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Effects of Apolipoprotein E Genotype on Blood Cholesterol in Adolescent Girls

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Abstract

Background—Few investigations have examined whether associations between the apolipoprotein E genotype (apo E) and total cholesterol or LDL-C are modified or explained by other characteristics. The objective of this study was to explore effects of behavioral characteristics, physical growth, body composition, sexual maturation, and endocrine function on age trajectories of total cholesterol and LDL-C by apo E in adolescent girls.

Methods—Participants were 247 Caucasian adolescent girls followed for 4 years. Apo E genotyping and plasma lipid concentrations were determined from fasting blood samples using standard enzymatic methods. Age; gender; fat-free mass (FFM); BMI; percent body fat (PBF); sexual maturation (pubic hair, Tanner Stages 1–5); estradiol concentration (EST); energy intake; and physical activity were collected or calculated with standard methods.

Results—In models including the proposed explanatory variables, apo E genotype remained strongly associated with total cholesterol and LDL-C. Girls with the epsilon (ϵ)3/3 and ϵ 3/4 genotypes (where ϵ is the protein isoform of the apo E gene), relative to those with ϵ 2/3, had total cholesterol and LDL-C values 16–23 mg/dL higher throughout adolescence. Age–apo E interaction terms remained significant. FFM, BMI, PBF, pubic-hair stage, and EST showed a significant effect on total cholesterol and LDL-C. When the combination of pubic-hair stage, EST, and one of FFM, BMI, and PBF was included in total cholesterol or LDL-C models, only EST was significant.

Conclusions—Adolescent girls with ϵ 3/3 and ϵ 3/4 genotypes had higher total cholesterol and LDL-C and showed different patterns of change, compared to those with ϵ 2/3 genotype. These apo E effects were independent of behavioral characteristics, physical growth, body composition, sexual maturation, and endocrine function. Girls with ϵ 3/3 or ϵ 3/4 genotypes may be at risk for elevated total cholesterol and LDL-C later in life.

Introduction

Apolipoprotein E (apo E) plays a major role in chylomicron remnant, low-density lipoprotein, and high-density lipoprotein metabolism. In humans, apo E exhibits three protein isoforms ($\epsilon 2$, $\epsilon 3$, and $\epsilon 4$) encoded by three haploalleles at codon positions 112 and 158.¹ Relative to individuals with an $\epsilon 3$ allele, the presence of an $\epsilon 4$ allele has been associated with development of coronary heart disease in adults^{2,3} and the presence of atherosclerotic lesions in young people.⁴ Presence of the $\epsilon 4$ allele also has been associated with altered total cholesterol and LDL cholesterol (LDL-C) levels in adults^{5,6} and children.⁷⁻⁹ Individuals with an $\epsilon 2$ allele tend to have lower total cholesterol and LDL-C levels; those with an $\epsilon 4$ allele tend to have higher levels.¹⁰

Apolipoprotein E also appears to affect the pattern of change in lipoproteins with age. Studies of youth^{11,12} and adults¹³ show that those with genotypes $\epsilon 3/3$ or $\epsilon 3/4$ (compared with $\epsilon 2/3$) exhibit different patterns of change in total cholesterol,¹¹ LDL-C,^{11,12} high-density lipoprotein cholesterol (HDL-C),¹² and triglycerides.¹³ Although an apo E-specific pattern of longitudinal change in lipids has been described, few investigators have examined the extent to which associations with apo E can be modified or explained by other characteristics.

In a longitudinal study of girls (aged 8–18 years), it was previously observed that the absolute values and the patterns of change in total cholesterol and LDL-C varied by apo E genotype.¹¹ It was suggested that reproductive hormones, body composition, and sexual maturation may play roles in cholesterol changes during adolescence. The current analysis explores these issues by examining the associations of behavioral characteristics, physical growth, body composition, sexual maturation, and endocrine function on changes in total and LDL-C by apo E genotype in girls aged 8–18 years. It was hypothesized that the associations previously observed of apo E genotype and age with total cholesterol and LDL-C are independent of behavioral characteristics, physical growth, body composition, sexual maturation, and endocrine function. This analysis is important and contributory as it seeks to determine the independent contributions of the apo E genotype, behavioral measures of energy intake and physical activity, and characteristics of growth and sexual maturation on changes in cholesterol values in adolescent girls.

Methods

Sample

Project HeartBeat! is a longitudinal study of cardiovascular disease (CVD) risk factor development in children and adolescents. To assess the natural course of development of CVD risk factors, three cohorts of children were observed from October 1991 until August 1995. The cohorts were aged 8, 11, and 14 years at study entry. Extensive assessments of the CVD risk factors, including lipoprotein determinations, were conducted three times per year at 4-month intervals. The target population consisted of residents of The Woodlands and Conroe TX. Households were invited to visit the project's field center for a tour and recruitment interview. A total of 678 children enrolled in the project. The complete design and methods of Project HeartBeat! have been described previously.^{14,15}

The DNA analyses in Project HeartBeat! were restricted to female participants. The population for analysis consisted of 247 Caucasian girls who underwent baseline examinations. None had missing lipid data over time or excessively high (>250 mg/dL) total cholesterol levels at baseline. Informed consent and parental consent were documented for each participant and approved by The University of Texas at Houston and the CDC Committee for the Protection of Human Subjects.

Laboratory Methods

Lipid concentrations were determined in plasma after an overnight fast using standard enzymatic methods.^{16–18} Samples were obtained and processed as previously reported¹⁵ at the Lipid Research Laboratory at Baylor College of Medicine. A Cobas Fara II analyzer was used for the enzymatic process of cholesterol determination. Values are reported in mg/dL. LDL-C was calculated using the equation: $\text{LDL-C} = [\text{total cholesterol} - (\text{triglycerides}/5 + \text{HDL-C})]$.¹⁶

Apolipoprotein E genotyping was carried out following polymerase chain reaction (PCR)–assisted amplification of a region of the apo E gene, using the primers described by Emi et al.¹⁹ and digestion with HhaI.²⁰ Following polyacrylamide gel electrophoresis and ethidium bromide staining, the gels were scored independently by two laboratory personnel.

Estradiol concentration (EST, ng/ml) is a marker for female gonadal activity and was determined from 100 micro-liters of serum analyzed in duplicate by ¹²⁵I radioimmunoassay designed for in vitro use. The inter-assay and intra-assay coefficients of variation for the kit were 11.9% and 10.6%, respectively.

Sexual Maturation

Three times per year at 4-month intervals, trained observers visually categorized each participant's sexual maturation stage (Tanner stage,^{21,22} based on the work of Reynolds and Wines^{23,24}) between Stages 1 (prepubertal) and 5 (adult). Assessments were based on genitalia and pubic-hair development for boys and breast and pubic-hair development for girls. Staging of secondary sexual characteristics has been well described and is a simple and straightforward means to classify the sexual development of children and adolescents on the basis of a 5-point scale.^{21,22} Pubic-hair stage was used in the analysis to estimate stage of sexual maturation, as it was the measure collected on both boys and girls.

Body Mass Index

Three times per year at 4-month intervals, anthropometric measurements were obtained for each participant by two trained and certified observers working together using standardized protocols.^{25,26} Weight was measured using a beam-balance scale to the nearest 0.1 kg; height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. BMI was calculated by standard formula (kg/m^2).

Percent Body Fat (PBF)

Bioelectric impedance and anthropometric measurements were used to estimate PBF. Bioelectric impedance assessments were obtained every 4 months following standardized

procedures using an RJL Systems bioelectric impedance analyzer BIA-101-A to measure reactance and resistance.²⁷ PBF was calculated indirectly by a formula²⁸ for girls using resistance; anthropometry (weight, height); and lateral calf, triceps, and subscapular skinfold measurements: Fat-free mass (FFM [kg]) = $4.3383 + 0.6819 (\text{weight, kg}) - 0.1846 (\text{lateral calf skinfold, mm}) - 0.2436 (\text{triceps skinfold, mm}) - 0.2018 (\text{subscapular skinfold, mm}) + 0.1822 (\text{height}^2 [\text{cm}^2] / \text{resistance} [\text{ohms}])$.

Energy Intake

Energy intake (kcal/day) was estimated from a food-frequency questionnaire (FFQ) designed for school-aged children.²² Trained interviewers questioned participants about the frequency and quantity of 137 foods consumed during the preceding week. The food list was based on an extensive database available from prior studies of food intake among children and adults in The Woodlands TX.²² The database included important food sources of nutrients associated with CVD. Dietary interviews were conducted in the home of the participant or at the field center. The parent who was involved with food preparation was asked to be available to help participants aged <11 years. Dietary intake was assessed at baseline and yearly thereafter. Nutrient amounts were calculated with the use of nutrient and gram weight information from the U.S. Department of Agriculture Survey Nutrient Database, version 4.0, and were expressed in terms of the amount of the average daily nutrient intake consumed during the preceding week. Total energy intake was calculated as described elsewhere.²⁵

Physical Activity and Sedentary Behavior

Physical activity was assessed using a 24-hour, interviewer-administered recall questionnaire adapted from a 7 day–recall instrument modified for use with pre-adolescent children. This questionnaire has been previously validated among 3rd- and 5th-grade children; in fifth graders, correlations between the questionnaire and two validation standards ranged from 0.63 (accelerometer) to 0.72 (heart rate monitor).¹⁹ Using a segmented-day approach, trained interviewers asked participants to recall the physical activities and sedentary behaviors in which they participated during the preceding 24 hours. For each recalled physical activity, participants estimated three time segments: (1) total time spent in the activity (e.g., 2-hour baseball game); (2) time spent truly participating in the activity (e.g., 1 hour spent participating in the baseball game); and (3) time spent in vigorously intense activity (activity to make one breathe hard or sweat; e.g., 10 minutes of the baseball game). Time segments were added as a modification to the original questionnaire. Based on published estimates of physical activity energy expenditure,^{20,21} time spent in physical activities of moderate or greater intensity (i.e., 3.0 METs) defined moderate-to-vigorous physical activity (MVPA, min/day). The amount of time spent truly participating in an activity was used for analysis. As part of the questionnaire, participants also recalled time spent in three sedentary behaviors (min/day): TV viewing, reading, and computer use. Physical activity was assessed at baseline and yearly thereafter.

Statistical Analysis

Longitudinal multilevel modeling of the trajectories of total cholesterol and LDL-C by age, apo E genotype, BMI, FFM, PBF, Tanner stage, and EST was conducted using MLwiN

software.²⁹ Because of non-normal distribution, the $\log_{10}$ of EST was used in multi-level modeling analyses. To examine the additional contribution of behavioral characteristics to the models for total cholesterol and LDL-C, energy intake, sedentary behavior, and physical activity were added to the models containing the variables listed above. The MLwiN regression analysis program computed maximum likelihood estimates of the parameters for mixed linear models. To indicate significance, the parameter estimate divided by its SE was assumed to follow a t distribution, so that if the test statistic was greater than 1.96, then $p < 0.05$.

Repeated measurements of total cholesterol and LDL-C were regressed on age, age², age³, apo E genotype, age $\times$ genotype interaction terms, and FFM, BMI, PBF, Tanner stage, and EST either alone or together in all of the models presented. Age was centered first by subtracting the M from the original value before entering it into the total cholesterol and LDL-C multilevel models. The inclusion of quadratic and cubic terms of age in describing the trajectories for total cholesterol and LDL-C concentrations was considered essential, based on previous work showing that the age-related trajectories of lipid values are polyphasic from ages 8 to 18 years.^{11,15}

The hypothesis for this analysis was that the significance of the apo E and age $\times$ genotype interaction terms would remain unchanged (i.e., statistically significant) from previous findings¹¹ when the potential explanatory variables (representing behavioral characteristics, physical growth, body composition, sexual maturation, and endocrine function) were each added to the base model. Here, the base model refers to the models presented for total cholesterol and LDL-C that contain the age and genotype terms and their interactions. In separate models, then, each of the five potential explanatory variables was added to the base model, and the significances of the apo E genotype terms and the age $\times$ genotype interaction term were tested.

Results

The Ms and SDs of baseline values for age, plasma lipids and lipoproteins, body composition, energy intake, sedentary behavior, physical activity, and sexual maturation by apo E genotype are shown in Table 1. As demonstrated previously,¹¹ mean levels of total cholesterol and LDL-C were significantly different among the apo E genotypes. Mean total cholesterol and LDL-C values (mg/dL) were higher ($p < 0.001$) for genotype $\epsilon 3/4$ (165.9 mg/dL, 98.7 mg/dL) than for genotype $\epsilon 2/3$ (141.7 mg/dL, 74.6 mg/dL). Mean FFM and mean PBF also were higher ($p = 0.04$) for genotype $\epsilon 3/4$ (12.3 kg, 27.8%) than for genotype $\epsilon 2/3$ (9.7 kg, 23.9%). There were no differences ($p > 0.05$) by apo E genotype for age, BMI, energy intake, sedentary behavior, MVPA, EST, or Tanner stage.

Multilevel modeling analyses were based on the numbers of total and LDL-C determinations ranging from 1324 to 1960, depending on the combinations of variables included in different models. Multilevel longitudinal models for total cholesterol are shown in Table 2. In models including age, age², age³, apo E genotype ($\epsilon 2/3$, $\epsilon 3/3$, and $\epsilon 3/4$), age $\times$ apo E interaction terms, and one of FFM, BMI, PBF, Tanner stage, and EST, girls with $\epsilon 3/3$ genotypes had total cholesterol levels 16.09–16.91 mg/dL higher than girls with $\epsilon 2/3$ genotypes at the

centered age of 12.3 years. In the same model, girls with $\epsilon 3/4$ genotypes had total cholesterol levels 20.18–21.95 mg/dL higher (about 1 SD of the total cholesterol distribution) at age 12.3 years. When FFM; BMI; PBF; Tanner Stages 3, 4, or 5; or EST were in separate multilevel models for total cholesterol, each was significant. The apo E ($\epsilon 3/3$ and $\epsilon 3/4$) and $\text{age}^2 \times \text{apo E}$ interaction terms remained significantly associated with total cholesterol ($p < 0.05$) when each potential explanatory variable was included in the multilevel model, revealing that measures of physical growth, body composition, sexual maturation, and endocrine function did not remove the associations between apo E and the serial change in total cholesterol.

Additional models were examined (compared to the models presented) that included energy intake, sedentary behavior, and MVPA and revealed similar findings. Compared to girls with $\epsilon 2/3$ genotype, girls with either $\epsilon 3/3$ or $\epsilon 3/4$ genotype had approximately 14–18 mg/dL higher total cholesterol levels. Similar associations between apo E and total cholesterol levels were observed when breast-development stage (1–5) rather than pubic-hair development was included in the multilevel longitudinal models (data not shown).

Multilevel longitudinal models for LDL-C are shown in Table 3. In models including age; age^2 ; age^3 ; apo E genotype ($\epsilon 2/3$, $\epsilon 3/3$, and $\epsilon 3/4$); $\text{age} \times \text{apo E}$ interaction terms; and one of FFM, BMI, PBF, Tanner stage, and EST, girls with $\epsilon 3/3$ genotypes had LDL-C levels that were 18.46–18.79 mg/dL higher than girls with $\epsilon 2/3$ genotypes. Girls with $\epsilon 3/4$ genotypes had LDL-C levels of 21.81–22.87 mg/dL at the centered age of 12.3 years. Each of the potential explanatory variables (FFM, BMI, PBF, Tanner Stage 3 or 4, and EST), included separately, was significant in the multilevel models for LDL-C. The apo E terms remained significant after the potential explanatory variables (noted above) were included, except for the $\text{age}^2 \times \text{genotype}$ interaction terms in the LDL-C model with EST (coefficient for $\text{age}^2 \times \epsilon 3/3$ was 0.2108 and for age^2 by $\epsilon 3/4$ was 0.5704, $p > 0.05$). Disappearance of $\text{age}^2 \times \text{genotype}$ interaction terms in the model with EST indicates that the interaction may not be independent of EST.

Additional models were examined (compared to the models presented) that included energy intake, sedentary behavior, and MVPA and revealed similar findings. Compared to girls with $\epsilon 2/3$ genotype, girls with either $\epsilon 3/3$ or $\epsilon 3/4$ genotype had approximately 17–21 mg/dL higher LDL-C levels. Similar associations between apo E and LDL-C levels were observed when breast-development stage (1–5) rather than pubic-hair development was included in the multilevel longitudinal models (data not shown).

Multilevel models for total cholesterol and LDL-C that include the age terms, apo E genotype, $\text{age} \times \text{genotype}$ interactions, and measures of physical growth, body composition, sexual maturation, and endocrine function are shown in Table 4. When the combination of Tanner stage, EST, and one of FFM, BMI, or PBF was included in total cholesterol or LDL-C models, only EST was significant. In the LDL-C model, when EST was included, the $\text{age}^2 \times \text{apo E}$ interaction terms were no longer significant. In additional models, when energy intake, sedentary behavior, and MVPA were added to the full model, EST was no longer significant in total cholesterol and LDL-C models. This result, however, must be interpreted with caution, as the sample size was reduced by more than two thirds of the observations

because energy intake, sedentary behavior, and physical activity were assessed annually, whereas the other variables in the model were assessed three times per year. All two-way interactions between apo E and FFM, BMI, PBF, Tanner stage, or EST were not significant ($p>0.05$) in either the total cholesterol or the LDL-C model.

Discussion

This study demonstrates that the associations between apo E and changes in total cholesterol and LDL-C from ages 8 to 18 years among healthy adolescent girls are independent of behavioral characteristics, physical growth, body composition, sexual maturation, and endocrine function. When energy intake, sedentary behavior, MVPA, FFM, PBF, BMI, Tanner stage, and EST measurements were included either separately or together in multilevel longitudinal models of total cholesterol and LDL-C, the apo E genotype terms ($\epsilon 2/3$, $\epsilon 3/3$, $\epsilon 3/4$) remained significant. Girls with the $\epsilon 3/3$ and $\epsilon 3/4$ genotypes, relative to those with $\epsilon 2/3$, had total cholesterol and LDL-C values that were 16–23 mg/dL higher throughout adolescence. These findings support the hypothesis that the apo E genotype exhibits a strong, independent association with changes in total cholesterol and LDL-C during adolescence in girls.

It is difficult to compare the current findings to those from other studies, most of which^{8,9,12,13} do not control for multiple growth and developmental characteristics in a longitudinal design. Some studies^{12,13} do take into account the effects of body size or composition on changes in cholesterol values. In a longitudinal study of participants from childhood to young adulthood, investigators¹² showed that increases in adiposity lowered HDL-C in adulthood to a larger extent in $\epsilon 2$ carriers compared with $\epsilon 3$ carriers, but not in $\epsilon 4$ carriers. This finding was in contrast to an earlier study from France¹³ that found a larger increase in β lipoprotein levels and triglycerides with weight gain in adult $\epsilon 4$ carriers, compared with $\epsilon 2$ carriers. In children in that study, however, weight gain in $\epsilon 4$ carriers was marginally associated with an increase in β lipoprotein, but not triglycerides.

Differences in cholesterol levels by apo E genotype have been observed in many studies,¹⁰ although the extent to which these age-related patterns of change in cholesterol are influenced by characteristics other than apo E, to our knowledge, has not been examined. Longitudinal patterns of change in total cholesterol values in this cohort of adolescent girls has been described previously,¹⁵ showing a polyphasic pattern similar to those observed when stratified by apo E genotype. In a previous report,¹¹ it was observed that not only was there an independent effect of apo E on the age-related changes in total cholesterol and LDL-C, but also that the patterns of change with age were different for the $\epsilon 2/3$ genotype compared with the $\epsilon 3/3$ or $\epsilon 3/4$ genotypes, noting a significant $\text{age}^2 \times \text{apo E}$ interaction.

In analyses of all the hypothesized explanatory variables, when behavioral characteristics were not included in the models, EST was the only variable independently associated with age-related changes in total cholesterol and LDL-C. Increases in EST were associated with decreases in total and LDL-C. Although the addition of EST diminished the significance of the $\text{age}^2 \times \text{genotype}$ interaction terms in the multilevel model for LDL-C, it did not remove the significance of the main effect of apo E. Nonetheless, studies have found favorable

effects of part of estrogen replacement therapy (ERT)^{30–32} on lipid and heart disease outcomes, and these effects often vary by apo E genotype. In cross-sectional analyses, postmenopausal women receiving ERT had lower total cholesterol and LDL-C levels than women not on therapy.³³ In prospective studies of Finnish postmenopausal women,^{30,32} women without an $\epsilon 4$ allele who were receiving ERT showed favorable changes in atherosclerosis progression³⁰ and in total cholesterol and LDL-C levels, compared with a similar group of women not receiving ERT.³² In a cohort of U.S. women with an $\epsilon 2$ allele, women receiving ERT had higher HDL-C levels than those not on ERT.³¹

The effect of apo E genotype on cholesterol levels may be modulated by behavioral factors such as dietary intake or physical activity, although the evidence to support these hypotheses is inconclusive. In the present analyses, when energy intake, sedentary behavior, and MVPA were included in the multilevel models, the associations between apolipoprotein E and total cholesterol and LDL-C remained. In a cross-sectional analysis³⁴ among Finnish men and boys aged 9–24 years, apo E significantly influenced the effects of physical activity on total cholesterol and LDL-C levels. Physical activity lowered cholesterol levels in men with an $\epsilon 3$ allele, but it had the opposite effect in men with the $\epsilon 4/4$ genotype. In women, no associations were observed between physical activity and total cholesterol or LDL-C levels. Measures of dietary intake in this study did not account for the observed associations. Some investigators^{35,36} have shown a greater cholesterol response to a diet low in total fat, whereas others have not.³⁷ Apo $\epsilon 4$ carriers may exhibit more variation in cholesterol presumably because they are more sensitive to diet.³⁸ Using an experimental design, the apo E gene did not have a major effect on lipid levels when dietary cholesterol was increased.³⁷ In addition, in Finnish children with a family history of CVD who received 33 months of a family-based health education intervention, the effect on plasma lipids did not vary by apo E genotype.³⁹

There are at least two limitations to the current study. One is the inability to generalize the current findings beyond white, adolescent girls. In this Project HeartBeat! substudy, apo E genotyping was conducted only for girls; however, genotyping is being planned for all samples of boys and girls to examine the association between genetic variation and coronary heart disease risk factors in children. Another limitation is the lack of standardization of the timing of the EST measurement for menarcheal girls. Estradiol concentrations are known to fluctuate during the menstrual cycle; therefore, standardizing EST collection to occur systematically during the cycle (pre- or post-ovulation) would have allowed us to rule out normal EST fluctuation (among the 39% of girls that had obtained menarche) as a possible explanation for the association between EST and total cholesterol and LDL-C. Standardizing EST assessment in this manner, however, would have placed a burden on the participants that may have reduced overall study compliance.

Conclusion

The apo E effects on age-related serial changes in total cholesterol and LDL-C are independent of behavioral characteristics, physical growth, body composition, sexual maturation, and EST in healthy adolescent girls. Girls with $\epsilon 3/3$ or $\epsilon 3/4$ genotypes may be at higher risk for elevated total cholesterol and LDL-C later in life. Population screening for

apo E is not recommended because of poor predictive value when screening for atherosclerosis,¹⁰ although these genotypes may be important to identify in targeted intervention studies or in clinical practice to evaluate responsiveness to lipid-lowering therapy.

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Table 1Variables by apo E genotype,^a Project HeartBeat!, 1991–1995, M±SD unless otherwise noted

Parameter	Genotype			Total (N=247)	p-value ^b
	2/3 (n=23)	3/3 (n=157)	3/4 (n=54)		
Age (years)	10.9±2.5	10.8±2.5	11.4±2.5	10.9±2.5	0.32
Cholesterol (mg/dL)					
Total	141.7±22.0	161.6±22.2	165.9±25.1	161.0±23.8	<0.001
LDL	74.6±22.0	94.8±22.2	98.7±25.1	94.0±23.8	<0.001
HDL	51.3±12.9	50.1±10.7	49.1±11.6	49.9±11.0	0.71
Triglycerides (mg/dL)	79.5±28.8	83.4±41.6	90.6±42.1	85.4±41.2	0.44
BMI (kg/m²)	18.1±2.6	18.5±3.6	19.0±3.3	18.6±3.5	0.55
Fat mass (kg)	9.7±4.6	10.1±5.7	12.3±5.9	10.6±5.7	0.04
Fat-free mass (kg)	29.6±8.8	28.3±9.0	30.3±9.4	28.9±9.0	0.37
EST (ng/ml)	31.9±48.1	30.4±40.4	36.6±50.0	32.0±43.4	0.66
Energy intake (kcal/day)	2121.8±504.4	2140.3±670.3	2123.3±471.6	2134.3±610.8	0.98
Sedentary behavior (min/day)	118.2±109.0	129.7±106.4	158.8±103.0	135.6±106.2	0.18
MVPA (min/day)	49.4±47.5	61.8±57.5	67.5±70.5	62.0±60.0	0.52
Pubic-hair stage (%)					
1	52.2	53.5	34.8	49.3	0.48
2	21.7	19.4	28.3	21.6	
3	0.0	6.3	8.7	6.1	
4	17.4	10.4	17.4	12.7	
5	8.7	10.4	10.9	10.3	
Breast stage (%)					
1	56.5	50.3	40.4	48.8	0.41
2	13.0	19.0	19.1	18.4	
3	13.0	12.9	6.4	11.5	
4	4.3	8.2	12.8	8.8	
5	13.0	9.5	21.3	12.4	

EST, estradiol concentration; MVPA, moderate-to-vigorous physical activity

^aTwo, five, and six girls had apo E genotypes 2/2, 2/4, and 4/4, respectively. The following *n*'s were used for measures of energy intake (*n*=233); sedentary behavior (*n*=230); and MVPA (*n*=228).^b*p*-value determined from one-way ANOVA using F-ratio, except for pubic and breast stages, which were determined by chi-square tests.

Table 2

Multilevel longitudinal models for total cholesterol, Project HeartBeat!, 1991–1995

Parameter	Fat-free mass		BMI		Percent body fat		Tanner stage		EST	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Fixed parameters										
Constant	143.1	4.373	142.2	4.553	142.4	4.435	145.8	4.593	142	4.671
Age	-2.745	1.249	-5.6	1.084	-4.55	1.055	-3.139	1.232	-3.346	1.475
Age ²	-0.7929	0.2369	-0.6986	0.2332	-0.7087	0.2326	-0.8367	0.2406	-0.83	0.3524
Age ³	0.07162	0.02428	0.1212	0.0229	0.1046	0.02259	0.07564	0.02823	0.08553	0.03077
3/3 genotype	16.73	4.678	16.71	4.881	16.91	4.755	16.09	4.747	16.73	4.992
3/4 genotype	20.73	5.229	21.24	5.451	20.18	5.316	20.94	5.357	21.95	5.561
Age×3/3 genotype	0.9224 ^{ns}	1.083	0.6803 ^{ns}	1.074	0.7144 ^{ns}	1.07	0.889 ^{ns}	1.095	0.3657 ^{ns}	1.526
Age×3/4 genotype	0.2126 ^{ns}	1.218	0.2683 ^{ns}	1.203	0.4205 ^{ns}	1.203	0.029 ^{ns}	1.244	-1.094 ^{ns}	1.719
Age ² ×3/3 genotype	0.7636	0.2515	0.7283	0.2482	0.7068	0.248	0.8693	0.254	0.877	0.3559
Age ² ×3/4 genotype	0.7193	0.2774	0.6768	0.2734	0.6648	0.2735	0.8148	0.283	0.9718	0.392
Selected variable ^a	-0.5295	0.1634	1.141	0.306	0.6071	0.1096			-4.231	1.363
SV-Pubic-Hair Stage 2 ^b							-2.012 ^{ns}	1.344		
SV-Pubic-Hair Stage 3 ^b							-4.497	1.945		
SV-Pubic-Hair Stage 4 ^b							-5.134	2.316		
SV-Pubic-Hair Stage 5 ^b							-5.621	2.719		
Between-subjects variance/covariance										
Constant	370.9	40.05	411	43.44	387	41.34	372	41.61	392	43.38
Age/constant	-7.95 ^{ns}	6.114	-6.22 ^{ns}	6.301	-7.379 ^{ns}	6.148	-3.743 ^{ns}	6.151	-7.596 ^{ns}	7.069
Age	7.167	2.175	6.419	2.109	6.679	2.122	5.731	2.172	2.781	2.384
Within-subjects variance										
Error	155.9	5.601	156.1	5.545	153.6	5.52	159.1	6.02	157.7	7.005

EST, estradiol concentration; ns, not significant

^a Selected variable represents each of fat-free mass, BMI, percent body fat, Tanner stage, and EST, respectively, in corresponding models.^b Selected variable (SV) representing Tanner stage, which is Pubic-Hair Stages 2–5.

Table 3

Multilevel longitudinal models for LDL-C, Project HeartBeat!, 1991–1995

Parameter	Fat-free mass		BMI		Percent body fat		Tanner stage		EST ^d	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Fixed parameters										
Constant	72.34	3.959	71.69	4.048	71.9	3.936	74.17	4.181	71.5	4.196
Age	-3.526	1.098	-5.947	0.9408	-4.957	0.9134	-3.882	1.104	-5.252	1.352
Age ²	-0.5193	0.2069	-0.4266	0.2026	-0.443	0.202	-0.5372	0.2147	-0.132 ^{ns}	0.3248
Age ³	0.02523 ^{ns}	0.02161	0.06382	0.02025	0.04971	0.01997	0.0242 ^{ns}	0.02534	0.0165 ^{ns}	0.02824
3/3 genotype	18.46	4.236	18.48	4.34	18.58	4.221	18.77	4.325	18.79	4.484
3/4 genotype	22.46	4.733	22.85	4.847	21.81	4.719	22.87	4.882	22.79	4.996
Age×3/3 genotype	1.25 ^{ns}	0.9418	0.9574 ^{ns}	0.9281	1.048 ^{ns}	0.9241	1.151 ^{ns}	0.9808	2.629 ^{ns}	1.399
Age×3/4 genotype	0.5217 ^{ns}	1.059	0.4371 ^{ns}	1.041	0.6735 ^{ns}	1.039	0.3799 ^{ns}	1.114	1.128 ^{ns}	1.578
Age ² ×3/3 genotype	0.5662	0.2196	0.5165	0.2156	0.5099	0.2154	0.5679	0.2266	0.2108 ^{ns}	0.3283
Age ² ×3/4 genotype	0.7644	0.2421	0.7057	0.2375	0.7131	0.2375	0.7904	0.2526	0.5704 ^{ns}	0.3617
Selected variable ^a	-0.3673	0.1464	1.127	0.2717	0.5694	0.0979			-3.495	1.241
SV-Pubic-Hair Stage 2 ^b							-1.615 ^{ns}	1.208		
SV-Pubic-Hair Stage 3 ^b							-3.998	1.746		
SV-Pubic-Hair Stage 4 ^b							-4.399	2.078		
SV-Pubic-Hair Stage 5 ^b							-3.693 ^{ns}	2.439		
Between-subjects variance/covariance										
Constant	306	32.65	325.1	34.13	304.9	32.41	311	34.59	314	35.09
Age/constant	-6.156 ^{ns}	4.784	-6.697 ^{ns}	4.808	-7.15 ^{ns}	4.683	-5.102 ^{ns}	5.042	-8.183 ^{ns}	5.886
Age	4.602	1.646	3.952	1.577	4.134	1.585	4.48	1.744	2.984 ^{ns}	2.034
Within-subjects variance										
Error	126.7	4.549	127.1	4.512	125.2	4.491	128.2	4.848	130.2	5.788

EST, estradiol concentration; ns, not significant

^a Selected variable represents each of fat-free mass, BMI, percent body fat, Tanner stage, and EST, respectively, in corresponding models. Due to non-normal distribution, the log10 of EST was used in multilevel modeling analyses.

Selected variable (SV) representing Tanner stage, which is Pubic-Hair Stages 2–5.
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Table 4

Multilevel longitudinal models for total cholesterol and LDL-C, Project HeartBeat!, 1991–1995

Parameter	Total cholesterol model		LDL-C model	
	Estimate	SE	Estimate	SE
Fixed parameters				
Constant	143.2	4.89	71.65	4.216
Age	−3.001	1.636	−5.109	1.244
Age ²	−0.9468	0.3971		
Age ³	0.07912	0.03513		
3/3 genotype	15.79	5.238	18.93	4.533
3/4 genotype	21.5	5.888	23.48	5.122
Age×3/3 genotype	0.2955 ^{ns}	1.703	3.085	1.323
Age×3/4 genotype	−1.049 ^{ns}	1.964	2.884	1.489 ^{ns}
Age ² ×3/3 genotype	0.947	0.3997		
Age ² ×3/4 genotype	1.015	0.4475		
FFM/BMI/PBF ^a				
Pubic-Hair Stage 2				
Pubic-Hair Stage 3				
Pubic-Hair Stage 4				
Pubic-Hair Stage 5				
EST ^b	−3.8	1.497	−3.256	1.341
Between-subjects variance/covariance				
Constant	397.4	47.77	308.9	37.83
Age/constant	−10.46 ^{ns}	8.454	−9.817 ^{ns}	6.998
Age	6.35	3.097	6.474	2.7
Within-subjects variance				
Error	151.9	7.546	126.4	6.282

EST, estradiol concentration; FFM, fat-free mass; PBF, percent body fat; ns, not significant

^aFFM, BMI, and PBF are mutually exclusive in any total cholesterol or LDL-C models.^bEST was transformed using base 10 log scale.