Changes in Fasting Lipids during Puberty

Mona A. Eissa, MD, MPH, PhD[1], Nicole L. Mihalopoulos, MD, MPH[2], Richard Holubkov, PhD[2], Shifan Dai, MD, PhD[3], and Darwin R. Labarthe, MD, MPH, PhD[4]

Objective To describe longitudinal changes in plasma lipid levels and pubertal stage in youths from age 8-18 years, in Project HeartBeat!

Study design Fasting blood samples and pubertal stage, using physical assessment of secondary sex characteristics, were obtained every 4 months for up to 4 years in a mixed longitudinal study of 633 children (49.1% female, 20.1% black), initially aged 8, 11, and 14 years. Total cholesterol, low density lipoprotein-cholesterol, high density lipoprotein-cholesterol, triglycerides (TG), and nonhigh density lipoprotein-cholesterol measurements were obtained. Data were collected from 1991-1995.

Results Pubertal stage correlations with age varied among all race-sex groups (range, r = 0.61-0.70), and a given pubertal stage could represent a range of 5 years or more of chronological age. Throughout puberty, levels of total cholesterol, low density lipoprotein-cholesterol, and nonhigh density lipoprotein-cholesterol decreased, TG in males increased, and high density lipoprotein-cholesterol and TG in females showed no changes. Within a given pubertal stage, plasma lipid levels tended to differ by race, sex, or both.

Conclusions Lipid levels change markedly by pubertal stage, and patterns differ by sex and race. Chronological age ranges widely within a given pubertal stage and is an insensitive indicator of pubertal stage and the related changes in lipid levels. Pubertal development should be considered when determining screening criteria to identify youths with adverse blood lipid levels. *(J Pediatr 2016;170:199-205)*.

See editorial, p 7 and related article, p 315

Adverse levels of blood lipids constitute a well-established cardiovascular disease risk factor.[1] Studies in children and young adults have documented tracking of lipid levels over time,[2-4] and their association with early atherosclerotic lesions.[5-7] Several cross-sectional and longitudinal studies have described the changes of lipid levels by age during childhood and adolescence[8-21]; fewer studies have taken pubertal stage into account.[15-21] Recently, the Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents recommended universal screening with nonfasting nonhigh density lipoprotein-cholesterol (non-HDL-C) at ages 9-11 and 17-21 years.[22] These ages are within the time of the greatest changes of lipid levels, after infancy, where hormonal changes and associated physical and sexual maturation occur.[23] The 9- to 11-year-old age group has the greatest variation in sexual maturation.[24]

Children of the same age, sex, and race vary considerably in their degree of sexual and somatic maturation. The influence of hormonal changes associated with puberty on lipid levels is documented, and several studies have described lipid levels during these pubertal changes.[17,19,20,25,26] However, the pattern of changes of blood lipid components varied among these studies and changes of non-HDL-C were not described. These data are derived from studies where sexual maturation was assessed once in cross-sectional studies and annually or semiannually, at most, in longitudinal studies. Sexual maturation data were based on the age of menarche, self-assessment using photographs for pubertal stage, or by physical examination. Observations have commonly been reported in terms of 2 or 3 categories (eg, early, middle, and late puberty). This report describes the changes in blood lipid levels (total cholesterol [TC], low density lipoprotein-cholesterol [LDL-C], high density lipoprotein-cholesterol [HDL-C], and non-HDL-C, and triglycerides [TG]) in relation to changes in pubertal stage during puberty in a primarily normal weight cohort of black and nonblack youths, aged 8-18 years. Pubertal staging, using physical assessment of secondary sex characteristics, was obtained every 4 months for up to 4 years in 633 participants.

BMI	Body mass index
HDL-C	High density lipoprotein-cholesterol
LDL-C	Low density lipoprotein-cholesterol
non-HDL-C	Nonhigh density lipoprotein-cholesterol
TC	Total cholesterol
TG	Triglycerides

From the [1]Department of Pediatrics, The University of Texas Medical School, Houston, TX; [2]Department of Pediatrics, University of Utah, Salt Lake City, UT; [3]Division of Nutrition, Physical Activity, and Obesity, Centers for Disease Control and Prevention, Atlanta, GA; and [4]The University of Northwestern Feinberg School of Medicine, Chicago, IL

Supported by the National Heart, Lung, and Blood Institute (through Cooperative Agreement U01-HL-41166) and the Centers for Disease Control and Prevention through the Southwest Center for prevention Research (U48/CCU609653). N.M. was supported by the National Heart, Lung, and Blood Institute (HL#092069). The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2015.11.018

Methods

Project HeartBeat! was designed to evaluate dynamics of change in cardiovascular disease risk factors among children and adolescents. Design and methods of the study have been described in detail elsewhere.[27,28] Briefly, 3 cohorts of children, initially aged 8, 11, and 14 years, were enrolled between October 1991 and July 1993 from The Woodlands and Conroe, Texas. The total study sample consisted of 678 participants: 49.1% female, 74.6% white, 20.1% black, and 5.3% other (Hispanic, Asian, and American Indian). For data analysis, race/ethnicity was categorized as black or nonblack based on questionnaire responses provided by the participants' parents because separate analysis for Hispanic, Asian, and American Indian respondents was not advisable because of small sample size. A participant's exact age was calculated on each occasion of data collection. Participants were examined 3 times per year through August 1995 (mean number of 8.3 examinations per participant). Because the study design included overlapping ages between cohorts, it was possible to estimate a consecutive 10-year developmental pattern (for ages 8-18 years) over the period of 4 years. For the primary analysis, 633 participants who had pubertal stage, lipids, and body mass index (BMI) z-score for at least 1 encounter were included. Analysis excluded observations when age and sex specific BMI was ≥95th percentile (BMI z-score of 1.645) leaving 587 participants and a total of 4229 observations. Secondary analysis was performed excluding all participants who ever had BMI ≥95th percentile leaving 533 participants and 3986 observations. The differences between lipid levels calculated in the primary and secondary analyses were compared at each pubertal stage for all sex-race groups. Clinically important difference of lipid levels was arbitrarily set to be equal or greater than 3 mg/dL. The study protocol was approved by the institutional review boards of the University of Texas at Houston Health Science Center and of Baylor College of Medicine; the University of Utah gave an exemption to the current secondary data analysis. For each participant, informed consent or assent and parental consent were obtained.

Plasma lipid concentrations were determined in the Lipid Research Laboratory of Baylor College of Medicine. At each examination, the participant's blood was drawn, after an overnight fast, into powdered ethylenediaminetetraacetic acid-containing tubes by a trained phlebotomist at the participant's home. The blood was kept at 4°C and was separated within 1 hour of collection. Aliquots were held at −70°C until laboratory testing. TC, HDL-C, and TG levels were determined using standard enzymatic methods.[29,30] The Cobas Fara II analyzer (Roche Diagnostics, Switzerland) was used for the determination. Standards of performance for the inter- and intra-assay required that coefficients of variation not exceed 3%. LDL-C was calculated as LDL-C = (TC − [TG/5 + HDL-C]).[31] Non-HDL-C was calculated as non-HDL-C = TC − HDL-C.[32]

Physical assessment of secondary sex characteristics, was performed through direct visual assessment of pubic hair and breast or testicular volume/scrotal texture/penile growth according to the method of Tanner,[33,34] based on earlier work by Reynolds and Wines.[35,36] The Project HeartBeat! protocol was implemented under evaluation by James M. Tanner, co-investigator on the project. The observed pubertal stage ranged from 1 (prepubescent) to 5 (adult) for each characteristic. For this report, we used breast staging for females and genital staging for males to reflect pubertal development.

Statistical Analyses

We fit linear mixed models of serum lipids on pubertal stage, sex, and race using SAS Proc Mixed (SAS Institute Inc, Cary, North Carolina)[37] to account for repeated measurements across visits for the same subject. We report least squares means and SEs derived from the fitted models. A heterogeneous variance autoregressive covariance structure was found to be appropriate for all measurements considered in this report. For each measurement, we considered main effects and 2-way interactions for sex, race (black vs nonblack), and pubertal stage treated as a 5-level categorical variable. We retained interactions statistically significant at $\alpha = 0.05$ level in final models along with the corresponding main effects. The interaction pubertal stage-race was not significant for any model, which may be due to smaller numbers of black females. Notably, after the addition of pubertal stage to stepwise models, age at examination was no longer significant.

Concordance between breast/genital and pubic hair staging was 81.2%, with the highest concordance at pubertal stage 1 and pubertal stage 5 (95.3% and 92.4%, respectively). The correlation of age with pubertal stage, within the same individual observed over time because there are repeated measurements for each individual over time, was calculated using the approach of Bland and Altman.[38] Correlation calculations were also performed stratifying by sex and race.

We adjusted the type 1 error using Bonferroni correction, where $\alpha = 0.05$ was divided by the number of tests ($0.05/225 = 0.00022$), and the level of significance was conservatively set to 0.0001. We arrived at 225 tests by conducting 5 pair wise comparisons between 4 race-sex categories at each of the 5 time points (pubertal stage) for each of the 5 outcomes (lipid components). For each race-sex category, we compared means at adjacent pubertal stage and between pubertal stage 1 and pubertal stage 5. Thus, the total number of comparisons is $5 \times [(5 \times 5) + (4 \times 5)] = 225$.

Results

Table I addresses the composition of the study population and the relation between pubertal stage and chronological age. For every race-sex group, pubertal changes started in some participants before age 9 years; within each group other than black males, some individuals completed puberty before age 12 years. In each group, the majority of participants attained pubertal stage 5 by age 17 years. Correlation coefficients for age and pubertal stage were 0.65