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Vitamin D Status And Age Of Menarche:

Alexandra Hua
Yale University, alexandra.hua@yale.edu

Yale School of Public Health

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NHANES 2001-2010 Analysis of Adolescent and Early Adult Females in the United States

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ABSTRACT

Early age of menarche (AOM) increases risk for chronic disorders, including cardiovascular disease and related mortality, type 2 diabetes, and breast cancer. Vitamin D deficiency may be linked to earlier onset of menstruation, but there is limited research examining vitamin D and its association with AOM. The objective of this study was to examine the association between vitamin D levels and AOM by analyzing the National Health and Nutrition Examination Survey (NHANES) data from 2001-2010. We hypothesized that that vitamin D deficiency is associated with earlier age of menarche in adolescent and early adult females in the United States. Cross-sectional data on serum 25-hydroxy vitamin D (25OHD) levels and self-reported AOM were available on a sample of 3,572 females between the ages of 12-19 in the 2001-2010 NHANES. Multinomial logistic regression analysis was undertaken to estimate adjusted odds ratios (AORs) for the association between vitamin D status as reflected by serum 25OHD levels with both early (≤9 years) and late (≥14 years) AOM, with adjustment for sampling design and controlling for potential confounders (race, body mass index [BMI], socio-economic status and age at time of participation). Girls with evidence of vitamin D deficiency (serum 25OHD <50nmol/L) were nearly twice as likely to report early AOM compared with the non-vitamin D deficient population (OR: 1.89; 95% CI 1.04-3.41). On multivariable analyses, however, this association between vitamin D deficiency and early AOM no longer held after controlling for age at interview, race/ethnicity, BMI, and poverty income ratio. Racial and BMI related predisposition to early AOM were evident. There was no interaction between vitamin D and the covariates of interest with the outcome AOM. Overall, in a representative sample of the US population, vitamin D status was not associated with AOM after adjusting for potential confounders.

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BACKGROUND

With adolescents in the United States reaching puberty at an earlier age, understanding factors that influence the onset of menstruation is critical in learning how to adjust pubertal timing (Chumela et al. 2003). According to existing research, most girls in the U.S. are menstruating by 13.8 years of age; with a median of 12.4 years, and only less than 10% reach menarche before age 11 (Chumela et al. 2003). Beyond the onset of sexual maturation, age of menarche (AOM) represents other characteristics of a population, including nutritional status and environmental factors (Chumela et al. 2003).

Younger AOM has been shown to be associated with a number of adverse health outcomes, including increased risk of cardiovascular disease, cardiovascular disease mortality (Lakshman et al. 2009), overall mortality, ischemic heart disease, stroke mortality (Jacobsen et al. 2009), and type 2 diabetes (Lakshman et al. 2008; He et al. 2010). On the other hand, late AOM may also have adverse implications, particularly for bone health. During puberty, bone development is at its peak as the bones lengthen and accrues mineral deposition and gains in bone density (Gilsanz 2011). Women with later onset of menarche have demonstrated significantly decreased peak bone mass (Armamento-Villareal R et al. 1992), and bone mass and bone density at skeletal maturity exhibit an inverse relationship with pubertal timing in healthy adolescents (Gilsanz et al. 2011). Later AOM has also been associated with increased fracture risk (Cooper & Sandler 1997). It is thus important to understand modifiable factors that are associated with pubertal timing so as to optimize health in the adolescence period that may potentially promote the healthiest age for onset of menstruation.

Although prior epidemiological studies have suggested an association between some lifestyle factors that are recognized to relate to vitamin D status (e.g. body mass, physical

activity) with AOM, the existing data have almost entirely focused on vitamin D-related variables, rather than vitamin D itself (Chew & Harris 2013). The only study that has directly examined a relationship between circulating vitamin D levels and AOM is a prospective cohort study of pre-menarcheal girls (n=242), conducted in Bogota, Columbia. The investigators found that girls with evidence of vitamin D deficiency (25OHD<50 nmol/L) were twice as likely to reach menarche during the observation period of 30 months compared with girls who were vitamin D sufficient (Villamor et al. 2011). Despite a burgeoning interest within the US in health implications of vitamin D in general, association between lifestyle factors and AOM in the US population remains hitherto unexplored.

The purpose of this study therefore is to examine the association between vitamin D status, as reflected by serum levels of its metabolite 25-hydroxy vitamin D (25OHD), with AOM in adolescent and young reproductive age females in the United States, using a nationally representative population sample enrolled in the National Health and Nutrition Examination Survey (NHANES). Ours is the first study to examine an association between AOM and serum 25OHD levels in the U.S. population.

METHODS

Study Participants

The National Center for Health Statistics of the Centers for Disease Control and Prevention (CDC) conducts the multistage, complex cluster design, cross-sectional NHANES survey each year to provide a nationally representative snapshot of the health and nutritional status of children and adults in the United States (CDC 2016). Comprehensive health information such as smoking habits, alcohol consumption, sexual practices, drug use, physical activity, weight, and dietary intake is collected on approximately 5,000 respondents each year. This information is analyzed to determine the prevalence and risk factors for chronic diseases in the United States. Information on demographics and health-related behaviors is collected during an initial interview in the participants' homes. Following the interview, a physical examination is conducted in the Mobile Examination Center (MEC) to obtain physiological measurements and conduct a laboratory evaluation.

Data from five waves of the NHANES survey for which information on laboratory values for vitamin D and information on reproductive health variables were available, 2001-2002, 2003-2004, 2005-2006, 2007-2008, 2009-2010, were aggregately analyzed for this study. Data prior to 2001 and after 2010 do not contain information for vitamin D, and reproductive health data are not publicly available for the most recent, 2013-2014, dataset. Low-income persons, adolescents 12-19 years old, persons over 60 years of age, African Americans, and Mexican-Americans are oversampled in these survey cycles. Our analyses are restricted to females between ages 12 and 19 years at the time of screening in an attempt to reduce potential for recall bias from inclusion of older populations, and to evaluate exposures closer to the AOM of survey respondents. Females were considered for inclusion in the study if they had already reached menarche, and

had complete information for AOM (n=4,075). Serum 25OHD laboratory values (nmol/L) were available for 3,572 (88%) (Figure 1).

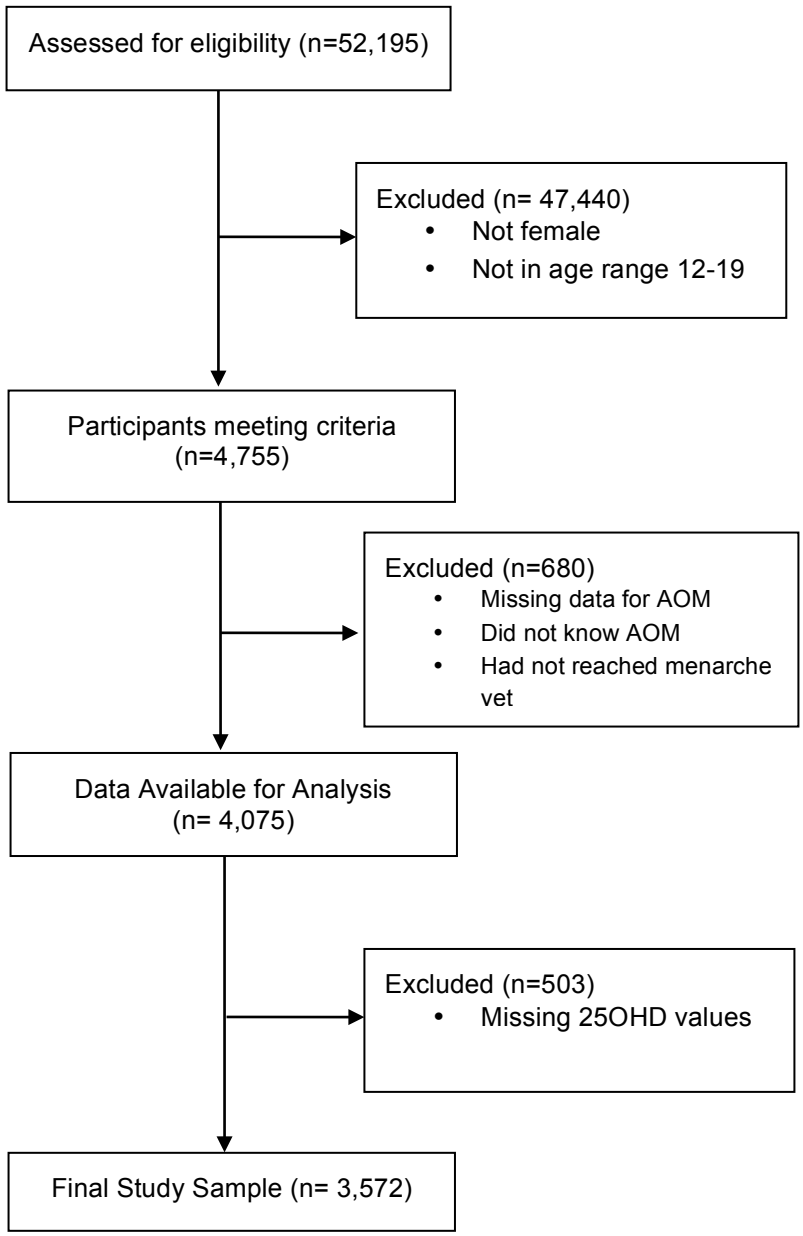

Figure 1. Study Sample Selection

Assessment of Vitamin D Status

Vitamin D status can be reliably determined by serum 25OHD levels. Circulating 25OHD primarily reflects endogenously synthesized vitamin D in the skin following sun exposure, with lesser contributions from the diet (Heaney 2011). Both vitamin D2, from plants, and vitamin D3, from animals, are represented in 25OHD values. In NHANES, serum 25OHD levels were assessed in participants aged one and older who were examined in the Mobile Examination Center (MEC). In accordance with the Endocrine Society's classification, vitamin D status was categorized as follows: deficient (<50), insufficient (50-<75), and normal (≥75); for analyses, normal vitamin D status was taken as the referent group. For conversion from SI (nmol/L) units to conventional units (ng/ml), values were divided by 2.5.

Assessment of Age of Menarche (AOM)

All female participants aged 12 and older were asked the question, "How old were you when you had your first menstrual period?" Anyone who responded that they had not begun menstruating, did not know, or refused to answer the question was excluded from analysis. The AOM was available in whole numbers, ranging from 7 to 17. For analysis, AOM is categorized as: early (7-9 years), normal (10-13 years), and late (≥14 years); for analyses, normal AOM was taken as the referent group.

Covariates

Potential confounders for the relationship between AOM and vitamin D were identified from the literature and include: age at screening (continuous in years), race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American/other Hispanic, other), poverty income

ratio (PIR: ratio of income to the family's appropriate poverty threshold, dichotomized as <1 for extreme poverty and ≥1 for above the poverty threshold), and BMI (continuous in kg/m^2). Earlier studies concluded that non-Hispanic black and Mexican-American girls attained menarche at a significantly earlier age than non-Hispanic white girls, signifying that race is associated with our outcome of interest (Chumela et al. 2003). Race is also associated with our exposure of interest (vitamin D level), as vitamin D insufficiency is more prevalent among non-Hispanic blacks and Mexican-Americans as compared with Americans of other races and ethnicities (Harris 2006). Socioeconomic status has been found to be positively associated with both AOM and vitamin D levels (James-Todd et al. 2010; Weishaar & Vergili 2012) deeming its inclusion as a covariate in multivariable analyses. BMI was controlled for as it has been shown to exhibit an inverse relationship with both AOM (Al-Awadhi et al. 2013; Mohamad et al. 2013), and with serum 25OHD levels (Lagunova et al. 2009).

Data Analysis

The analysis for this study proceeded in four steps. First, descriptive statistics were used to characterize the sample by categorized vitamin D status and AOM (Tables 1 and 2, respectively). Distribution for continuous variables (age, BMI, serum 25OHD level) was assessed and geometric means were computed using PROC SURVEYMEANS, while design-adjusted chi-square analysis (PROC SURVEYFREQ) was used to calculate associations between categorical demographic factors (race and PIR) and AOM, and to assess differences in frequencies across categories of AOM (early, normal, and late) and of vitamin D status (normal, insufficiency, and deficiency). For unadjusted associations between categorized outcome variables and sample characteristics, a parametric analysis of variance (ANOVA) F-test was used for continuous variables, while the Pearson's chi-square test for independence was used for

categorical variables. Second, multivariate linear regression was conducted with AOM and vitamin D as continuous variables to test for trend, using PROC SURVEYREG. Corresponding coefficients are discussed in the results.

Third, to estimate adjusted and unadjusted odds ratios (OR) and corresponding confidence intervals for the magnitude of association between categorized AOM and vitamin D level, logistic regression analyses were conducted. PROC SURVEYLOGISTIC was used to determine whether vitamin D levels affected the probability of early or late AOM (normal AOM was taken as the referent group). A multinomial logistic regression compared the independent association of vitamin D status with AOM (early, normal, and late) after controlling for race/ethnicity, BMI, age at screening, and PIR. Table 4 shows results for the unadjusted regression, Table 5 lists OR for adjusted analyses.

Lastly, to examine if any association between AOM and vitamin D was modified by BMI, race, or PIR, interaction terms of vitamin D and each of these covariates were included in separate logistic regression models. All analyses were conducted through survey procedures to account for the weighted sample in SAS 9.4 (SAS Institute, Cary, NC). To account for the complex survey design, non-response, oversampling, and post-stratification of NHANES, weights were incorporated to ensure that data is representative of the U.S. civilian non-institutionalized population. As mentioned by the CDC's National Health Center for Statistics (2013), the sample weight assigned to each sample person is a measure of the number of people in the population represented by that individual. As 10 survey years of NHANES were included in this analysis, a weight was constructed to combine weights across all five survey cycles, and thus all presented results are based on the weighted sample. Statistical significance was defined by an alpha of <0.05.

RESULTS

Characteristics of the study sample by vitamin D status are presented in Table 1. The final study sample was composed of 3,572 adolescent and young adult females between the ages of 12 and 19 in the 2001-2010 NHANES. The mean age of the sample was 15.6±0.1 years, with mean BMI of 23.9±0.1 kg/m^2; average AOM was 12.1 years. Approximately 31% of the population was vitamin D deficient; another 42% demonstrated vitamin D insufficiency, with the remaining 27% exhibiting normal vitamin D status. Vitamin D deficiency was greatest in non-Hispanic blacks (38.0% vs. 25.1% in non-Hispanic whites and 28.2% in Mexican-Americans). Multivariate linear regression analyses showed no relationship between serum 25OHD levels with AOM (β=0.007, $p<0.001$).

Age of Menarche (AOM)

Table 2 presents characteristics of the study sample by categorical AOM. In the weighted sample, early and late AOM was reported by 2.7% and 13.2% respectively. Serum 25OHD levels were the lowest in the group reporting early AOM, and were the highest for those with late AOM ($p<0.001$). The prevalence of vitamin D deficiency was highest amongst the early AOM and the lowest for the late AOM group (41.6% versus 24.3%, $p<0.001$); conversely, prevalence of normal vitamin D status was highest in the late AOM and least in the early AOM population (36.9% versus 18.2%, $p<0.001$). Table 4 presents results of multinomial univariate logistic regression analyses demonstrating associations between vitamin D and the covariates with AOM, presented as OR (95% CI). On unadjusted analyses, females who were vitamin D deficient had nearly twice the odds of early AOM compared with those with normal levels of vitamin D (OR 1.89, 95% CI 1.04-3.41).

Racial and ethnic differential in AOM was noted. Both Mexican-Americans and non-Hispanic blacks were three times more likely to have experienced early AOM compared with non-Hispanic whites (Table 4), and the Mexican-American population was significantly less likely to experience late AOM compared with the Non-Hispanic whites. Each unit increase in BMI was associated with an 11% increased odds of early AOM (OR 1.11, 95% CI 1.08-1.14).

Results from multivariable multinomial logistic regression analysis are presented in Table 5. The directionality of relationship between vitamin D deficiency and early AOM observed on univariate analyses (OR: 1.89, 95% CI 1.04-3.41) changed after controlling for age at screening, race/ethnicity, BMI, and PIR (OR: 0.69, 95% CI 0.28-1.70). There was no evidence of interaction between vitamin D and BMI, race, or PIR for the outcome of AOM.

DISCUSSION

Our analyses of vitamin D status and AOM among adolescents and young adult women identify BMI and race/ethnicity as independent predictors of AOM. While individuals with evidence of vitamin D deficiency were significantly more likely to report early AOM, this relationship did not hold after controlling for BMI, race/ethnicity, SES and age at screening. Our data therefore identify confounding influence of race/ethnicity and BMI for association between vitamin D status and AOM. Relationship between higher BMI and early AOM is well described (Al-Awadhi et al. 2013; Mohamad et al. 2013) and this impression was corroborated in our study.

The cross-sectional design of NHANES is a limitation that restricts our ability to establish causality and temporality of observed associations. Although AOM was self-reported, however, recall bias was minimized in our study by age restriction from 12 to 19 year olds. The

greatest strength of this analysis includes the use of NHANES, which incorporates a large sample size representative of the national population over a span of a decade. The only other study looking at vitamin D in relation to AOM is Villamor et al.'s prospective cohort study conducted in Bogota, Columbia, making our analysis the second to directly examine the association between vitamin D and AOM, and the first to incorporate a national sample of the U.S. Villamor et al.'s prospective cohort study (n=242) followed girls (mean age: 8.8±1.6 years) over 2.5 years and found that 57% of vitamin D deficient girls reached menarche during follow-up compared with only 23% of those who were vitamin D sufficient. After adjusting for baseline age and BMI, investigators found that the probability of menarche for vitamin D deficient girls was two times higher than the probability for vitamin D sufficient girls (p=0.04).

The difference between our null results and the outcomes of Villamor et al. may be attributed to racial and underlying genetic heterogeneity in the two populations. While categorization of AOM may have limited our study power to detect relationship between vitamin D deficiency with early and late AOM (given that only 2.7% and 13.2% of the sample reported early and late AOM respectively), we reexamined this association between vitamin D and AOM utilizing multivariable linear regression analysis that exhibited a similar non-statistically significant trend in relating lower vitamin D levels with earlier AOM result after adjustment for potential confounders (data not shown); thus small sample sizes in the extreme AOM groups are unlikely to explain the discrepancy between our results and those reported by Villamor et al.

CONCLUSION

In a nationally representative sample of adolescent and early adult females of ages between 12 and 19 years from the 2001-2010 NHANES, vitamin D status at time of screening did not relate to AOM. Although females who were vitamin D deficient (25OHD<50nmol/L) were more likely to report AOM at or before 9 years of age, this relationship disappeared after controlling for age at screening, race/ethnicity, BMI and SES. Future research on this subject should utilize a prospective approach, estimating both total and bioavailable 25OHD levels in the pediatric age predating onset of menses. Regardless of the focus of interest (AOM in this case), the importance of sufficiency for both skeletal and non-skeletal targets for the adolescents and the early adults must be kept in perspective (Alsharani & Aljohani 2013) and strategies to optimize vitamin D status encouraged to prevent chronic health disorders such as osteoporosis.

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APPENDIX

Characteristic	Total	Vitamin D Level: Deficient (<50 nmol/L)	Insufficient, (50-<75 nmol/L)	Normal (≥75 nmol/L)	p[c]
Total	3572	1768 (31.0%)	1269 (41.7%)	535 (27.4%)	
Age (years)	15.6±0.1	15.7±0.1	15.3±0.1	16.1±0.1	0.012
Age of menarche (years)	12.1±0.0	11.9±0.0	12.1±0.0	12.4±0.1	<0.001
Age of menarche (Row %)					<0.001
Early (6-9)	129 (2.7%)	72 (3.6%)	43 (2.6%)	14 (1.8%)	
Normal (10-13)	3026 (84.1%)	1503 (86.0%)	1093 (85.1%)	430 (80.4%)	
Late (≥14)	417 (13.2%)	193 (10.3%)	133 (12.3%)	91 (17.8%)	
Race/ethnicity					<0.001
Non-Hispanic white	1019 (61.2%)	136 (25.1%)	479 (68.0%)	404 (91.6%)	
Non-Hispanic black	1067 (14.6%)	864 (38.0%)	181 (6.0%)	22 (1.2%)	
Mexican-American/Other Hispanic	1336 (18.6%)	688 (28.2%)	550 (19.9%)	98 (5.8%)	
Other	150 (5.7%)	80 (8.7%)	59 (6.1%)	11 (1.5%)	
Annual household income					<0.001
<$20,000	727 (11.7%)	458 (18.2%)	211 (10.0%)	58 (6.7%)	
≥$20,000	1855 (46.8%)	879 (43.1%)	697 (51.2%)	279 (44.1%)	
Missing	990 (41.6%)	431 (38.6%)	361 (38.8%)	198 (49.2%)	
Poverty income ratio					<0.001
<1.0	1288 (26.4%)	754 (37.6%)	397 (21.8%)	137 (20.7%)	
≥1.0	2284 (73.6%)	1014 (62.4%)	872 (78.2%)	398 (79.3%)	
Body mass index (kg/m^2)	23.9±0.1	25.7±0.3	23.4±0.2	22.7±0.2	<0.001

Table 1. Sample characteristics across vitamin D categories in 2001-2010 NHANES

*Percentages are weighted based on the study sample

	Age of Menarche				
Characteristic	Total N (%)	Early (6-9 years)	Normal (10-13 years)	Late (≥14 years)	p[c]
Total	3572	129 (2.7%)	3026 (84.1%)	417 (13.2%)	
Age (years)	15.6±0.1	15.8±0.2	15.4±0.1	17.2±0.1	<0.001
Race/ethnicity					<0.001
Non-Hispanic white	1019 (61.2%)	21 (37.7%)	845 (60.5%)	153 (70.2%)	
Non-Hispanic black	1067 (14.6%)	52 (26.5%)	888 (14.4%)	127 (13.1%)	
Mexican-American/Other Hispanic	1336 (18.6%)	50 (31.9%)	1163 (19.1%)	123 (12.5%)	
Other	150 (5.7%)	6 (4.0%)	130 (5.9%)	14 (4.2%)	
Annual household income					
<$20,000	727 (11.7%)	31 (13.0%)	591 (11.4%)	105 (13.1%)	0.555
≥$20,000	1855 (46.8%)	60 (40.5%)	1599 (47.6%)	196 (42.9%)	
Missing	990 (41.6%)	38 (46.5%)	836 (41.0%)	116 (44.0%)	
Poverty income ratio					
<1.0	1288 (26.4%)	56 (36.6%)	1060 (25.6%)	172 (29.4%)	0.138
≥1.0	2284 (73.6%)	73 (63.4%)	1966 (74.4%)	245 (70.6%)	
Body mass index (kg/m^2)	23.9±0.1	28.8±0.8	23.8±0.2	23.6±0.4	0.004
Vitamin D (nmol/L)	63.6±1.0	55.1±2.4	62.9±1.0	70.0±2.1	<0.001
Vitamin D					
Deficient (<50 nmol/L)	1768 (31.0%)	72 (41.6%)	1503 (31.7%)	193 (24.3%)	<0.001
Insufficient (50-<75 nmol/L)	1269 (41.7%)	43 (40.2%)	1093 (42.2%)	133 (38.8%)	
Normal (≥75 nmol/L)	535 (27.4%)	14 (18.2%)	430 (26.1%)	91 (36.9%)	

Table 2. Sample characteristics across age of menarche categories in 2001-2010 NHANES

*Percentages are based on the weighted study sample

Characteristic	Early (<9 years)	Normal (10-13 years)	Late (≥14 years)
Vitamin D			
Deficient (<50 nmol/L)	**1.89** (1.04, 3.41)**	1.00	**0.54** (0.40, 0.74**)
Insufficient (50-<75 nmol/L)	1.37 (0.76, 2.47)	1.00	**0.65** (0.48, 0.89)**
Normal (≥75 nmol/L)	1.00	1.00	1.00
BMI (kg/m^2)	**1.11** (1.08, 1.14)**	1.00	0.99 (0.96, 1.03)
Race/ethnicity			
Non-Hispanic white	1.00	1.00	1.00
Non-Hispanic black	**2.95** (1.68, 5.17)**	1.00	0.78 (0.58, 1.06)
Mexican-American	**2.68** (1.31, 5.51)**	1.00	**0.56** (0.40, 0.78)**
Other	1.07 (0.34, 3.42)	1.00	0.61 (0.28, 1.34)
Age (years)	1.10 (0.99, 1.22)	1.00	**1.61** (1.49, 1.74)**
Poverty income ratio			
<1.0	1.68 (0.98, 2.88)	1.00	1.21 (0.85, 1.71)
>=1.0	1.00	1.00	1.00

Table 3. Association of covariates and age of menarche (unadjusted), NHANES, 2001-2010

** = significant at p<0.05

Characteristic	Early (<9 years)	Normal (10-13 years)	Late (≥14 years)
Vitamin D			
Deficient (<50 nmol/L)	0.69 (0.28, 1.70)	1.00	0.78 (0.49, 1.23)
Insufficient (50-<75 nmol/L)	1.09 (0.44, 2.71)	1.00	1.05 (0.71, 1.54)
Normal (≥75 nmol/L)	1.00	1.00	1.00
BMI (kg/m^2)	**1.10** (1.07, 1.14)**	1.00	0.97 (0.94, 1.00)
Race/ethnicity			
Non-Hispanic white	1.00	1.00	1.00
Non-Hispanic black	**2.93** (1.45, 5.91)**	1.00	1.05 (0.71, 1.55)
Mexican-American	**2.95** (1.34, 6.51)**	1.00	**0.63** (0.44, 0.91)**
Other	1.60 (0.49, 5.21)	1.00	0.71 (0.31, 1.64)
Age (years)	1.02 (0.92, 1.14)	1.00	**1.66** (1.52, 1.81)**
Poverty income ratio			
<1.0	1.06 (0.62, 1.81)	1.00	0.93 (0.64, 1.36)
>=1.0	1.00	1.00	1.00

Table 4. Association of covariates and age of menarche (adjusted), NHANES, 2001-2010

Note: adjusted for vitamin D, race, age, BMI, PIR
** = significant at p<0.05