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Blood Lead Concentration and Delayed Puberty in Girls

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ABSTRACT

BACKGROUND

Environmental lead exposure has been linked to alterations in growth and endocrine function. It is not known whether such exposure affects pubertal development.

METHODS

We analyzed the relations between blood lead concentration and pubertal development among girls (defined as females 8 to 18 years of age) who were enrolled in a cross-sectional study (the third National Health and Nutrition Examination Survey) in which race was self-reported or proxy-reported: 600 were non-Hispanic white, 805 were non-Hispanic African-American, and 781 were Mexican-American girls. Puberty was measured on the basis of the age at menarche and Tanner stage for pubic-hair and breast development.

RESULTS

Geometric mean lead concentrations were less than 3 μg per deciliter (0.144 μmol per liter) in all three groups. As compared with concentrations of 1 μg per deciliter (0.048 μmol per liter), lead concentrations of 3 μg per deciliter were associated with decreased height ($P<0.001$), after adjustment for age, race, and other factors, but not with body-mass index or weight. Blood lead concentrations of 3 μg per deciliter were associated with significant delays in breast and pubic-hair development in African-American and Mexican-American girls. The delays were most marked among African-American girls; in this group, the delays in reaching Tanner stages 2, 3, 4, and 5 associated with a lead concentration of 3 μg per deciliter as compared with 1 μg per deciliter were 3.8, 5.3, 5.8, and 2.1 months, respectively, for breast development and 4.0, 5.5, 6.0, and 2.2 months, respectively, for pubic-hair development; the associated delay in age at menarche was 3.6 months. In white girls, there were nonsignificant delays in all pubertal measures in association with a lead concentration of 3 μg per deciliter.

CONCLUSIONS

These data suggest that environmental exposure to lead may delay growth and pubertal development in girls, although confirmation is warranted in prospective studies.

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EXPOSURE TO ENVIRONMENTAL CONtaminants may accelerate[1,2] or delay[3] pubertal development in girls. Lead is a ubiquitous environmental contaminant associated with a variety of health effects.[4] Although the average body burdens in U.S. children have decreased markedly since the removal of lead from gasoline in 1979,[5] current body burdens nonetheless remain appreciably higher than preindustrial levels.[6]

Exposure to lead could indirectly affect the timing of puberty in girls through effects on growth. Prenatal or postnatal exposure to lead is associated with growth restriction in laboratory animals[7] and humans.[8,9] In large cross-sectional studies of young children, increased lead concentrations were associated with decreased height, weight, or both.[10,11] In turn, increased height, body-mass index (the weight in kilograms divided by the square of the height in meters), and weight were directly associated with earlier pubertal development.[12,13]

In addition to effects on growth, prenatal and postnatal exposure to lead may influence pubertal development through the hypothalamic–pituitary–gonadal axis. Studies of rats exposed to lead found altered hormone concentrations and delayed puberty.[7,14,15] We assessed the relations between blood lead concentrations and puberty in girls in the third National Health and Nutrition Examination Survey (NHANES III), conducted from 1988 to 1994.[16,17]

METHODS

NHANES III was a cross-sectional, nationally representative, complex-sample survey of the U.S. noninstitutionalized population two months of age or older, designed to provide estimates of the health status of the general population.[16,17] About 40,000 civilian, noninstitutionalized persons were surveyed with use of a stratified, multistage, clustered-probability design. Race and ethnic background were self-reported or proxy-reported in NHANES III. Mexican Americans and African Americans were oversampled to provide more precise estimates for these groups.

During the household interview, 2741 girls, defined as 8 to 18 years of age at screening, were selected for the study. Of these, 2585 completed the survey, including the physical examination and biologic-sample collection. Data on pubertal development and lead concentrations were available for 2186 girls: 600 non-Hispanic white girls, 805 non-Hispanic African-American girls, and 781 Mexican-American girls. Measures of puberty included pubic-hair stage for 1964 girls, breast-development stage for 1986 girls, and age at menarche for 1796 girls (limited to those 8 to 16 years of age). Nine percent declined to be examined for pubic-hair stage, and 8 percent for breast-development stage.[17] The 113 girls belonging to other racial or ethnic groups were not analyzed owing to their small numbers. Written informed consent was obtained from all participants.

NHANES data were weighted to adjust for unequal selection probabilities and nonresponse and adjusted to match estimates of the Current Population Survey for the U.S. noninstitutionalized population.[16,17] To accommodate the complex sample design when calculating variance estimates, we used SUDAAN (release 8.0.1) and SAS (release 8.1) software. In comparing characteristics across racial and ethnic groups, we used the median test, adapted for complex survey data, for continuous data[18] and the Cochran–Mantel–Haenszel test for categorical data.[19] Since blood lead concentrations were not distributed normally, the data were log-transformed. A log (base 3) transformation was used to compare blood lead concentrations of 3 μg per deciliter (0.144 μmol per liter) with those of 1 μg per deciliter (0.048 μmol per liter), to approximate the 75th and 25th percentiles, respectively. Multivariable linear regression was used to model relations between growth and blood lead concentrations. Measures of body size included height, weight, and body-mass index.

Associations between the Tanner stage and body size and between the Tanner stage and blood lead concentrations were examined. Tanner staging for pubic-hair or breast development classifies girls into five progressively more mature stages, ranging from stage 1 (no development) to stage 5 (fully mature).[20] Tanner stages were assessed by trained clinicians without knowledge of the girls' blood lead concentrations.[16] Ordinal logistic regression (logistic regression for ordered responses) was used to assess the progression of the Tanner stage, which calculated odds ratios of the likelihood of reaching higher Tanner stages with changes in lead concentrations, after controlling for age and other factors. Odds ratios greater than 1.0 indicate accelerated pubertal development, whereas ratios of less than 1.0 indicate a delay, relative to the reference group. The mean age for each Tanner stage was estimated from the model by generating age-specific probabilities and computing the weighted mean age for that stage.

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The age at menarche of the 8-to-16-year-old girls was obtained by interviewing girls who were 10 years of age or older or, for girls younger than 10 years, adults (usually the girls' mothers). To reduce the interval between the onset of menarche and a subsequent blood lead assessment, girls older than 16 years of age were not included in menarche analyses. The relation between the lead concentration and the age at menarche was analyzed with use of Cox proportional-hazards modeling after the exclusion (censoring) of data on each premenarchal girl at her age during the survey. The age at examination was recorded in months, whereas the age at menarche was recorded in years. To reduce downward bias, 0.5 year was added to age at menarche.

Pubertal onset has been reported to occur earlier in African-American girls than in whites.[21-23] In an earlier analysis of data from NHANES III, pubertal development in Mexican-American girls was intermediate between that of African-American girls and white girls.[23] Thus, analyses were performed separately for each of the three groups. Delays were estimated by comparing the modeled median age at each blood lead concentration with the median age for a blood lead concentration of 1 μg per deciliter.

In all analyses, age and potential risk factors or confounders were examined, including age at examination (in months), smoking status, dietary information (a 24-hour report of dietary calcium, iron, vitamin C, and total fat), presence or absence of anemia,[4,11,24] and measures of socioeconomic status (urban vs. rural residence and family income of less than $20,000 per year vs. $20,000 or more). Variables remained in the model if they reached borderline statistical significance ($P<0.1$) or were potential confounders (i.e., if the coefficient for the log blood lead concentration changed by more than 10 percent when that factor was included in the final model). For consistency, any factor included for one racial or ethnic group was included in analyses for the other groups.

RESULTS

The mean ages of the girls were similar in the three groups (Table 1). As compared with whites, African-American girls were significantly taller, and both African Americans and Mexican Americans had a higher average body-mass index. The average stages of breast and pubic-hair development and blood lead concentrations were highest among African Americans, a finding consistent with prior analyses of a subgroup of these girls.[23] African-American girls were significantly younger at menarche than whites, and Mexican Americans were intermediate between and not significantly different from African-American girls and white girls. There were also differences between groups in some measures of socioeconomic status (income and level of education of the head of household) and some personal factors (ever having smoked or consumed alcohol and dietary calcium levels).

Overall, blood lead concentrations declined with age (Fig. 1). The geometric mean blood lead concentrations were below 3 μg per deciliter for all groups. Few girls had blood lead concentrations of more than 5 μg per deciliter (0.24 μmol per liter): 2.7 percent of whites, 11.6 percent of African Americans, and 12.8 percent of Mexican Americans. Very few had concentrations of more than 10 μg per deciliter (0.48 μmol per liter) — the Centers for Disease Control and Prevention's level of concern for children[25] — 0.3 percent of whites, 1.6 percent of African Americans, and 2.3 percent of Mexican Americans.

Each stage of pubertal development occurred earliest among African-American girls. Among girls whose blood lead concentration was 1 μg per deciliter or below, the average ages for Tanner stages 2, 3, 4, and 5 of breast development were 10.1, 11.7, 13.2, and 15.8 years, respectively, among African Americans; 10.7, 12.5, 14.1, and 16.4 years, respectively, among Mexican Americans; and 11.2, 12.9, 14.7, and 16.4 years, respectively, among whites. African Americans were also the youngest at each stage of pubic-hair development (10.1, 11.3, 13.4, and 16.0 years at Tanner stages 2, 3, 4, and 5, respectively). The average ages for each of these stages in white girls (11.2, 12.6, 14.8, and 16.8 years, respectively) and Mexican-American girls (11.1, 12.7, 14.8, and 16.7 years, respectively) were similar. African-American girls were also the youngest at menarche, with an average age of 12.0 years, as compared with 12.4 years in whites and 12.2 years in Mexican Americans.

To explore whether pubertal development was associated with measures of growth, we assessed relations between pubertal development and body size and between lead concentration and body size. Greater height, weight, and body-mass index were each individually associated with a higher breast-development stage. When height, body-mass index, and weight were included in models simultaneously, however, only height and body-mass index

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Table 1. Characteristics of the Participants According to Racial and Ethnic Group.*

Characteristic	Non-Hispanic Whites (N=600)	African Americans (N=805)	Mexican Americans (N=781)	P Value
Age at physical examination (yr)				0.79
Mean	13.4	13.4	13.4	
95% CI	13.0–13.8	13.1–13.7	13.1–13.7	
Height — in.				<0.001
Mean	60.5	61.0	59.6	
95% CI	59.9–61.1	60.5–61.4	59.2–60.0	
Body-mass index				0.02
Mean	20.5	21.7	21.7	
95% CI	20.0–21.1	21.2–22.1	20.9–22.5	
Urban residence (%)	43.0	50.0	54.5	0.24
Family income <$20,000/yr (%)	22.0	58.6	57.7	<0.001
Head of household's level of education (yr)				<0.001
Mean	13.1	11.6	8.6	
95% CI	12.8–13.5	11.3–11.8	8.1–9.1	
Any history of smoking 100 cigarettes (%)	11.0	1.5	3.6	<0.001
No. of cigarettes smoked/day (among smokers)				0.02
Mean	11.8	5.8	7.1	
95% CI	10.6–13.0	NA	NA	
Any history of alcohol consumption (among those >12 yr of age) (%)	37.9	17.9	29.7	<0.001
Dietary calcium (mg/day)				<0.001
Mean	854.7	735.2	923.3	
95% CI	811.2–898.2	692.6–777.9	856.3–990.2	
Dietary iron (mg/day)				0.93
Mean	13.1	12.7	13.4	
95% CI	12.1–14.1	12.0–13.5	12.4–14.5	
Dietary vitamin C (mg/day)				0.18
Mean	89.6	110.0	106.2	
95% CI	80.7–98.5	100.7–119.2	97.9–114.6	

remained associated with greater breast development, owing to the high correlation between body-mass index and weight (odds ratio for each 1-in. [2.5-cm] increase in height, 1.17 [95 percent confidence interval, 1.09 to 1.25], and odds ratio for each 1-unit increase in body-mass index, 1.08 [95 percent confidence interval, 1.01 to 1.15], after adjustment for age, age squared, dietary iron, family income [less than $20,000 per year vs. $20,000 or more], and race). Increased height was associated with earlier pubic-hair development; the odds ratio for reaching a given Tanner stage associated with a 1-in. increase in height was 1.23 (95 percent confidence interval, 1.14 to 1.33), after adjustment for age, age squared, dietary vitamin C, family income, and race and ethnic group. No measure of growth was significantly associated with age at menarche. Higher blood lead concentrations (3 μg per deciliter vs. 1 μg per deciliter) were associated with decreased height (regression slope = −0.51, $P<0.001$) but not with changes in body-mass index or weight, after adjustment for age, age squared, race and ethnic group, family income, presence or absence of anemia at examination, and dietary vitamin C, iron, and calcium — a finding that was similar to those in girls younger than eight years of age.[11]

Higher blood lead concentrations were associated with significant delays in all pubertal measures among African Americans and in breast and pubic-hair development among Mexican Americans (Table 2 and Fig. 2 and 3), after adjustment for relevant physical, demographic, and socioeconomic characteristics. For white girls, the relations between lead concentrations and pubertal development were in

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Table 1. (Continued.)

Characteristic	Non-Hispanic Whites (N=600)	African Americans (N=805)	Mexican Americans (N=781)	P Value
Anemia (%)†	4.5	23.1	7.2	<0.001
Blood lead concentration (μg/dl)				<0.001
Geometric mean	1.4	2.1	1.7	
95% CI	1.2–1.5	1.9–2.3	1.6–1.9	
Age at menarche (yr)‡				0.02
Mean	12.4	12.0	12.2	
95% CI	12.2–12.6	11.9–12.2	12.1–12.4	
Breast-development stage§				0.02
Mean	3.3	3.7	3.4	
95% CI	3.0–3.5	3.5–3.8	3.3–3.6	
Pubic-hair stage§				<0.001
Mean	3.2	3.6	3.2	
95% CI	2.9–3.4	3.5–3.8	3.1–3.3	

* P values among the three racial and ethnic groups were calculated with use of the Cochran–Mantel–Haenszel chi-square test for categorical outcomes[19] and the median test[18] for continuous variables. For outcomes significant at a P value of less than 0.05, comparisons examined differences among pairs of groups: all three groups differed from the other two with respect to the level of education of the head of household and blood lead concentration; African Americans differed from both other groups with respect to drinking status, anemia status, and dietary calcium levels; whites differed from the other two groups with respect to family income, ever having smoked 100 cigarettes, and body-mass index; Mexican Americans differed from the other two groups with respect to height; whites and Mexican Americans differed with respect to the number of cigarettes smoked per day; and whites differed from African Americans with respect to pubic-hair development and age at menarche. To convert values for height to centimeters, multiply by 2.54. To convert values for lead to micromoles per liter, multiply by 0.048. CI denotes confidence interval, and NA not available.

† The presence of anemia at examination was defined with the use of age-specific hemoglobin cutoff values.[24]

‡ The midpoint of the year of age at menarche was used for girls 8 to 16 years of age (for girls whose age was less than the midpoint of the year of age at menarche, current ages were used). Of all the girls who were 8 to 16 years of age, the following had reached menarche: 237 of 490 non-Hispanic whites (48.4 percent), 345 of 654 African Americans (52.8 percent), and 312 of 652 Mexican Americans (47.9 percent).

§ These values represent the average stage for all girls in each group. Tanner stages[20] were scored separately. For both, stage 1 represented preadolescence, with no development. Tanner stages for pubic-hair development are as follows: stage 2 is defined by sparse growth of nonadult hair in a limited area, stage 3 by darkening hair and greater coverage, stage 4 by hair of adult quality but limited to the pubic area, and stage 5 by full distribution of hair to medial aspect of thigh (fully mature). Tanner stages for breast development are as follows: stage 2 is defined by breast buds, stage 3 by further enlargement and elevation of breast and aureola, stage 4 by projection of aureola and papilla above breast, and stage 5 by fully mature breasts.

the same direction as in the other groups but were not significant.

The greatest delays in breast development associated with higher lead concentrations were in African Americans. As compared with a lead concentration of 1 μg per deciliter, a lead concentration of 3 μg per deciliter was associated with delays in reaching Tanner stages 2, 3, 4, and 5 of breast development (delays of 3.8, 5.3, 5.8, and 2.1 months, respectively). The likelihood of reaching each stage of breast development decreased as blood lead concentrations increased (Table 2). Smaller delays were observed for Mexican-American girls (for Tanner stages 2, 3, 4, and 5, the respective delays were 2.4, 2.8, 3.0, and 1.3 months). The delays were nonsignificant for white girls (respective delays of 1.7, 2.2, 2.3, and 0.7 months).

The delays in pubic-hair development associated with lead concentrations of 3 μg per deciliter as compared with 1 μg per deciliter were also largest among African Americans: 4.0, 5.5, 6.0, and 2.2 months for Tanner stages 2, 3, 4, and 5, respectively. As for breast development, the likelihood of reaching consecutive stages of pubic-hair development decreased as blood lead concentrations increased (Table 2). There were smaller, stage-specific delays in pubic-hair development for the same lead concentrations among Mexican Americans (3.5, 4.0, 3.7, and 1.5 months for Tanner stages 2, 3,4, and 5, respectively) and nonsignificant delays among whites (2.6, 3.0, 3.0, and 1.1 months, respectively).

A significant association between higher blood lead concentration and age at menarche was found only in African-American girls, with a delay of 3.6 months for blood lead concentrations of 3 μg per deciliter as compared with 1 μg per deciliter (Table

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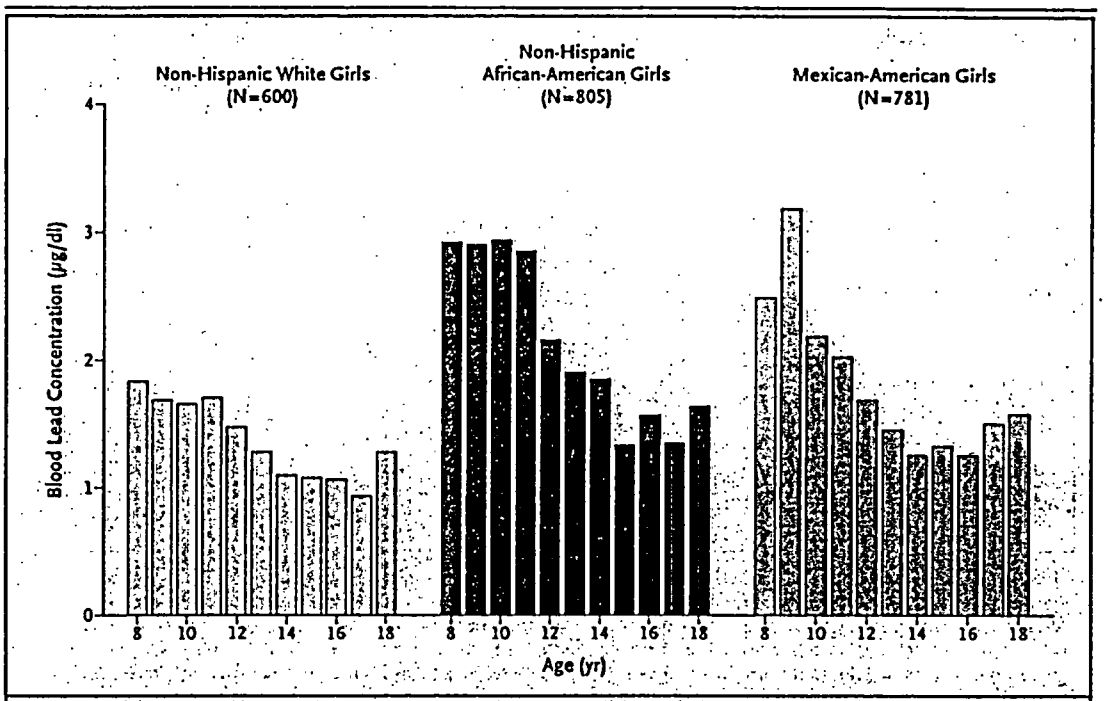

Figure 1. Blood Lead Concentrations among Non-Hispanic White, Non-Hispanic African-American, and Mexican-American Girls.

Age-specific geometric means were calculated with the use of sampling weights to adjust for unequal probabilities of selection. The distribution among the three groups was significantly different (P=0.006). To convert values for lead to micromoles per liter, multiply by 0.048.

2). The difference in age at menarche with a blood lead concentration of 3 μg per deciliter as compared with 1 μg per deciliter was smaller and not significant in whites (2.9-month delay) and Mexican Americans (0.4-month delay).

DISCUSSION

As compared with a blood lead concentration of 1 μg per deciliter, a blood lead concentration of 3 μg per deciliter was associated with delayed pubertal development, after adjustment for body size and other factors. Delays were statistically significant for all three measures in African-American girls and for breast and pubic-hair development in Mexican-American girls. For white girls, the relations between lead concentration and measures of puberty were in the same direction as in the other groups but were not significant.

We also found that higher blood lead concentrations were associated with decreased height, but not weight or body-mass index, after adjusting for racial and ethnic group and other factors. Our analyses of girls 8 to 18 years of age extend findings from previous studies of younger children.[10,26,27] In two studies, increased blood lead concentration was associated with reduced height but not weight or body-mass index.[10,11] In a third study, decreased height and weight were independently associated with increased blood lead concentration.[27] Prenatal and postnatal exposure to lead also increased pituitary growth hormone and suppressed plasma insulin-like growth factor 1 in rats.[15,28]

In our study, relations between measures of body size and pubertal development, adjusted for racial and ethnic group, varied with the measure of puberty. Only increased height was associated with a more advanced pubic-hair development; weight and body-mass index were not. All three body-size measures were individually associated with breast development, but in analyses including all three, only height and body-mass index were significantly associated with breast development, owing

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Table 2. Effect of Blood Lead Concentrations of 3 μg per Deciliter as Compared with 1 μg per Deciliter on Measures of Pubertal Development.*

	Non-Hispanic Whites	African Americans	Mexican Americans
8-to-18-year-old girls			
Breast development — odds ratio (95% CI)			
Adjusted for age only	0.82 (0.53–1.27)	0.60 (0.42–0.84)†	0.77 (0.61–0.97)†
Fully adjusted‡	0.82 (0.47–1.42)	0.64 (0.42–0.97)†	0.76 (0.63–0.91)†
Pubic-hair development — odds ratio (95% CI)			
Adjusted for age only	0.64 (0.36–1.13)	0.58 (0.41–0.81)†	0.60 (0.45–0.80)†
Fully adjusted§	0.75 (0.37–1.51)	0.62 (0.41–0.96)†	0.70 (0.54–0.91)†
8-to-16-year-old girls			
Age at menarche — hazard ratio (95% CI)§			
Adjusted for lead only	0.81 (0.61–1.08)	0.79 (0.64–0.99)†	0.91 (0.76–1.10)
Fully adjusted¶	0.74 (0.55–1.002)	0.78 (0.63–0.98)†	0.90 (0.73–1.11)

* Odds ratios reflect the likelihood of reaching a successive stage of pubertal development for girls with a log-transformed lead concentration of 3 μg per deciliter as compared with 1 μg per deciliter. Hazard ratios were calculated with the use of Cox proportional-hazards models. CI denotes confidence interval.

† $P<0.05$.

‡ Analyses were adjusted for age, age squared, height, body-mass index, family income (<$20,000 per year vs. $20,000 or more), ever having smoked 100 cigarettes (vs. never having done so), and dietary intake of iron, vitamin C, and calcium.

§ Analyses were adjusted for age, age squared, height, family income (<$20,000 per year vs. $20,000 or more), ever having smoked 100 cigarettes (vs. never having done so), presence or absence of anemia (defined on the basis of age-specific hemoglobin cutoff values[24]), and dietary intake of iron and vitamin C.

¶ Analyses were adjusted for height, body-mass index, family income (<$20,000 per year vs. $20,000 or more), presence or absence of anemia (defined on the basis of age-specific hemoglobin cutoff values[24]), and dietary intake of calcium.

to a high correlation between body-mass index and weight. No body-size measures were associated with the age at menarche. Body-fat measurements, including body-mass index, and height were associated with pubertal development in other studies.[12,13,29-31]

We found differences in the timing of pubertal development in girls of different racial and ethnic groups, findings consistent with the results of previous studies.[21-23] For each measure of puberty, African Americans were younger than whites at every stage of pubertal development. Mexican-American girls reached breast-development stages earlier than whites, but pubic-hair development and menarche occurred at ages similar to those of whites.

Higher blood concentrations of lead were associated with delayed puberty in African-American and Mexican-American girls, but these associations were not significant in white girls. The reasons for these differences are unclear. Nonsignificant pubertal delays in white girls with higher lead concentrations may result from a decreased power to detect an association owing to the examination of fewer white girls. Alternatively, differences among the groups may result from genetic or other environmental factors. For example, African-American girls reach skeletal maturity earlier than whites, although this may be accounted for by increased adiposity among African Americans.[13,32] White girls have longer menstrual cycles and durations of menstrual bleeding than African Americans, suggesting differences in hypothalamic–pituitary–gonadal regulation.[33,34] White and African-American girls also have differences in hypothalamic–pituitary–adrenal function, as evidenced by a greater corticotropin response,[35] which may be affected by exposure to lead.[36]

Developmental exposure to lead affects growth and sex hormones in animals. Prenatal, lactational, and prepubertal exposure to lead delayed the age at which vaginal opening and first estrus occurred in rats.[7,14,15,37] Rats exposed to lead prenatally or during lactation and rats with low postnatal lead concentrations had decreased serum concentra-

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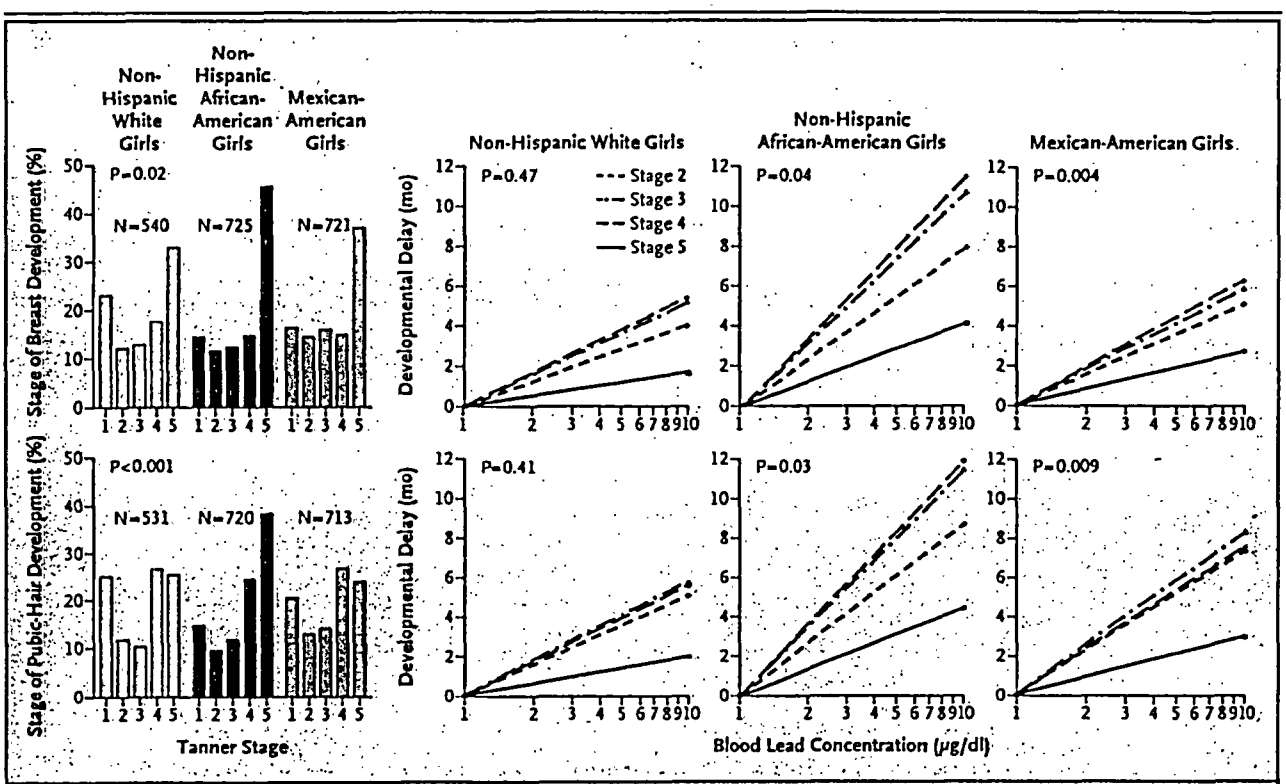

Figure 2. Association between Tanner Stage and Blood Lead Concentrations.
The two left-most panels show the Tanner stages of all girls. The other panels show the model-based estimates of the delay in each stage, calculated with use of the results of ordinal logistic regression of Tanner stages, and on solving the equation, with the use of age-specific means for other risk factors and confounders. To convert values for lead to micromoles per liter, multiply by 0.048.

tions of insulin-like growth factor 1, luteinizing hormone, and estradiol in the absence of effects on body weight.[15] Decreases in these puberty-related hormones might delay the onset or progression of puberty.

The delays in puberty associated with blood lead concentrations are striking given the low concentrations recorded during the survey. An increase in blood lead concentrations from 1 μg per deciliter to 3 μg per deciliter was associated with delays in breast and pubic-hair development ranging from two to six months among African-American girls, depending on the stage of puberty. Smaller delays were observed in these measures in the other two groups. However, we do not have data on earlier concentrations in these girls, and we cannot exclude the possibility that higher lead concentrations early in life may explain or contribute to observed pubertal delays.

In children, blood lead concentrations typically peak at about one to two years of age and gradually decrease thereafter,[5,11] data consistent with the decline in blood lead concentrations with age observed in this study. Younger children have higher blood lead concentrations than older children in the same environment because of greater exposure and lead absorption from the gastrointestinal tract.[38] In addition, environmental concentrations of lead have decreased markedly over the past two decades.[4] Some girls in this cross-sectional study would have been young children at the time of NHANES II (1976 to 1980), in which the geometric mean lead concentration among children one to five years of age was 15.0 μg per deciliter (0.72 μmol per liter), with 88.2 percent having concentrations above 10 μg per deciliter. Although our findings cannot prove a causal relation between mildly elevated lead concentrations and delayed puberty, they suggest that even a relatively low level of exposure to lead may influence growth and sexual development in girls.

In addition, other factors associated with body lead burden and pubertal development that we did

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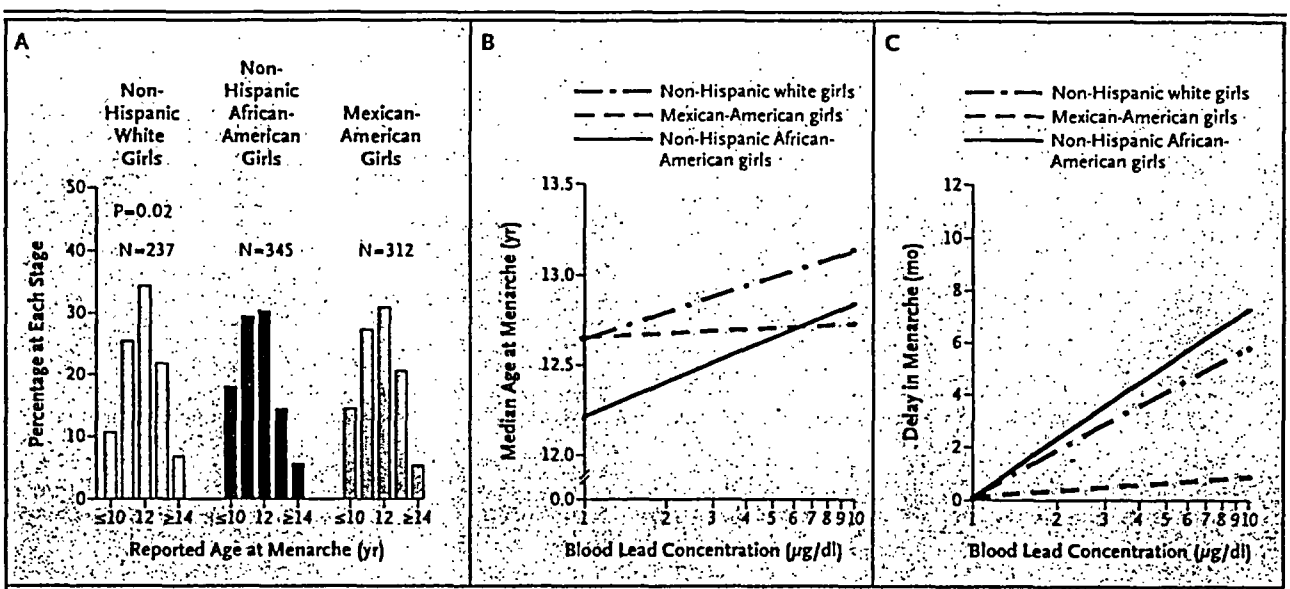

Figure 3. Association between Age at Menarche and Blood Lead Concentrations.
Panel A shows the reported age at menarche for all girls. Delays in menarche were estimated with the use of PROC LIFEREG software (SAS). In Panel B, the age at menarche was modeled with the use of the same potential risk factors and confounding variables in the SUDAAN PROC SURVIVAL analysis. In Panel C, model-based estimates of delays in menarche were obtained by evaluating each model at blood lead concentrations ranging from 1 to 10 μg per deciliter, with the use of mean values for each group (shown in Panel B). Then delays were calculated by subtracting the median age for a blood lead concentration of 1 μg per deciliter from the median age for lead concentrations of 2 to 10 μg per deciliter. Since the PROC LIFEREG software cannot accommodate the complex sampling weights used in the third National Health and Nutrition Examination Survey and this analysis was not available in SUDAAN software, only the ages at menarche, and not the confidence intervals or significance levels, were estimated. To convert values for lead to micromoles per liter, multiply by 0.048.

not assess may be responsible for the observed associations. As with any cross-sectional study, reporting of past events, such as age at menarche and dietary history, is subject to errors in recall. We adjusted for several potential confounders measured at the time of the study, but these factors may have differed during periods critical for pubertal development or other unmeasured confounders may have affected the results. Our study documents associations between the blood lead concentration and delayed pubertal development after adjustment for measures of body size, age, and potential confounders. These findings suggest that delays in pubertal development may be due at least in part to mechanisms independent of effects on growth, conceivably to alterations in endocrine function.

The views expressed in this report are those of the authors and do *not necessarily reflect the views or policies of the Environmental Protection Agency.*

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REFERENCES

1. Blanck HM, Marcus M, Tolbert PE, et al. Age at menarche and Tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. Epidemiology 2000;11:641-7.
2. Krstevska-Konstantinova M, Charlier C, Craen M, et al. Sexual precocity after immigration from developing countries to Belgium: evidence of previous exposure to organochlorine pesticides. Hum Reprod 2001;16:1020-6.
3. Den Hond E, Roels HA, Hoppenbrouwers K, et al. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. Environ Health Perspect 2002;110:771-6.
4. Toxicological profile for lead. Atlanta: Agency for Toxic Substances and Disease Registry, July 1999.
5. Prikle JL, Brody DJ, Gunter EW, et al. The decline in blood lead levels in the United States: the National Health and Nutrition Examination Survey (NHANES). JAMA 1994;272:284-91.
6. Patterson CC. British mega exposure to industrial lead. In: Rutter M, Jones RR, eds. Lead versus health. New York: John Wiley, 1983:17-32.
7. Ronis MJ, Badger TM, Shema SJ, Roberson PK, Shaikh F. Effects on pubertal growth and reproduction in rats exposed to lead perinatally or continuously throughout devel-

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opment. J Toxicol Environ Health A 1998;53:327-41.
8. Bellinger D, Leviton A, Rabinowitz M, Allred E, Needleman H, Schoenbaum S. Weight gain and maturity in fetuses exposed to low levels of lead. Environ Res 1991;54:151-8.
9. Shukla R, Dietrich KN, Bornschein RL, Berger O, Hammond PB. Lead exposure and growth in the early preschool child: a follow-up report from the Cincinnati Lead Study. Pediatrics 1991;88:886-92.
10. Frisancho AR, Ryan AS. Decreased stature associated with moderate blood lead concentrations in Mexican-American children. Am J Clin Nutr 1991;54:516-9.
11. Ballew C, Khan LK, Kaufmann R, Mokdad A, Miller DT, Gunter EW. Blood lead concentration and children's anthropometric dimensions in the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. J Pediatr 1999;134:623-30.
12. Rubin K, Schirduan V, Gendreau P, Sarfarazi M, Mendola R, Dalsky G. Predictors of axial and peripheral bone mineral density in healthy children and adolescents, with special attention to the role of puberty. J Pediatr 1993;123:863-70.
13. Kaplowitz PB, Slora EJ, Wasserman RC, Pedlow SE, Herman-Giddens ME. Earlier onset of puberty in girls: relation to increased body mass index and race. Pediatrics 2001;108:347-53.
14. Kimmel CA, Grant LD, Sloan CS, Gladen BC. Chronic low-level lead toxicity in the rat. I. Maternal toxicity and perinatal effects. Toxicol Appl Pharmacol 1980;56:28-41.
15. Dearth RK, Hiney JK, Srivastava V, Burdick SB, Bratton GR, Dees WL. Effects of lead (Pb) exposure during gestation and lactation on female pubertal development in the rat. Reprod Toxicol 2002;16:343-52.
16. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988–94. Vital and health statistics. Series 1. No. 32. Hyattsville, Md.: National Center for Health Statistics, 1994. (DHHS publication no. (PHS) 94-1308.)
17. National Health and Nutrition Examination Survey III, 1988-1994. NHANES III series II. No. 1A. Hyattsville, Md.: National Center for Health Statistics, 1997 (CD-ROM).
18. Mood AM. Introduction to the theory of statistics. New York: McGraw-Hill, 1950.
19. Selvin S. Statistical analysis of epidemiologic data. New York: Oxford University Press, 1991.
20. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child 1969;44:291-303.
21. Biro FM, McMahon RP, Striegel-Moore R, et al. Impact of timing of pubertal maturation on growth in black and white female adolescents: the National Heart, Lung, and Blood Institute Growth and Health Study. J Pediatr 2001;138:636-43.
22. Herman-Giddens ME, Slora EJ, Wasserman RC, et al. Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. Pediatrics 1997;99:505-12.
23. Wu T, Mendola P, Buck GM. Ethnic differences in the presence of secondary sex characteristics and menarche among US girls: the Third National Health and Nutrition Examination Survey, 1988-1994. Pediatrics 2002;110:752-7.
24. Recommendations to prevent and control iron deficiency in the United States. MMWR Morb Mortal Wkly Rep 1998;47(RR-3):1-29.
25. Preventing lead poisoning in young children: a statement by the Centers for Disease Control. Atlanta: Centers for Disease Control, October 1991.
26. Pounds JG, Long GJ, Rosen JF. Cellular and molecular toxicity of lead in bone. Environ Health Perspect 1991;91:17-32.
27. Schwartz J, Angle C, Pitcher H. Relationship between childhood blood lead levels and stature. Pediatrics 1986;77:281-8.
28. Ronis MJ, Badger TM, Shema SJ, et al. Endocrine mechanisms underlying the growth effects of developmental lead exposure in the rat. J Toxicol Environ Health A 1998;54:101-20.
29. Vizmanos B, Marti-Henneberg C. Puberty begins with a characteristic subcutaneous body fat mass in each sex. Eur J Clin Nutr 2000;54:203-8.
30. Magarey AM, Boulton TJ, Chatterton BE, Schultz C, Nordin BE, Cockington RA. Bone growth from 11 to 17 years: relationship to growth, gender and changes with pubertal status including timing of menarche. Acta Paediatr 1999;88:139-46.
31. Wattigney WA, Srinivasan SR, Chen W, Greenlund KJ, Berenson GS. Secular trend of earlier onset of menarche with increasing obesity in black and white girls: the Bogalusa Heart Study. Ethn Dis 1999;9:181-9.
32. Russell DL, Keil MF, Bonat SH, et al. The relation between skeletal maturation and adiposity in African American and Caucasian children. J Pediatr 2001;139:844-8.
33. Harlow SD, Campbell B. Ethnic differences in the duration and amount of menstrual bleeding during the postmenarcheal period. Am J Epidemiol 1996;144:980-8.
34. Harlow SD, Campbell B, Lin X, Raz J. Ethnic differences in the length of the menstrual cycle during the postmenarcheal period. Am J Epidemiol 1997;146:572-80.
35. Yanovski JA, Yanovski SZ, Boyle AJ, et al. Hypothalamic-pituitary-adrenal axis activity during exercise in African American and Caucasian women. J Clin Endocrinol Metab 2000;85:2660-3.
36. Kim D, Lawrence DA. Immunotoxic effects of inorganic lead on host resistance of mice with different circling behavior preferences. Brain Behav Immun 2000;14:305-17.
37. Ronis MJ, Gandy J, Badger T. Endocrine mechanisms underlying reproductive toxicity in the developing rat chronically exposed to dietary lead. J Toxicol Environ Health A 1998;54:77-99.
38. Exposure factors handbook. Vol. 1. General factors. Washington, D.C.: Environmental Protection Agency, 1997:4-16. (EPA/600/P-95/002.)

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