3,000 rpm for 10 minutes in order to remove mucin and then screened for pH and possible blood contamination. Any samples that tested positive for blood contamination (> 1.0 mg/dl blood protein) were excluded from further analysis. Samples that tested outside the pH range of 4-9 were diluted to correct pH prior to salivary testing. A double-antibody radioimmunoassay (RIA) developed at the Pennsylvania State University Behavioral Endocrinology Laboratory was used to assay all samples for salivary DHEA (48). This test utilizes 100 μL of saliva, has a minimum

detection limit of 4 pg/mL, an average intra-assay coefficient of variation less than 4.05%, and an average inter-assay coefficient of variation less than 12.57%. Values from matched serum and saliva samples show a strong linear relationship for males, $r = 0.82$, $p < 0.0001$, and for females, $r = 0.90$, $p < 0.0001$. The range of sensitivity for this assay is high, capturing 100% of developmental and individual differences in the DHEA levels found in the saliva of males and females, ages 7 to 45 years old.

Each saliva sample with up to 1.8 mL of saliva was analyzed and two tests for DHEA level was performed per submitted sample. The acceptable coefficient of variation limits for duplicate DHEA testing was less than 15% unless the absolute difference between the duplicate sample 1 and duplicate sample 2 values was less than or equal to 5 pg/mL. Any sample with duplicate results not meeting the acceptable coefficient of variation limits was retested. In this study, only one saliva sample required retesting and the quantity of this sample was not sufficient for retesting to occur.

Data Analysis Plan

Descriptive statistics for the study group were computed for the following continuous variables: age (years), DHEA (pg/mL), height (cm), weight (kg), and body mass index (BMI) (kg/m^2). The average time spent in the study was also calculated for the study population. Descriptive statistics were computed for categorical variables, Tanner stage, gender, school grade, status of child's birth (single or multiple birth), preterm birth, diagnosis of birth defect, and low birth weight. Additionally, parental descriptive statistics were presented including father's height, mother's height, age at mother's first menstrual period, parental education, race, and mother's behaviors during

pregnancy. Frequencies and percentages were calculated for all categorical variables while means, standard deviations, skewness and kurtosis were calculated for all continuous variables.

Further exploratory analyses were performed, including boxplots and scatterplots, to assess variable distributions and identify non-normality, possible outliers, or the need for potential transformation. The previous medical conditions and current medication usage was examined for all individuals (Appendix). If a prior condition or medication was believed to affect pubertal development or DHEA levels, the possible exclusion of that participant was explored. One child was excluded from further analyses due to the prior diagnosis of a non-specified birth defect. The distribution of DHEA in pg/ML was calculated overall and based on gender status. Due to the non-normality of the distribution of DHEA, a natural logarithm transformation of DHEA was performed. Additionally, DHEA observations below the limit of detection of 5 pg/mL were replaced with the recommended value of the limit of detection divided by the square root of 2 (49). Thus, in this study, a total of 13 observations were below the limit of detection and replaced with the imputed value. A total of 5 observations had only one DHEA sample with a readable level of DHEA. The quantity was not sufficient or no level of DHEA was detected in the remaining duplicate sample for these individuals. The correlation between sample 1 and sample 2 was investigated for all observations in the study. As the coefficient of correlation between the sample 1 and sample 2 values was extremely high, one viable sample was used to measure the DHEA instead of the two averaged samples for these five observations (Figure 1). A total of 7 participants had a total of five saliva samples analyzed while 21 participants had a total of 4 saliva samples analyzed due to the

recollection of samples at repeat visits. For the purpose of these analyses, only three total saliva samples were included per child and the replacement DHEA sample from the repeat study visit was chosen over the initial DHEA sample collected for these individuals.

To determine the inter-rater reliability for Tanner stage between child self-assessments and parental assessment in children ages 7-10, the overall percent agreement between the raters was calculated. Furthermore, Cohen's kappa statistic was also calculated to measure the level of inter-rater reliability (50).

Bivariate analyses were performed to examine the relationship between DHEA and other study variables using independent t-test, Analysis of Variance (ANOVA), and Pearson's correlation coefficient. Additional analyses were performed to investigate the relationship between Tanner stage and other study variables using ANOVA and Chi Square. Additional analyses were performed to investigate the relationship between age and other study variables using independent t-test, ANOVA, and Pearson's correlation coefficient.

The relationship between Tanner Stage and DHEA was modeled using multivariate linear regression for longitudinal data, unadjusted for other study variables associated with the outcome. Separately, the relationship between age and DHEA was investigated using multivariate linear regression for longitudinal data, unadjusted for other study variables associated with the outcome.

The relationship between two predictors, age and Tanner stage, on the level of DHEA was modeled using multivariate linear regression for longitudinal data.

Additionally, the relationships between age and Tanner stage, on the level of DHEA, adjusted for birth weight, height, father's education level, or BMI were modeled using multivariate linear regression for longitudinal data.

All data analysis for this paper was performed using SAS Software, Version 9.3 for Windows (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The continuous characteristics of the study population are presented in Table 1. The children ranged in age from 7 to 16 years old and on average, were 12 years of age. The average height of a participant was 151 cm while the average child weighed 47 kg. The mean birth weight of all participants was 3,442 grams and ranged from 1,446 to 4,819 grams. On average, the time through the study for each individual child was approximately 7 months. The range of participant time in the study was relatively large, with one individual taking 13 months to complete the study. Table 2 presents the categorical characteristics of the study population. The majority of study participants were male (55%). The most common school grades of the population were sixth grade (18%) and seventh grade (18 %). In this population, 4% of the population was born a twin, 7% were born preterm, and 8% were born with a low birth weight. Additionally, 2 of the children enrolled in the study were born with a birth defect.

Table 3 displays the continuous characteristics of the parents of children enrolled in the study. The average height of the father of a child in the study was 72 inches while the average height of the mother of a child in the study was 65 inches. The categorical characteristics of the parental population are shown in Table 4. The race of the mothers of all participants was Caucasian. While the majority of the fathers of participants were Caucasian (97%), 2 of the fathers were multiracial. The mothers of participants were highly educated, with 42% of mothers having graduated college. The fathers of this population were slightly less educated, with only 32% having completed college.

The distribution of the natural logarithm of DHEA level at visit 1 and visit 3 is presented in Table 5. The average level of DHEA in the population did not change significantly from visit 1 to visit 3. The mean natural logarithm of DHEA by self-assessed Tanner Stage for female breast development for visit 1 and visit 3 is presented in Table 6. At visit 1, the predominant stage of breast development for female children was stage 1 (34%). By visit 3, the most common stage of breast development was found to be stage 4 (40%). None of the female children in this study reached Tanner stage 5 for breast development at any of the study visits. The average level of DHEA is greatest in stage 4 of breast development at visit 1 and visit 3. Additionally, Figure 2 depicts the natural logarithm of DHEA by Tanner stage for female breast development for all visits. The mean natural logarithm of DHEA by self-assessed Tanner Stage for female pubic hair development at visit 1 and visit 3 is shown in Table 7. At visit 1, the most common stage of pubic hair development for female children was stage 1 (37%). By visit 3, the most common stages of female pubic hair development were found to be stage 1 (29%) and stage 5 (29%). The average level of DHEA is greatest in stage 5 of female pubic hair development at visit 1 and visit 3. Figure 3 depicts the natural logarithm of DHEA by Tanner stage for female pubic hair development for all visits. The mean natural logarithm of DHEA by self-assessed Tanner Stage for male genital development for visit 1 and visit 3 is presented in Table 8. At visit 1, the predominant stage of genital development for male children was stage 2 (35%). By visit 3, the most common stages of male genital development were found to be stage 2 (23%) and stage 3 (23%). At visit 1, the average DHEA level is greatest in stage 4 of male genital development. By visit 3, the average DHEA level is greatest in stage 3 of male genital development. Figure 4

depicts the natural logarithm of DHEA by Tanner stage for male genital development for all visits. The mean natural logarithm of DHEA by self-assessed Tanner Stage for male pubic hair development for Visit 1 and Visit 3 is presented in Table 9. At visit 1, the most common stage of pubic hair development for male children was stage 1 (43%). By visit 3, the largest portion of the male children were in found to be in stage 2 (28%) of pubic hair development. At both visit 1 and visit 3, the average DHEA level is greatest in stage 4 of male pubic hair development. Additionally, Figure 5 depicts the natural logarithm of DHEA by Tanner stage for male pubic hair development. On average, the children were 7 months older at visit 3 when compared to the age at the initial visit (Table 10).

Table 11 displays the association of the natural logarithm of DHEA with continuous covariates. DHEA concentration was found to be significantly associated with increasing age, height, weight and BMI. Additionally, Figure 6 depicts the distribution of the natural logarithm of DHEA levels by age at all three visits for males. Figure 7 depicts the distribution of the natural logarithm of DHEA levels by age at all three visits for females. Figures 8, 9, and 10 portray spaghetti plots of the natural logarithm of DHEA levels by age group for males. Figures 11, 12, and 13 include spaghetti plots of the natural logarithm of DHEA levels by age group for females. Table 12 presents the association of the natural logarithm of DHEA with categorical covariates. The level of DHEA varied significantly based on mother's education level and father's education level. Additionally, DHEA was significantly associated with low birth weight status. However, the level of DHEA was not found to vary significantly based on gender, father's race, or preterm birth.

Table 13 depicts the association of Tanner stage for female breast development at visit 1 with continuous covariates, age, height, weight and BMI. All covariates were found to differ significantly with female breast development. Table 14 shows the association of Tanner stage for female pubic hair development at visit 1 with continuous covariates. All covariates except for BMI were found to vary significantly with female pubic hair development. In Table 15, the association of Tanner stage for male genital development at visit 1 with the continuous covariates is shown. All covariates except for BMI were found to be associated with male genital development. Table 16 presents the association of Tanner stage for male pubic hair development at visit 1 with continuous covariates. All covariates except for BMI were found to be associated with male pubic hair development. Associations between tanner stage and categorical covariates could not be calculated due to small population size and very low cell counts.

Table 17 depicts the association of age with continuous covariates. Age was found to be significantly associated with increasing height, weight and BMI. Table 18 presents the association of age with categorical covariates. Age was found to differ significantly based on father's education level. However, gender, father's race, mother's education, preterm birth, and low birth weight were not significantly associated with age.

Table 19 displays the inter-rater reliability of the parental Tanner stage assessment and the child Tanner self-assessment for female breast development in the children aged 7 to 10 years. The percent of overall agreement between parent and child was extremely high at both visit 1 (100%) and visit 3 (79%) for female breast development. The kappa statistic of inter-rater reliability for Tanner female breast development is 0.76. Table 20 displays the inter-rater reliability of the parental Tanner

stage assessment and the child Tanner self-assessment for female pubic hair development in the children aged 7 to 10 years. The percent of overall agreement between parent and child was extremely high at both visit 1 (86%) and visit 3 (92%) for female pubic hair development. The kappa statistic of inter-rater reliability for Tanner female pubic hair development is 0.62. Table 21 displays the inter-rater reliability of the parental Tanner stage assessment and the child Tanner self-assessment for male genital development in the children aged 7 to 10 years. The percent of overall agreement for male Tanner stage was slightly lower than for the female Tanner stage. However, the percent of overall agreement between parent and child was still relatively high at visit 1 (71%) and visit 3 (69%) for male genital development. The kappa statistic of inter-rater reliability for Tanner male genital development is 0.37. Table 22 displays the inter-rater reliability of the parental Tanner stage assessment and the child Tanner self-assessment for male pubic hair development in the children aged 7 to 10 years. The percent of overall agreement between parent and child was very high at visit 1 (87%) for male pubic hair development. However, the percent of overall agreement between parent and child was much lower for visit 3 (57%) for male pubic hair development. The kappa statistic of inter-rater reliability for Tanner male pubic hair development is 0.10. Overall, good to excellent inter-rater reliability was found between parental assessment and child self-assessment for female Tanner stage assessment for breast development and pubic hair development. Poor inter-rater reliability was found between parental and child self-assessment for male Tanner stage assessment for genital development and pubic hair development.

The association between Tanner stage for female breast development and age with DHEA is presented in Table 23. With the addition of age to the model, all levels of

Tanner stage are no longer significantly associated with DHEA. However, age remains significantly associated with DHEA.

The association between Tanner stage for female pubic hair development and age with DHEA is shown in Table 24. In this model, age remains significantly associated with DHEA. Additionally, Tanner stage 2 is significantly associated with DHEA when compared to the referent stage 1. After adjusting for age, Tanner stage 2 for female pubic hair development was associated with a significant increase in DHEA concentration of 2.72 pg/mL (95% CI: 1.33 – 5.59). All remaining Tanner stages were not significantly associated with DHEA.

The association between Tanner stage for male genital development and age with DHEA is shown in Table 25. With the addition of age to the model, all levels of Tanner stage are no longer significantly associated with DHEA. However, age remains significantly associated with DHEA.

The association between Tanner stage for male pubic hair development and age with DHEA is depicted in Table 26. In this model, age remains significantly associated with DHEA. Additionally, both Tanner stage 2 and Tanner stage 4 are significantly associated with DHEA when compared to the referent stage 1. After adjusting for age, Tanner stage 2 for male pubic hair development was associated with a significant increase in DHEA concentration of 2.52 pg/mL (95% CI: 1.39 – 4.57). All remaining Tanner stages were not significantly associated with DHEA.

All Tanner stage models were run controlling for both age and height (Tables 27, 28, 29, 30). In the model for male genital development and male pubic hair development,

height was found to be significantly associated with DHEA concentration. However, even with the addition of age and height, Tanner stage 2 for female pubic hair development and Tanner stage 2 for male pubic hair development remained significant.

All Tanner stage models were run controlling for both age and BMI (Tables 31, 32, 33, 34). In all Tanner models, BMI was found to be significantly associated with DHEA level. Additionally, with the addition of BMI to the Tanner model for female pubic hair development, Tanner stage 2 no longer remained significant. However, in the Tanner model for male pubic hair development, Tanner stage 2 remained significantly associated with DHEA despite the addition of BMI.

All Tanner stage models were run controlling for both age and father's education level (Tables 35, 36, 37, 38). In all Tanner models, father's education was found to vary significantly with DHEA level. However, even with the addition of age and father's education, Tanner stage 2 for female pubic hair development and Tanner stage 2 for male pubic hair development remained significantly associated with DHEA.

Additionally, all Tanner stage models were run controlling for both age and low birth weight (Appendix). However, birth weight was not found to be significantly associated with DHEA concentration in any of the models.

DISCUSSION

In this longitudinal study of 77 children and adolescents, we found an association between age and the level of salivary DHEA. Additionally, an association was observed between Tanner stage and the level of DHEA. However, when the association between Tanner stage for female breast development and DHEA was investigated, controlling for age, Tanner stage was no longer found to be significantly associated with DHEA. Similar results were obtained when the association between the Tanner stage for male genital development and DHEA, controlling for age, was investigated. However, in the investigation of Tanner stage for female pubic hair development and DHEA, controlling for age, Tanner stage 2 remained significant. Similarly, in the investigation of male pubic hair development and DHEA, controlling for age, both Tanner stage 2 and Tanner stage 4 remained significantly associated with DHEA. In addition, a very high level of overall agreement was found between parental Tanner stage assessment and child self-assessment in female children 7 to 10 years of age.

According to the results obtained in this study, a significant increase in DHEA levels were seen in the progression from Tanner stage 1 to Tanner stage 2 in female pubic hair development and male pubic hair development. For female pubic hair development, the average age for stage 1 was 9.7 years while the average age for stage 2 was 9.6 years. For male pubic hair development, the average age for stage 1 was 10.1 years while the average age for stage 2 was 12.1. However, other studies have shown a marked increase in DHEA much earlier in puberty. In a study by Apter, a distinct increase in DHEA levels was seen in females beginning at age 7.5 years (24). Additionally, in an investigation conducted by Maruyama et al., the rise of DHEA was found to occur in

both males and females at approximately 8 years of age (25). Among this population, fewer young participants were enrolled in the study, potentially preventing the ability to witness a marked increase in DHEA between the ages of 7.5 and 8 years old.

Based on results obtained from the Third National Health and Nutrition Examination Survey (NHANES III), the population in this study showed similar age distributions by Tanner stage to the national sample (1). However, the average ages associated with the highest stage of female breast development, female pubic hair development, male genital development, and male pubic hair development, were much lower in our study. The NHANES III enrolled individuals up to 19 years of age while the oldest individual enrolled at the start of this study was 15.5 years old. As a result, the average ages for the final stages of all Tanner categories are much lower in our study compared to the NHANES III sample.

An increase in the level of DHEA and other related androgenic steroids is associated with the development of pubic hair in both males and females during puberty (51). The findings of our study support the role of DHEA, an androgen, in the transition into adrenarche. When controlling for age, Tanner stage 2 was found to be significantly associated with an increase in DHEA levels for both female pubic hair development and male pubic hair development. Additionally, clinical observations have suggested that the appearance of pubic hair occurs on average one year earlier in females than in males (38, 39). The distribution of age for female pubic hair development in this study were about 1 year earlier for Tanner stage 1 compared to the distribution for male pubic hair development. Additionally, the average age for Tanner stage 2 for female pubic hair

development remained younger than the average age for Tanner stage 2 for male pubic hair development.

While many studies have investigated the validity of maturation assessments performed by clinicians, a lack of studies have investigated the inter-rater reliability between parental assessment and child self-assessment of Tanner stage. The high level of overall agreement between parents and female children in this small study sample, suggests that utilizing parental assessment of a child's pubertal development may be a valid measurement in future studies. However, the lower level of overall agreement and inter-rater reliability found between parents and male children, indicates that further investigation is needed before utilizing only parental assessment of pubertal development in male children in the future.

There were several potential limitations to the study. The sample size of the study population was small with only 77 total participants enrolled. The small sample size may have led to low power and decreased precision in the estimates obtained in the study. The cross-sectional nature of the study prohibited the ability to determine whether an increase in age or Tanner stage preceded an increase in the level of DHEA. Additionally, the study lacked a true control group for comparisons between groups. All children and adolescents enrolled in the study were originally chosen due to their residence on a farm and the possibility of previous pesticide exposure. The inclusion of a control population not living on a farm and without pesticide exposure, would have allowed the study to explore the effect of pesticide exposure on pubertal development and DHEA level. By only including individuals from the farming community, it is difficult to determine

whether the effects seen on DHEA and pubertal development among this population may be due to pesticide exposure or the experience of living on a farm.

It is possible that there was residual confounding in the study. Recollection of biological samples of DHEA at a later date from assessment of Tanner stage occurred for a significant number of individuals in the study. As a result, multiple DHEA samples were not collected on the same day as the performance of the Tanner assessment for many participants, potentially confounding the association between Tanner stage and DHEA. Furthermore, of the 77 study participants, many individuals were enrolled from the same family. The familial connection between participants may have confounded the results of the study. Siblings and relatives are more likely to exhibit similar pubertal development. Additionally, the study population was racially and ethnically homogenous. The race of both parents of all but two study participants were Caucasian. The lack of racial and ethnic diversity in the study population prevents the ability to generalize the results to a more diverse population.

Several forms of bias may have been inherent in the study design. Specifically, selection bias may have been present in the enrollment of participants. First, all participants were originally eligible for enrollment into the study based on the participation of their parents in the Agricultural Health Study. As a result, certain individuals or families may have been more likely to choose to participate in the study. Additionally, families may have been more likely to enroll their children in the study if they had high pesticide exposure or had experienced altered pubertal development. Information bias in the form of recall bias may have been possible in the collection of data in the study. As parents completed the Tanner stage assessments based on memory,

recall bias is likely in the parental assessment of pubertal development of the children ages 7 to 10 years. As a result, parents may have either underestimated or overestimated their child's pubertal development. Additional recall bias is possible in the completion of the parental questionnaires. Several questions referred to the child's current and past medication use and medical conditions. If a medication or medical condition had a direct effect on pubertal development, the possible exclusion of the child from the final study analyses was evaluated. Due to possible recall bias, parents may have incorrectly completed this portion of the questionnaire. As a result, children who would have been excluded from the analyses based on medication use or medical condition were included in the population. With the small size of the study population, the inclusion of individuals with possible conditions or medications affecting pubertal development or DHEA levels could have a significant impact on the final results.

In conclusion, significant associations were individually found for both age and Tanner stage with DHEA. However, when controlling for age, a significant association only remained between Tanner stage and DHEA for female pubic hair development and male pubic hair development. These results support the integral role of the increase in DHEA and additional androgenic steroids in the development of pubic hair during the early stages of puberty. Additionally, the high inter-rater reliability seen between female child self-assessment and parental assessment of Tanner stage suggests that in future studies parental assessment provides a valid result of pubertal development in female children. However, due to the small sample size and the possibility of bias and confounding, the application of the results may be limited. Further research is needed in the form of prospective longitudinal studies, to minimize bias and possible residual

confounding. Additionally, in order to increase the generalizability of these results, similar studies should be conducted among a less homogeneous population and in the children of non-agricultural farm workers.

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TABLES

Table 1. Distribution of continuous covariates for cohort of children (n=77).

	Mean	Standard Deviation	Range
Age (Years)	11.80	2.17	7.17 - 15.50
Height (cm)	151.25	15.36	122.00 - 186.00
Weight (kg)	46.48	14.84	22.60 - 95.60
Body Mass Index (kg/m^2)	19.85	3.76	13.46 - 30.18
Birth Weight (grams)	3442.07	597.37	1445.83 - 4819.42
Time in Study (Months)	6.81	1.51	5.00 - 13.00

Table 2. Distribution of categorical covariates for cohort of children (n=77).

	No.	%
Gender		
Male	42	54.55
Female	35	45.45
School Grade		
Kindergarten	2	2.60
First Grade	7	9.09
Second Grade	4	5.19
Third Grade	13	16.88
Fourth Grade	4	5.19
Fifth Grade	12	15.58
Sixth Grade	14	18.18
Seventh Grade	14	18.18
Eighth Grade	7	9.09
Status of Child's Birth		
Single Birth	74	96.10
Twin	3	3.90
Preterm Birth		
No	72	93.51
Yes	5	6.49
Child Diagnosed With Birth Defect		
No	75	97.40
Yes	2	2.60
Low Birth Weight (<2500 grams)		
No	71	92.21
Yes	6	7.79

Table 3. Distribution of continuous covariates for parents of cohort of children (n=77).

	Mean	Standard Deviation	Range
Height of Father (Inches)	71.61	2.74	66.00 - 77.00
Height of Mother (Inches)	65.01	2.99	57.00 - 71.00
Age of Mother at First Menstrual Period (Years)	12.43	1.41	9.00 - 17.00

Table 4. Distribution of categorical covariates for parents of cohort of children (n=77).

	No.	%
Mother Smoked During Pregnancy		
No	74	96.10
Yes	3	3.90
Mother's Race		
Caucasian	77	100.00
Father's Race		
Caucasian	75	97.40
Multiracial	2	2.60
Mother's Education		
High School Graduate	6	9.09
Vocational School (1-3 years)	12	18.18
Some College	16	24.24
College Graduate	28	42.42
Postgraduate (1 or more years)	4	6.06
Unknown	11	
Father's Education		
High School Graduate	24	32.00
Vocational School (1-3 years)	7	9.33
Some College	18	24.00
College Graduate	24	32.00
Postgraduate (1 or more years)	2	2.67
Unknown	2	

Table 5. Mean natural logarithm of DHEA level (pg/mL) at Visit 1 and Visit 3.

	Mean	Standard Deviation	Range
Visit 1 (n=72)	4.16	1.33	1.26 - 6.83
Visit 3 (n=74)	4.40	1.16	1.26 - 6.86

Table 6. Mean natural logarithm of DHEA (pg/mL) by self-assessed Tanner stage for female breast development at Visit 1 and Visit 3.

	No. (%)	Mean (SD) DHEA	Range DHEA
Visit 1 (n = 33)			
Stage 1	12 (34.29)	3.55 (0.68)	2.45 - 4.61
Stage 2	10 (28.57)	4.66 (1.63)	1.26 - 6.83
Stage 3	5 (14.29)	4.77 (1.12)	2.91 - 5.86
Stage 4	8 (22.86)	4.79 (0.68)	4.02 - 5.87
Visit 3 (n = 33)			
Stage 1	11 (31.43)	3.68 (1.01)	1.70 - 5.00
Stage 2	3 (8.57)	3.20 (1.81)	1.26 - 4.86
Stage 3	7 (20.00)	4.45 (0.36)	3.98 - 4.79
Stage 4	14 (40.00)	4.95 (0.75)	2.67 - 5.69

Table 7. Mean natural logarithm of DHEA (pg/mL) by self-assessed Tanner stage for female pubic hair development at Visit 1 and Visit 3.

	No. (%)	Mean (SD) DHEA	Range DHEA
Visit 1 (n = 33)			
Stage 1	13 (37.14)	3.77 (0.80)	2.45 - 5.08
Stage 2	3 (8.57)	4.68 (1.71)	3.01 - 6.42
Stage 3	5 (14.29)	3.93 (1.56)	1.26 - 5.20
Stage 4	8 (22.86)	4.83 (1.20)	2.92 - 6.83
Stage 5	6 (17.14)	5.07 (0.81)	4.02 - 5.87
Visit 3 (n = 33)			
Stage 1	10 (28.57)	3.27 (1.19)	1.26 - 5.00
Stage 2	6 (17.14)	4.51 (0.54)	3.72 - 4.94
Stage 3	2 (5.71)	3.98	3.98 - 3.98
Stage 4	7 (20.00)	4.67 (0.94)	2.67 - 5.49
Stage 5	10 (28.57)	5.02 (0.53)	4.16 - 5.69

Table 8. Mean natural logarithm of DHEA (pg/mL) by self-assessed Tanner stage for male genital development at Visit 1 and Visit 3.

	No. (%)	Mean (SD) DHEA	Range DHEA
Visit 1 (n = 38)			
Stage 1	10 (25.00)	2.81 (1.50)	1.26 - 4.85
Stage 2	14 (35.00)	3.75 (1.36)	1.26 - 5.56
Stage 3	5 (12.50)	4.85 (1.03)	3.70 - 6.20
Stage 4	9 (22.50)	4.96 (0.64)	3.64 - 5.58
Stage 5	2 (5.00)	4.91 (0.28)	4.72 - 5.12
Visit 3 (n = 39)			
Stage 1	7 (17.95)	3.58 (1.82)	1.26 - 6.55
Stage 2	9 (23.08)	4.04 (0.96)	2.65 - 5.88
Stage 3	9 (23.08)	4.96 (0.88)	3.64 - 6.07
Stage 4	7 (17.95)	4.76 (0.97)	2.99 - 6.04
Stage 5	7 (17.95)	4.92 (0.46)	4.26 - 5.68

Table 9. Mean natural logarithm of DHEA (pg/mL) by self-assessed Tanner stage for male pubic hair development at Visit 1 and Visit 3.

	No. (%)	Mean (SD) DHEA	Range DHEA
Visit 1 (n = 38)			
Stage 1	17 (42.50)	2.96 (1.56)	1.26 - 5.13
Stage 2	9 (22.50)	4.34 (0.75)	3.35 - 5.33
Stage 3	5 (12.50)	4.58 (0.87)	3.70 - 5.56
Stage 4	5 (12.50)	5.24 (0.79)	4.25 - 6.20
Stage 5	4 (10.00)	4.95 (0.20)	4.72 - 5.13
Visit 3 (n = 39)			
Stage 1	8 (20.51)	3.51 (1.69)	1.26 - 6.55
Stage 2	11 (28.21)	4.55 (0.95)	3.03 - 5.88
Stage 3	4 (10.26)	4.17 (1.15)	2.99 - 5.68
Stage 4	9 (23.08)	4.97 (0.75)	4.01 - 6.07
Stage 5	7 (17.95)	4.96 (0.44)	4.26 - 5.68

Table 10. Mean age (years) at Visit 1 and Visit 3 (n=76).

	Mean	Standard Deviation	Range
Visit 1	11.81	2.18	7.17 - 15.5
Visit 3	12.38	2.17	7.58 - 16.00

Table 11. Association of continuous covariates and natural logarithm of DHEA (pg/mL) at Visit 1 (n=72).

	Pearson's Correlation Coefficient	P-value
Age (Years)	0.58	<0.0001
Height (cm)	0.59	<0.0001
Weight (kg)	0.58	<0.0001
Body Mass Index (kg/m^2)	0.39	0.0008

Table 12. Association of categorical covariates and natural logarithm of DHEA (pg/mL) at Visit 1.

	Mean	Standard Deviation	P-value
Gender			0.30[a]
Male	4.01	1.44	
Female	4.34	1.18	
Father's Race			0.73[a]
Caucasian	4.17	1.34	
Multiracial	3.83	0.94	
Mother's Education			0.05[b]
High School Graduate	5.32	0.43	
Vocational School (1-3 years)	3.23	1.31	
Some College	4.01	1.23	
College Graduate	4.12	1.23	
Postgraduate (1 or more years)	4.17	1.24	
Father's Education			0.0006[b]
High School Graduate	4.63	1.03	
Vocational School (1-3 years)	3.59	0.96	
Some College	3.25	1.54	
College Graduate	4.25	1.03	
Postgraduate (1 or more years)	6.31	0.15	
Preterm Birth			0.67[a]
No	4.14	1.35	
Yes	4.41	1.17	
Low Birth Weight (<2500 grams)			0.04[c]
No	4.12	1.36	
Yes	4.74	0.45	

[a] Independent T-Test, Pooled Method
[b] ANOVA F-Test
[c] Independent T-Test, Satterthwaite Method

Table 13. Association of continuous covariates and self-assessed Tanner stage for female breast development at Visit 1.

	Mean	Standard Deviation	P-value
Age (Years)			<.0001
Stage 1	9.41	1.09	
Stage 2	11.76	1.83	
Stage 3	13.68	0.95	
Stage 4	13.75	0.91	
Height (cm)			<.0001
Stage 1	136.45	9.00	
Stage 2	150.77	10.37	
Stage 3	159.44	6.22	
Stage 4	163.53	5.21	
Weight (cm)			<.0001
Stage 1	29.89	5.38	
Stage 2	47.64	6.88	
Stage 3	52.58	7.98	
Stage 4	57.59	11.19	
BMI (kg/m^2)			0.0004
Stage 1	15.96	1.90	
Stage 2	21.13	3.70	
Stage 3	20.64	2.98	
Stage 4	21.40	3.24	

Table 14. Association of continuous covariates and self-assessed Tanner stage for female pubic hair development at Visit 1.

	Mean	Standard Deviation	P-value
Age (Years)			<.0001
Stage 1	9.67	1.49	
Stage 2	9.61	0.67	
Stage 3	12.70	1.34	
Stage 4	13.49	0.93	
Stage 5	13.83	0.83	
Height (cm)			<.0001
Stage 1	139.85	11.35	
Stage 2	134.42	3.39	
Stage 3	153.02	7.20	
Stage 4	161.06	7.57	
Stage 5	162.60	4.39	
Weight (cm)			0.0005
Stage 1	34.34	11.63	
Stage 2	37.80	8.89	
Stage 3	46.71	4.11	
Stage 4	53.24	10.95	
Stage 5	56.55	10.60	
BMI (kg/m^2)			0.1041
Stage 1	17.15	3.48	
Stage 2	21.07	5.48	
Stage 3	20.09	3.38	
Stage 4	20.34	3.00	
Stage 5	21.33	3.55	

Table 15. Association of continuous covariates and self-assessed Tanner stage for male genital development at Visit 1.

	Mean	Standard Deviation	P-value
Age (Years)			<.0001
Stage 1	10.03	1.47	
Stage 2	11.02	1.77	
Stage 3	12.95	0.57	
Stage 4	13.75	1.17	
Stage 5	14.96	0.41	
Height (cm)			<.0001
Stage 1	139.13	9.49	
Stage 2	143.86	12.02	
Stage 3	155.84	1.28	
Stage 4	169.79	9.10	
Stage 5	183.80	1.06	
Weight (cm)			<.0001
Stage 1	37.73	9.08	
Stage 2	41.91	11.48	
Stage 3	51.36	13.31	
Stage 4	62.94	15.10	
Stage 5	73.20	2.55	
BMI (kg/m^2)			0.5866
Stage 1	19.15	2.43	
Stage 2	19.97	3.55	
Stage 3	21.24	5.58	
Stage 4	21.78	4.61	
Stage 5	21.65	1.06	

Table 16. Association of continuous covariates and self-assessed Tanner stage for male pubic hair development at Visit 1.

	Mean	Standard Deviation	P-value
Age (Years)			<.0001
Stage 1	10.05	1.42	
Stage 2	12.07	1.52	
Stage 3	13.22	0.88	
Stage 4	13.55	0.95	
Stage 5	14.88	0.28	
Height (cm)			<.0001
Stage 1	139.66	10.67	
Stage 2	152.32	12.49	
Stage 3	155.75	6.46	
Stage 4	165.38	9.31	
Stage 5	182.40	4.13	
Weight (cm)			<.0001
Stage 1	38.15	8.17	
Stage 2	49.01	15.07	
Stage 3	45.32	6.70	
Stage 4	67.06	17.78	
Stage 5	70.54	3.47	
BMI (kg/m^2)			0.0734
Stage 1	19.35	2.53	
Stage 2	20.78	4.81	
Stage 3	18.74	2.62	
Stage 4	24.42	5.39	
Stage 5	21.21	1.38	

Table 17. Association of continuous covariates and age (years) at Visit 1 (n=76).

	Pearson's Correlation Coefficient	P-value
Height (cm)	0.88	<.0001
Weight (kg)	0.71	<.0001
Body Mass Index (kg/m^2)	0.31	0.0071

Table 18. Association of categorical covariates and age (years) at Visit 1.

	Mean	Standard Deviation	P-value
Gender			0.65[a]
Male	11.91	2.16	
Female	11.68	2.23	
Father's Race			0.24[a]
Caucasian	11.86	2.18	
Multiracial	10.00	1.77	
Mother's Education			0.50[b]
High School Graduate	13.22	1.19	
Vocational School (1-3 years)	11.46	2.51	
Some College	11.47	1.82	
College Graduate	11.71	2.15	
Postgraduate (1 or more years)	11.60	2.78	
Father's Education			0.04[b]
High School Graduate	12.79	1.82	
Vocational School (1-3 years)	10.20	2.62	
Some College	11.35	2.16	
College Graduate	11.74	2.18	
Postgraduate (1 or more years)	10.88	1.71	
Preterm Birth			0.59[a]
No	11.77	2.14	
Yes	12.32	2.96	
Low Birth Weight (<2500 grams)			0.09[a]
No	11.68	2.19	
Yes	13.26	1.59	

[a] Independent T-Test, Pooled Method
[b] ANOVA F-Test

Table 19. Inter-rater reliability of parental assessment and child self-assessment of Tanner stage for female breast development (age 7-10 years).

	Percent of Overall Agreement	κ
Visit 1 (n = 14)	100.00	1.00
Visit 3 (n = 14)	78.57	0.57
All Visits (n = 42)	90.48	0.76

Table 20. Inter-rater reliability of parental assessment and child self-assessment of Tanner stage for female pubic hair development (age 7-10 years).

	Percent of Overall Agreement	κ
Visit 1 (n = 14)	85.71	0.44
Visit 3 (n = 13)	92.31	0.76
All Visits (n = 40)	90.00	0.62

Table 21. Inter-rater reliability of parental assessment and child self-assessment of Tanner stage for male genital development (age 7-10 years).

	Percent of Overall Agreement	κ
Visit 1 (n = 14)	71.43	0.42
Visit 3 (n = 13)	69.23	0.43
All Visits (n = 42)	66.67	0.37

Table 22. Inter-rater reliability of parental assessment and child self-assessment of Tanner stage for male pubic hair development (age 7-10 years).

	Percent of Overall Agreement	κ
Visit 1 (n = 15)	86.67	0.00
Visit 3 (n = 14)	57.14	0.14
All Visits (n = 44)	70.45	0.10

Table 23. Generalized linear model for the association between Tanner stage for female breast development and age (years) with DHEA (pg/mL) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.34	0.66 - 2.73
Tanner Stage 3	0.94	0.38 - 2.32
Tanner Stage 4	1.48	0.58 - 3.73
Age	1.24	1.04 - 1.47

Table 24. Generalized linear model for the association between Tanner stage for female pubic hair development and age (years) with DHEA (pg/mL) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.72	1.33 - 5.59
Tanner Stage 3	0.52	0.19 - 1.43
Tanner Stage 4	1.39	0.52 - 3.73
Tanner Stage 5	1.31	0.46 - 3.71
Age	1.27	1.06 - 1.51

Table 25. Generalized linear model for the association between Tanner stage for male genital development and age (years) with DHEA (pg/mL) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.54	0.84 - 2.81
Tanner Stage 3	1.84	0.82 - 4.15
Tanner Stage 4	1.42	0.57 - 3.50
Tanner Stage 5	0.99	0.33 - 2.99
Age	1.47	1.25 - 1.72

Table 26. Generalized linear model for the association between Tanner stage for male pubic hair development and age (years) with DHEA (pg/mL) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.52	1.39 - 4.57
Tanner Stage 3	1.88	0.84 - 4.19
Tanner Stage 4	2.79	1.17 - 6.63
Tanner Stage 5	1.51	0.52 - 4.38
Age	1.36	1.16 - 1.60

Table 27. Generalized linear model for the association between Tanner stage for female breast development and DHEA (pg/mL) controlling for age (years) and height (cm) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.31	0.63 - 2.70
Tanner Stage 3	0.91	0.37 - 2.28
Tanner Stage 4	1.39	0.52 - 3.68
Age	1.20	0.97 - 1.50
Height	1.01	0.98 - 1.04

Table 28. Generalized linear model for the association between Tanner stage for female pubic hair development and DHEA (pg/mL) controlling for age (years) and height (cm) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.73	1.33 - 5.61
Tanner Stage 3	0.53	0.19 - 1.46
Tanner Stage 4	1.33	0.50 - 3.60
Tanner Stage 5	1.23	0.43 - 3.53
Age	1.20	0.96 - 1.49
Height	1.01	0.98 - 1.04

Table 29. Generalized linear model for the association between Tanner stage for male genital development and DHEA (pg/mL) controlling for age (years) and height (cm) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.52	0.87 - 2.65
Tanner Stage 3	1.83	0.87 - 3.85
Tanner Stage 4	0.84	0.35 - 2.00
Tanner Stage 5	0.39	0.13 - 1.18
Age	1.01	0.82 - 1.26
Height	1.07	1.04 - 1.10

Table 30. Generalized linear model for the association between Tanner stage for male pubic hair development and DHEA (pg/mL) controlling for age (years) and height (cm) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.29	1.29 - 4.05
Tanner Stage 3	1.94	0.90 - 4.19
Tanner Stage 4	2.10	0.90 - 4.89
Tanner Stage 5	0.91	0.31 - 2.64
Age	1.04	0.84 - 1.31
Height	1.05	1.02 - 1.08

Table 31. Generalized linear model for the association between Tanner stage for female breast development and DHEA (pg/mL) controlling for age (years) and BMI (kg/m^2) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	0.77	0.35 - 1.67
Tanner Stage 3	0.43	0.16 - 1.18
Tanner Stage 4	0.60	0.20 - 1.77
Age	1.34	1.13 - 1.60
BMI	1.11	1.04 - 1.19

Table 32. Generalized linear model for the association between Tanner stage for female pubic hair development and DHEA (pg/mL) controlling for age (years) and BMI (kg/m^2) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.99	0.95 - 4.18
Tanner Stage 3	0.37	0.13 - 1.01
Tanner Stage 4	1.01	0.38 - 2.72
Tanner Stage 5	0.90	0.32 - 2.58
Age	1.28	1.08 - 1.52
BMI	1.08	1.02 - 1.15

Table 33. Generalized linear model for the association between Tanner stage for male genital development and DHEA (pg/mL) controlling for age (years) and BMI (kg/m^2) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.49	0.84 - 2.64
Tanner Stage 3	1.89	0.88 - 4.06
Tanner Stage 4	1.41	0.60 - 3.31
Tanner Stage 5	1.23	0.43 - 3.49
Age	1.36	1.17 - 1.59
BMI	1.10	1.05 - 1.16

Table 34. Generalized linear model for the association between Tanner stage for male pubic hair development and DHEA (pg/mL) controlling for age (years) and height (kg/m^2) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.51	1.44 - 4.40
Tanner Stage 3	1.96	0.92 - 4.19
Tanner Stage 4	2.43	1.08 - 5.51
Tanner Stage 5	1.72	0.63 - 4.71
Age	1.28	1.09 - 1.50
BMI	1.10	1.05 - 1.16

Table 35. Generalized linear model for the association between Tanner stage for female breast development and DHEA (pg/mL) controlling for age (years) and and father's education level at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.26	0.63 - 2.51
Tanner Stage 3	0.92	0.39 - 2.22
Tanner Stage 4	2.66	0.62 - 3.75
Age	1.26	1.06 - 1.49
Father's Education Level[1]	Referent	
Father's Education Level[2]	1.24	0.70 - 2.21
Father's Education Level[3]	1.83	1.16 - 2.88

[1] Father's Education Level defined as High school graduate or Vocational School (1-3 years).
[2] Father's Education Level defined as Some College.
[3] Father's Education Level defined as College Graduate or Postgraduate (1 or more years).

Table 36. Generalized linear model for the association between Tanner stage for female pubic hair development and DHEA (pg/mL) controlling for age (years) and father's education level at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.90	1.45 - 5.81
Tanner Stage 3	0.64	0.24 - 1.69
Tanner Stage 4	1.79	0.68 - 4.68
Tanner Stage 5	1.90	0.68 - 5.36
Age	1.21	1.02 - 1.44
Father's Education Level	Referent	
Father's Education Level	1.57	0.92 - 2.69
Father's Education Level	1.92	1.24 - 2.97

Table 37. Generalized linear model for the association between Tanner stage for male genital development and DHEA (pg/mL) controlling for age (years) and father's education level at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.60	0.95 - 2.70
Tanner Stage 3	2.04	1.01 - 4.12
Tanner Stage 4	1.16	0.53 - 2.54
Tanner Stage 5	0.87	0.34 - 2.28
Age	1.35	1.17 - 1.55
Father's Education Level	Referent	
Father's Education Level	0.26	0.17 - 0.41
Father's Education Level	0.84	0.56 - 1.27

Table 38. Generalized linear model for the association between Tanner stage for male pubic hair development and DHEA (pg/mL) controlling for age (years) and father's education level at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.94	1.11 - 3.40
Tanner Stage 3	1.88	0.90 - 3.92
Tanner Stage 4	2.17	0.98 - 4.82
Tanner Stage 5	1.21	0.46 - 3.24
Age	1.28	1.10 - 1.48
Father's Education Level	Referent	
Father's Education Level	0.33	0.21 - 0.52
Father's Education Level	0.90	0.57 - 1.41

FIGURES

Figure 1. Association of DHEA Result 1 (pg/mL) and DHEA Result 2 (pg/mL) values.

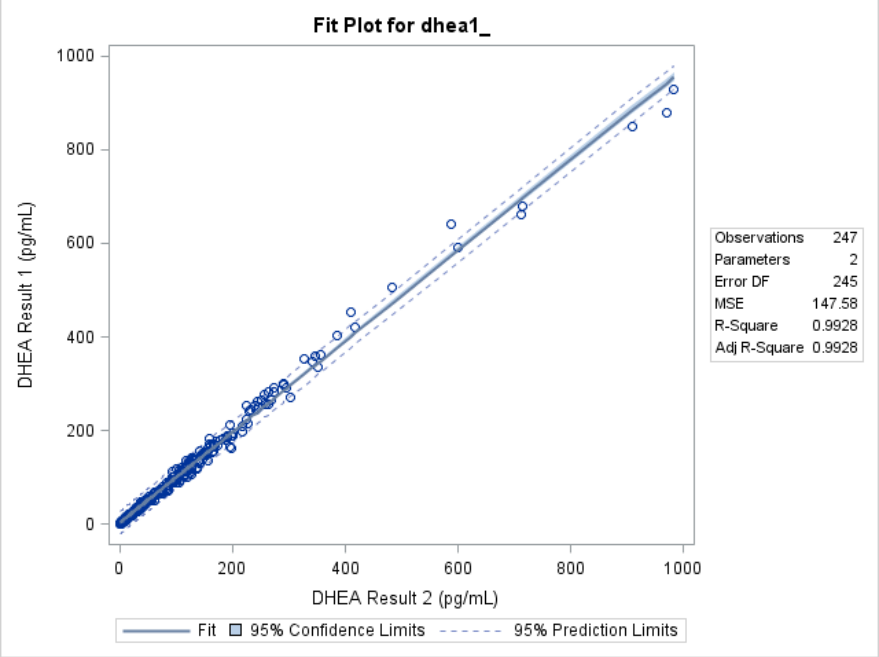


Figure 2. Box plot of the natural logarithm of DHEA (pg/mL) by Tanner stage for female breast development at all visits.

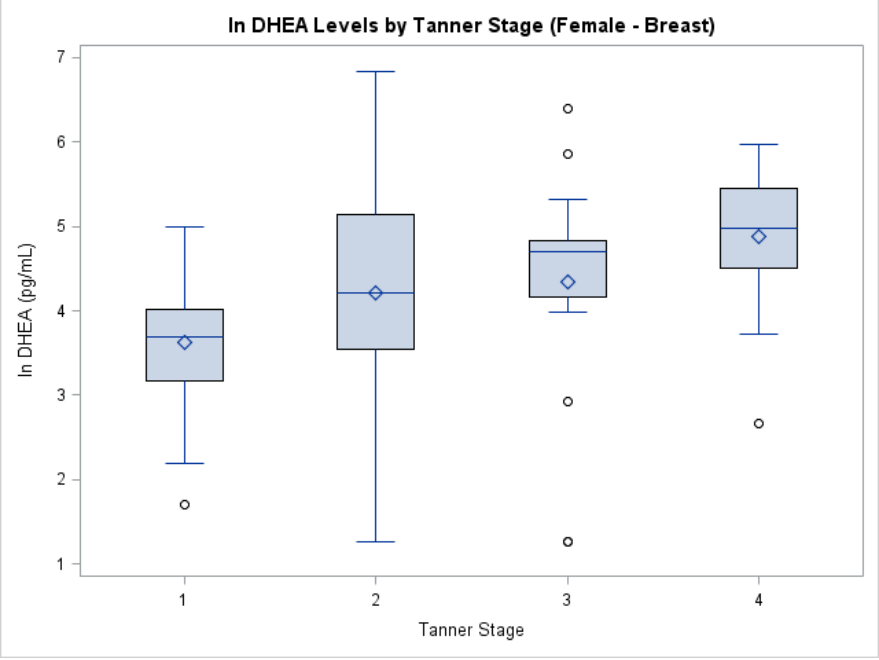


Figure 3. Box plot of the natural logarithm of DHEA (pg/mL) by Tanner stage for female pubic hair development at all visits.

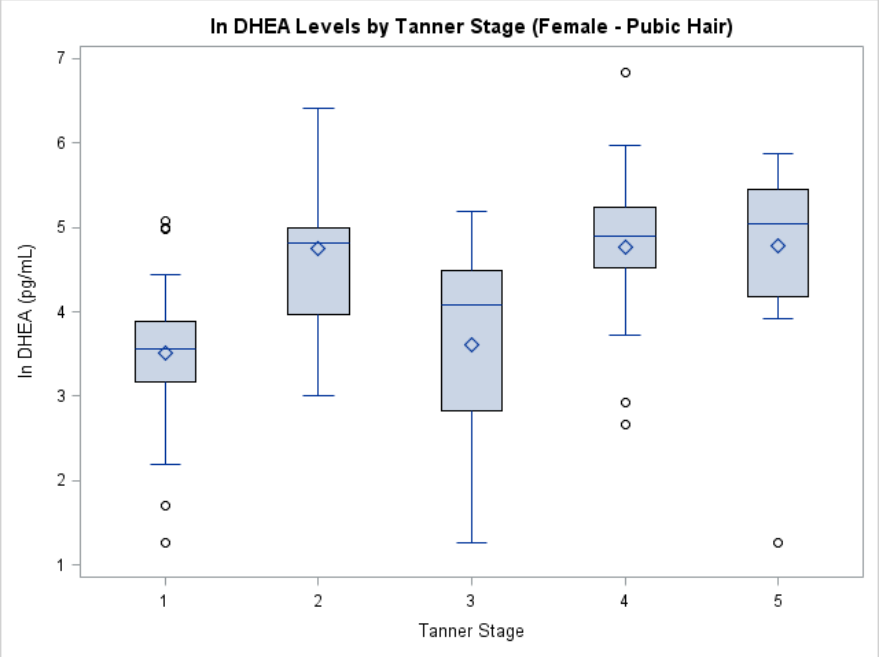


Figure 4. Box plot of the natural logarithm of DHEA (pg/mL) by Tanner stage for male genital development at all visits.

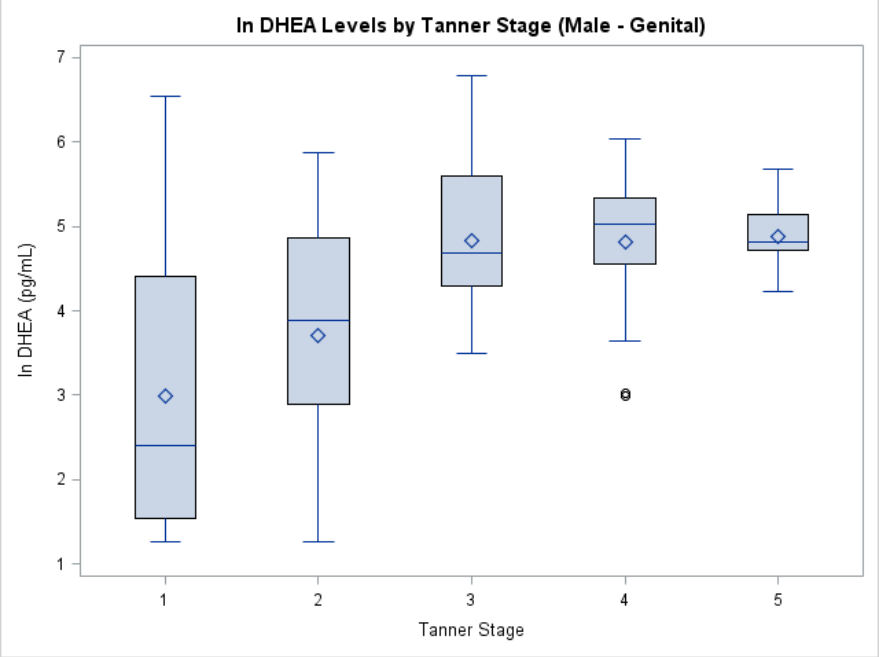


Figure 5. Box plot of the natural logarithm of DHEA (pg/mL) by Tanner stage for male pubic hair development at all visits.

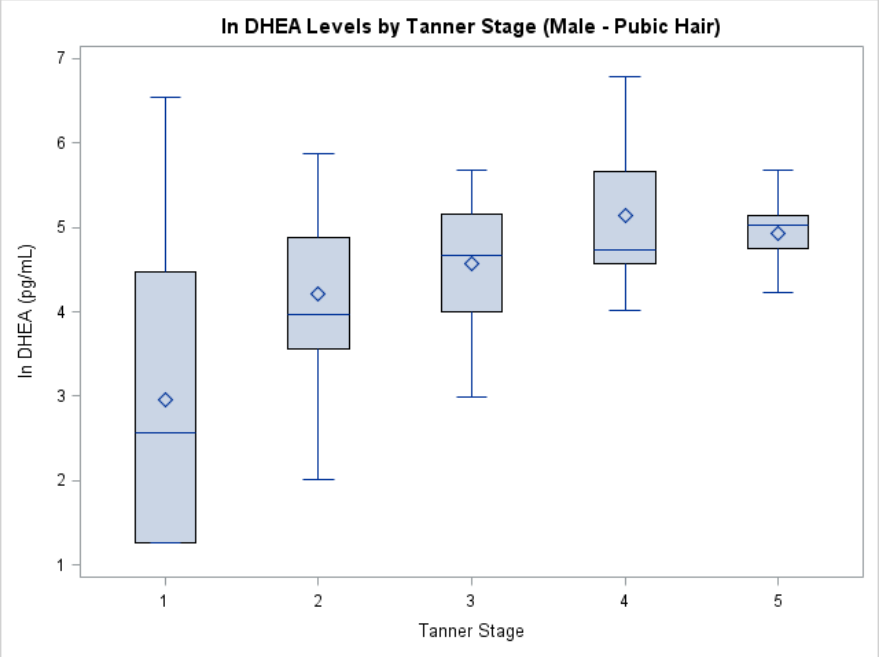


Figure 6. Distribution of the natural logarithm of DHEA (pg/mL) by age (years) in males at all visits.

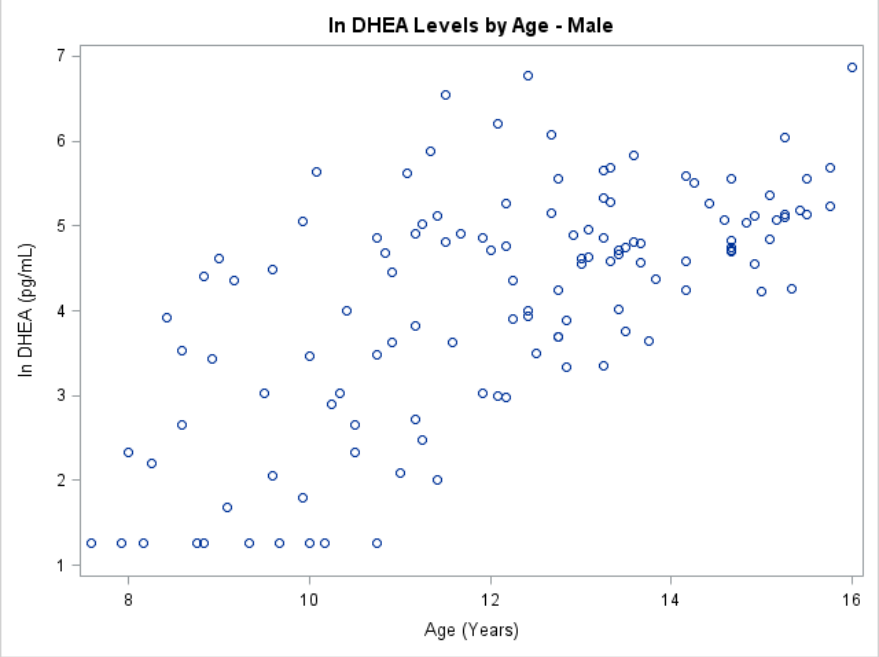


Figure 7. Distribution of the natural logarithm of DHEA (pg/mL) by age (years) in females at all visits.

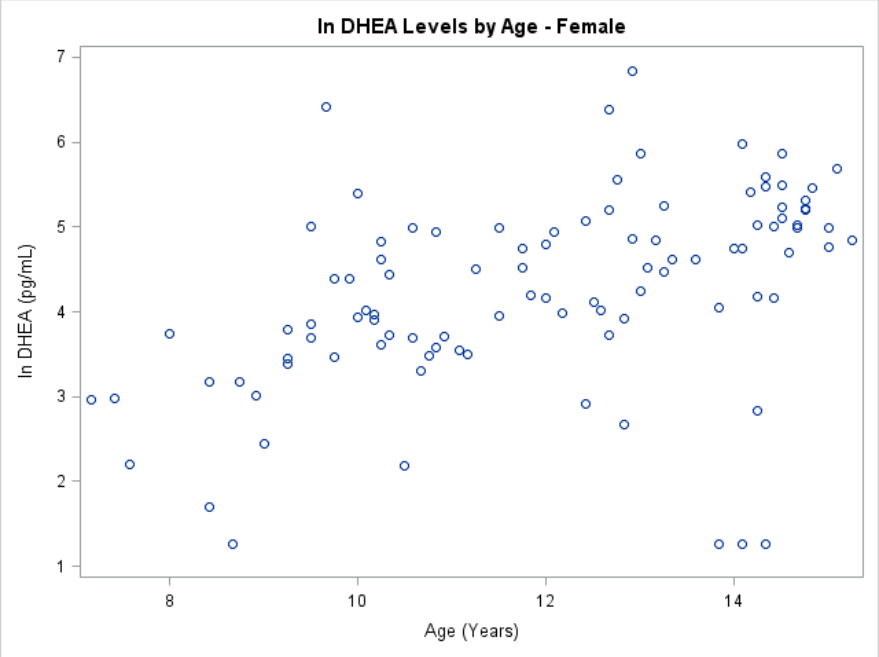


Figure 8. Spaghetti plot of the natural logarithm of DHEA (pg/mL) by age (years) for males 7 to 10 years old.

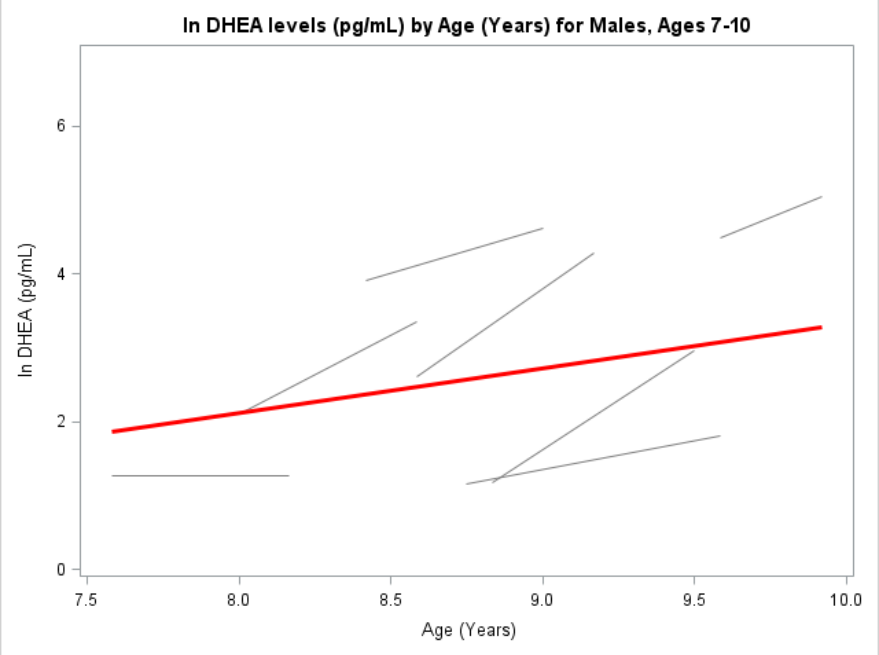


Figure 9. Spaghetti plot of the natural logarithm of DHEA (pg/mL) by age (years) for males 10 to 13 years old.

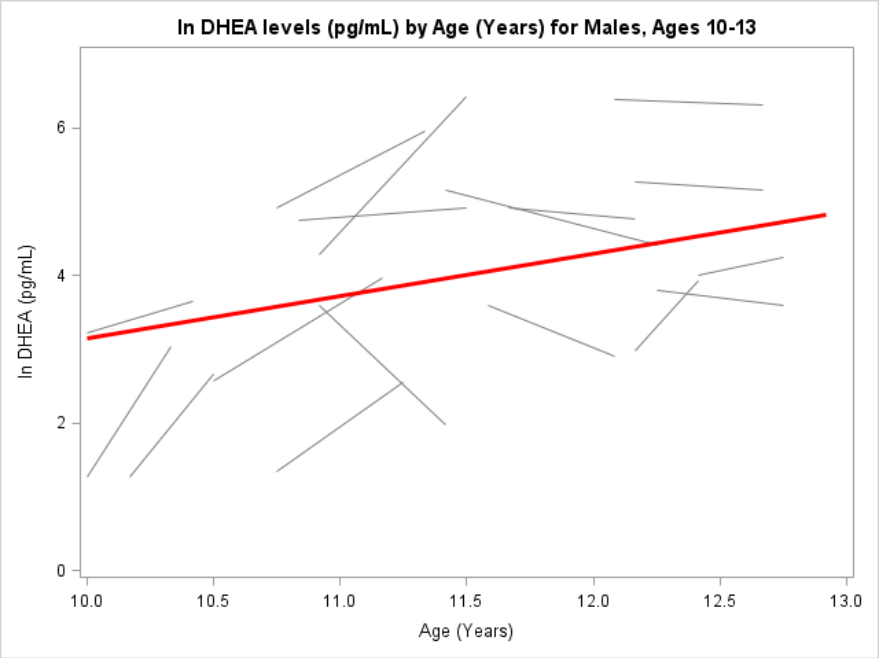


Figure 10. Spaghetti plot of the natural logarithm of DHEA (pg/mL) by age (years) for males 13 to 16 years old.

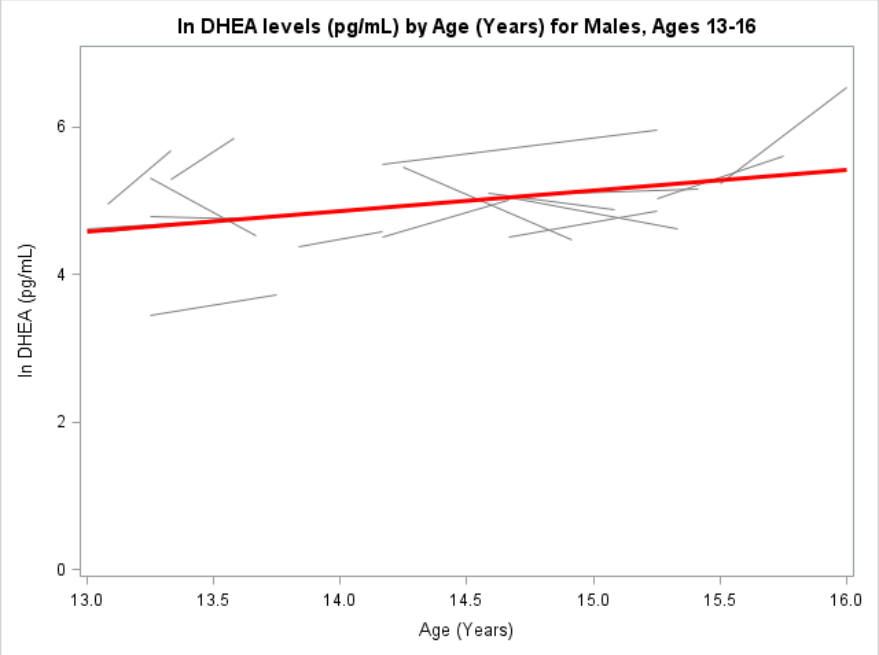


Figure 11. Spaghetti plot of the natural logarithm of DHEA (pg/mL) by age (years) for females 7 to 10 years old.

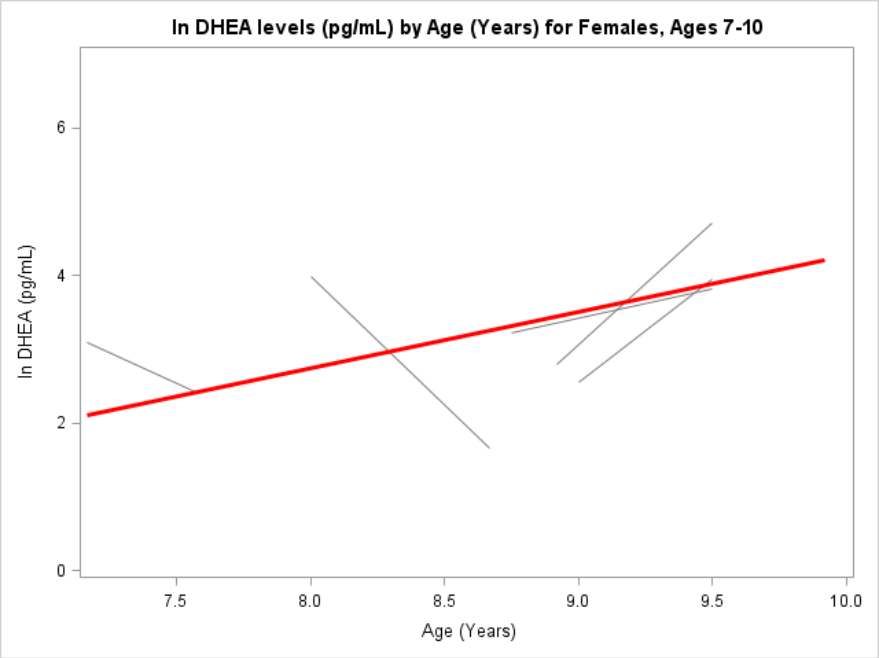


Figure 12. Spaghetti plot of the natural logarithm of DHEA (pg/mL) by age (years) for females 10 to 13 years old.

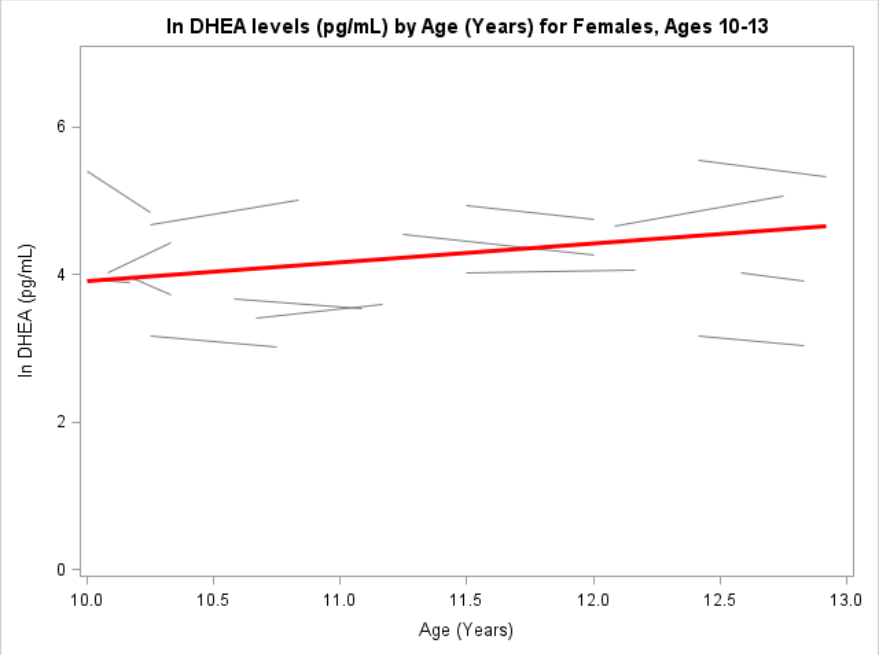
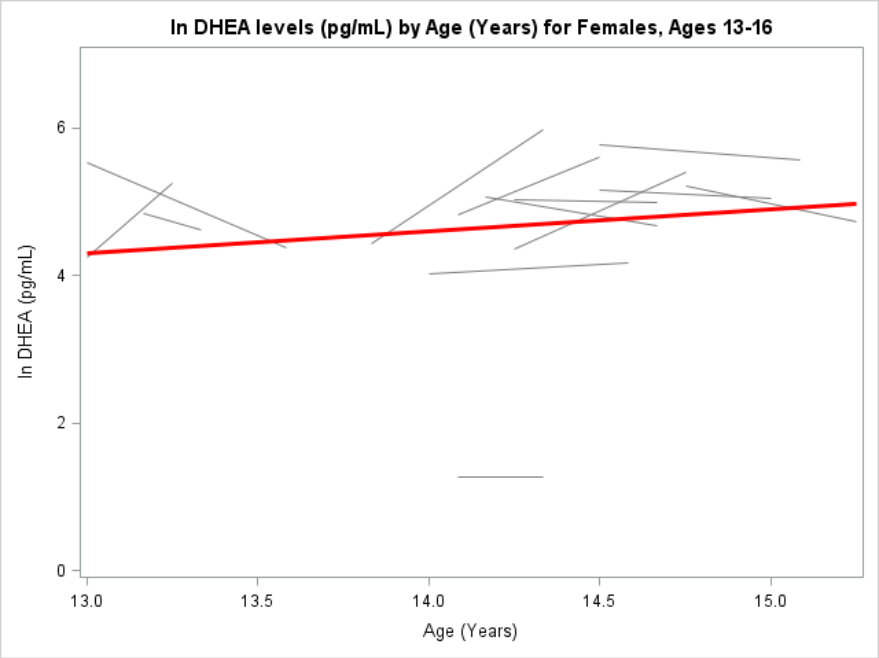


Figure 13. Spaghetti plot of the natural logarithm of DHEA (pg/mL) by age (years) for females 13 to 16 years old.



APPENDIX

Table 39. Possible exclusions due to preexisting medical conditions and current medication use in total population (n=77).

	Comment	Number of Children
Condition		
Small Left Kidney		1.0
Allergies		1.0
Bladder Infections When Little		1.0
Celiac Sprue		1.0
Eczema		1.0
Eczema. Allergy to milk and soy		1.0
Enlarged cup to disk ratio on optic fundi and high to normal eye pressure but no glaucoma.		1.0
Febrile Seizures		1.0
Fractured clavicle at 10 days of age. Fluid on brain at 3 months of age with retinal hemorrhage. Also Wolfe Parkinson White Syndrome.	Wolfe Parkinson White Syndrome - extra electrical circuit in heart, can lead to tachycardia, chest pain, dizziness	1.0
Had ear tubes when he was younger.		1.0
Jaundice		1.0
Birth Defect		
Unspecified		1.0
Vesicoureteral Reflux	Most common in children and infants, occurs in about 10% of children	1.0
Medication (Current)		
ADD or ADHD	Stimulant and non-stimulant types	1.0
Non-Steroidal Asthma Medication		3.0
Steroid Inhalers		1.0
Oral Steroids		2.0
Amoxicillin	Antibiotic	1.0
CRX RA Lot C 5% Sal Acid	Salicylic Acid - Topical treatment of acne	1.0
Hydrocodone	Synthetic Opioid, Pain Reliever	1.0
Hydroxyzine	Antihistamine	1.0
Hydroxyz HCL	Antihistamine	1.0
Ranitidine	Treatment of GERD	1.0
Septra	Antibiotic	1.0
Tetra Tannate	Antihistamine	1.0
Triotann Pediatric Susp	Antihistamine	1.0
45 Duac Gel	Topical treatment of acne	1.0
Nasonex	Allergy Medication	1.0
Benza Clin	Topical Antibiotic	1.0

Table 40. Generalized linear model for the association between age (years) and DHEA (pg/mL) at all visits (n = 76).

Variable	Coefficient	95% CI
Age	1.39	1.31 - 1.49

Table 41. Generalized linear model for the association between Tanner stage for female breast development and DHEA (pg/mL) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.81	0.92 - 3.57
Tanner Stage 3	2.05	1.10 - 3.82
Tanner Stage 4	3.55	2.03 - 6.22

Table 42. General linear model for the association between Tanner stage for female pubic hair development and DHEA (pg/mL) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	3.50	1.72 - 7.11
Tanner Stage 3	1.12	0.50 - 2.49
Tanner Stage 4	3.55	1.92 - 6.55
Tanner Stage 5	3.61	1.96 - 6.67

Table 43. General linear model for the association between Tanner stage for male genital development and DHEA (pg/mL) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.23	1.18 - 4.22
Tanner Stage 3	6.21	3.14 - 12.27
Tanner Stage 4	6.15	3.02 - 12.54
Tanner Stage 5	6.55	2.88 - 14.87

Table 44. General linear model for the association between Tanner stage for male pubic hair development and DHEA (pg/mL) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	4.23	2.43 - 7.36
Tanner Stage 3	5.11	2.74 - 9.53
Tanner Stage 4	8.89	4.77 - 16.58
Tanner Stage 5	7.21	3.67 - 14.16

Table 45. Generalized linear model for the association between Tanner stage for female breast development with DHEA (pg/mL) controlling for age (years) and birth weight (grams) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.35	0.66 - 2.75
Tanner Stage 3	0.95	0.38 - 2.40
Tanner Stage 4	1.48	0.58 - 3.80
Age	1.24	1.03 - 1.48
Birth Weight	1.00	1.00 - 1.00

Table 46. Generalized linear model for the association between Tanner stage for female pubic hair development with DHEA (pg/mL) controlling for age (years) and birth weight (grams) at all visits (n = 35).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.73	1.32 - 5.65
Tanner Stage 3	0.52	0.19 - 1.44
Tanner Stage 4	1.38	0.51 - 3.77
Tanner Stage 5	1.31	0.46 - 3.74
Age	1.27	1.06 - 1.52
Birth Weight	1.00	1.00 - 1.00

Table 47. Generalized linear model for the association between Tanner stage for male genital development with DHEA (pg/mL) controlling for age (years) and birth weight (grams) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	1.68	0.93 - 3.04
Tanner Stage 3	1.99	0.91 - 4.39
Tanner Stage 4	1.60	0.66 - 3.87
Tanner Stage 5	1.03	0.35 - 3.01
Age	1.43	1.22 - 1.70
Birth Weight	1.00	1.00 - 1.00

Table 48. Generalized linear model for the association between Tanner stage for male pubic hair development with DHEA (pg/mL) controlling for age (years) and birth weight (grams) at all visits (n = 40).

Variable	Coefficient	95% CI
Tanner Stage 1	Referent	
Tanner Stage 2	2.66	1.50 - 4.73
Tanner Stage 3	1.78	0.82 - 3.88
Tanner Stage 4	2.56	1.11 - 5.93
Tanner Stage 5	1.36	0.48 - 3.83
Age	1.36	1.16 - 1.59
Birth Weight	1.00	1.00 - 1.00