Blood Lead Levels and Sexual Maturation in U.S. Girls: The Third National Health and Nutrition Examination Survey, 1988–1994

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Using data from the Third National Health and Nutrition Examination Survey, we assessed measures of puberty in U.S. girls in relation to blood lead levels to determine whether sexual maturation may be affected by current environmental lead exposure. The study sample included 1,706 girls 8–16 years old with pubic hair and breast development information; 1,235 girls 10–16 years old supplied information on menarche. Blood lead concentrations (range = 0.7–21.7 µg/dL) were categorized into three levels: 0.7–2.0, 2.1–4.9, and 5.0–21.7 µg/dL. Sexual maturation markers included self-reported attainment of menarche and physician determined Tanner stage 2 pubic hair and breast development. Girls who had not reached menarche or stage 2 pubic hair had higher blood lead levels than did girls who had. For example, among girls in the three levels of blood lead described above, the unweighted percentages of 10-year-olds who had attained Tanner stage 2 pubic hair were 60.0, 51.2, and 44.4%, respectively, and for girls 12 years old who reported reaching menarche, the values were 68.0, 44.3, and 38.5%, respectively. The negative relation of blood lead levels with attainment of menarche or stage 2 pubic hair remained significant in logistic regression even after adjustment for race/ethnicity, age, family size, residence in metropolitan area, poverty income ratio, and body mass index. In conclusion, higher blood lead levels were significantly associated with delayed attainment of menarche and pubic hair among U.S. girls, but not with breast development. Key words: fecundity, lead, menarche, puberty, sexual maturation. Environ Health Perspect 111:737–741 (2003). doi:10.1289/ehp.6008 available via http://dx.doi.org/ [Online 4 February 2003]

Lead is a ubiquitous environmental contaminant whose toxicity, including reproductive and developmental effects in humans, is well known (Bellinger 1994; National Research Council 2000). Lead has been used as both a spermicide and abortifacient and has even been implicated in the fall of the ancient Roman Empire (Nriagu 1983). Although environmental and blood lead levels have declined over time (Pirkle et al. 1994), concern remains about the potential adverse impact of lead exposure on subtle aspects of child growth and development, including reproductive function. A recent study (Lanphear et al. 2000) reported cognitive deficits associated with blood lead concentration < 10 µg/dL in children and adolescents. Puberty—the onset of fecundity or the biologic capacity for reproduction—represents an important yet understudied health outcome that may be altered at relatively low levels of lead exposure.

Subtle lead-related effects on sexual maturation have been reported in animal studies. For example, laboratory animals exposed to lead prenatally and/or as juveniles were reported to have experienced delayed puberty or sexual maturation (Der et al. 1974; Kimmel et al. 1980; Ronis et al. 1998b). To address these findings, we assessed measures of puberty in U.S. girls in relation to blood lead levels to determine if there was any evidence in humans that sexual maturation may be adversely affected by current environmental lead levels. Methods Data. We used data from the Third National Health and Nutrition Examination Survey (NHANES III), 1988–1994, to assess the relation between blood lead levels and pubertal milestones in U.S. girls. Briefly, the NHANES III is a cross-sectional survey that used a stratified multistage probability sampling design to obtain nationally representative information on the health and nutrition of the U.S. population using interviews and physical examinations (n = 39,095). The NHANES III represents the noninstitutionalized U.S. population 2 months or older who reside in the 50 states and the District of Columbia. Detailed information on the sample design and conduct of the NHANES III is available elsewhere (National Center for Health Statistics 1994).

Study sample. There were 7,050 girls 1–10 years old who participated in the youth sample of NHANES III. Only girls 8 years or older were eligible for Tanner staging of pubic hair and breast development (National Center for Health Statistics 1998). Our study sample is restricted to the 1,706 girls 8–16 years old who could provide information on blood lead, Tanner staging of pubic hair and breast development, and other variables of interest. Menarche was ascertained in the NHANES III for girls 10 years or older. As such, among the 1,706 girls, menarche data were available for 1,235 girls. We refer to these two samples as study samples 1 and 2, respectively.

Markers of sexual maturation. Physicians assessed pubic hair and breast development using Tanner staging (Tanner 1986) as described in the Physician Examiners Training Manual (Centers for Disease Control and Prevention 1996), which required a chaperone to be in the room. Tanner stage 2 pubic hair was defined in the study protocol as “spare growth of long, slightly pigmented downy hair, straight or only slightly curled, appearing chiefly along the labia” stage 2 breast development was defined as the breast bud stage or the “elevation of breast and papilla as small mound . . . widening and elevation of the area ola with pigmentation.” For study purposes, we were interested in whether girls had reached stage 2 or more for these two puberty markers. Refusal rates for the Tanner staging varied by girl’s age, with the lowest rates encountered for girls 8 years old and the highest rates for girls 15 years old. Specifically, refusal rates ranged from 8 to 15% for pubic hair staging and from 6 to 13% for breast staging. Girls 10–16 years old were asked about whether they had had a period/menstrual cycle, and the attainment of menarche was defined in this study based on the self-reported data. Among girls 10–16 years old (n = 1,973) who participated in NHANES III, 1,580 (80.1%) had information on attainment of menarche.
Laboratory lead measurement. A blood specimen was obtained from children during the physical examination via venipuncture. Blood lead levels were measured by the laboratory at the National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), using a graphite furnace atomic absorption spectrophotometer based on the methodology of Miller et al. (1987). In the study sample, blood lead levels ranged from 0.7 to 21.7 µg/dL and were categorized into three levels for the analysis of level specific effect: 0.7–2.0 µg/dL, 2.1–4.9 µg/dL, and 5.0–21.7 µg/dL. Only 26 girls in the sample had blood lead levels greater than 10 µg/dL. Detailed information on blood lead levels for the entire NHANES III sample was reported elsewhere (CDC 1997; Pirkle et al. 1994).

Operational definitions. Blood lead level is our study exposure, and sexual maturation as measured by Tanner stage 2 for pubic hair and breast development, and attainment of menarche are our study outcomes. Other study covariates considered to be potential confounders for the analysis included race/ethnicity, age, family size, residence, poverty income ratio, and body mass index (BMI). These variables are based on the operational definitions set forth in NHANES III. Race/ethnicity referred to the following four categories as reported by the primary respondent in the screening and family interview portion of the survey: non-Hispanic white, non-Hispanic African American, Mexican American, and other (i.e., Hispanics not of Mexican origin or non-Hispanics from racial groups other than white or African American). Age referred to chronologic age (in completed months) at the time of the NHANES III exam and was converted (divided by 12) to years for analysis. Family size referred to the number of family members who were living in the household, and residency referred to the rural/urban code based on U.S. Department of Agriculture codes that described metropolitan and non-metropolitan counties by degree of urbanization and nearness to metropolitan areas (National Center for Health Statistics 1998). These codes were later recoded into two categories in the NHANES III data set to ensure confidentiality of data: 1, metro (i.e., central or fringe counties of metropolitan areas of ≥ 1 million population) or 2, nonmetro (all other areas). The poverty income ratio is the ratio of the reported family incomes divided by the poverty threshold, which was produced annually by the Census Bureau and adjusted for changes caused by inflation. The variable was categorized into < 1, 1–2, > 2, and a missing category was also created for analysis. Weight and height were measured in NHANES III, and BMI (kilograms per square meter) was computed by dividing weight (in kilograms) by height (in meters) squared.

Statistical analysis. The unweighted and weighted means and proportions were calculated to describe the study population/sample with respect to the covariates of interest. For each age, the unweighted percentages of girls with pubic hair, breast development, and attainment of menarche were computed for each category of blood lead. The Cochran-Mantel-Haenszel chi-square test controlling for age with the application of weights was used to test percentage differences by category of blood lead. Unweighted age-specific mean blood lead levels were computed for girls who had and had not attained each marker of sexual maturation. Girls who attained a pubertal marker were compared with those who did not with respect to the means in the natural logarithm–transformed values of blood lead after controlling for age using analysis of variance (ANOVA) with weighting. The sample size in each age is small, so age-specific weighted statistics were not computed. The attainment of puberty across the blood lead groups for all ages combined was also analyzed by logistic regression with weighting and the adjustment for race/ethnicity, age, family size, residence, poverty income ratio, and BMI. Specifically, the presence of a pubertal characteristic (pubic hair, breast development, or attainment of menarche) served as a dependent variable, whereas in addition to blood lead, race/ethnicity, age, family size, residence, poverty income ratio, and BMI were fit into logistic regression models to test whether their associations with the dependent variable were independent. To take into consideration the sampling strategy such as stratification and clustering employed in the NHANES III, all weighted analyses described above were performed using SUDAAN statistical software (SUDAAN 1995) using the weights established for the NHANES III examination sample. The SAS software package was used for the unweighted analyses (SAS 1992).

Results

Study sample 1 (n = 1,706) was composed of girls who ranged in age from 8 to 16 years (mean 12.2 ± 2.6 years). Reflecting the study design of NHANES III, the study sample included a nearly equal percentage of non-Hispanic African-American and Mexican-American girls (Table 1). A comparable percentage of girls resided in metropolitan and nonmetropolitan areas in both samples. Approximately 37% of girls lived in families with income levels below the poverty line. Blood lead concentration ranged from 0.7 to 21.7 µg/dL, with a mean (± SD) of 2.5 ± 2.2 µg/dL. Weighted descriptive statistics from samples 1 and 2 were provided for national estimates of the variables of interest.

The unweighted percentages of girls having attained menarche or Tanner stage 2 or higher for pubic hair and breast development are presented in Table 2. In general, a negative relation is observed between the percentage of girls having achieved each pubertal milestone and blood lead levels. This pattern is especially noticeable for younger girls. The Cochran-Mantel-Haenszel chi-square test, after controlling for age and with weighting, indicated significant differences across lead levels.
levels with respect to the percentage of girls who attained stage 2 pubic hair. The relation of blood lead levels with attainment of menarche had marginal significance in the weighted analysis. No significant differences were observed for breast development across the lead levels.

The unweighted mean blood lead levels for each age are presented in Table 3. As the data reflect, mean levels of blood lead in general tended to be higher for girls who did not reach puberty than for girls who did. After controlling for age using two-way ANOVA, a significant difference in mean blood lead levels was found for girls who reached stage 2 pubic hair compared with girls who did not. With respect to menarche, the weighted ANOVA shows a marginally significant difference in the mean blood lead levels. The difference in mean blood lead levels by breast development status was not significant.

Results from the logistic regression analyses are shown in Table 4. After adjustment for age, race/ethnicity, poverty income index, residence, family size, and BMI and after taking the NHANES III design features of stratification and cluster into account, using the weights and SUDAAN software, blood lead levels (both as a categorical and continuous variable) were significantly associated with the attainment of pubic hair and menarche. No significant association was observed for blood lead levels and breast development in this multivariate analyses.

**Discussion**

Blood lead levels that are considered “acceptable” have been changed continuously over the past three decades as new research underscores the human health hazards associated with environmental lead exposure. Currently, the CDC has defined elevated blood lead concentrations as blood lead levels > 10 µg/dL (CDC 1991). However, possible hazards at blood lead levels < 10 µg/dL still remain a concern (Landrigan 2000). This study found a significant negative association between blood lead concentrations even at relatively low levels and physical markers of sexual maturation in U.S. girls who participated in the NHANES III survey. This finding suggests that despite declining blood lead levels in the United States, current levels may still pose concerns for the growth and development of girls as measured by the three pubertal milestones considered in this study.

The potentially toxic effect of lead exposure on sexual maturation in humans is biologically plausible. Studies of lead workers (McGregor and Mason 1990; Rodamilans et al. 1988) have found chronic occupational lead exposure to be associated with a decrease in serum testosterone levels and/or alterations in circulating gonadotrophin levels. Such results suggest that lead has a direct effect on the onset of sexual maturation after lead exposure. What is surprising yet intriguing is that this finding, although preliminary in nature, may suggest an effect of blood lead at levels lower than those of concern currently established by the CDC. Therefore, these findings underscore the importance of prospective inquiry for assessing the health effects associated with low lead exposure by focusing on sensitive markers of human development such as puberty.

Our findings are consistent with many recent studies reporting a declining age at puberty and declining blood lead concentrations for more recently born cohorts of children. Specifically, several authors, including those reporting for boys in the NHANES III, have reported earlier ages at puberty for recent birth cohorts (Fredricks et al. 2000; Karpati et al. 2002), whereas others have not found much difference over time (Whincup et al. 2001). Complicating interpretation of these equivocal temporal trends is the observation that puberty appears to vary by race/ethnicity but only in some countries such as the United States (de Muinck et al. 2001; Herman-Giddens et al. 1997; Wu et al. 2002). Possible etiologic factors cited for the temporal pattern of declining age at puberty include both nutritional (body size) and environmental factors that either act independently or interactively with genes (Bray 1997; Treloar and Martin 1990).

The effect of the observed delays in puberty on children’s adult health status and fecundity is uncertain at this time. In the study by Kimmel et al. (1980), lead-exposed female rats that demonstrated delayed sexual maturation were no different from unexposed control animals with respect to the ability to conceive and carry a normal litter to term.

| Table 2. Unweighted percentage of girls at Tanner stage 2 for pubic hair and breast development and attainment of menarche, by age and blood lead level: NHANES III, 1988–1994. |

| Age (years) | 0.7–2.0 µg/dL | 2.1–4.9 µg/dL | 5.0–21.7 µg/dL | p-Value |
|------------|---------------|---------------|---------------|---------|
| Pubic hair |               |               |               |         |
| 8          | 75            | 18.7          | 70            | 15.7    | 43 | 7.0 |
| 9          | 88            | 36.4          | 102           | 36.3    | 41 | 19.5 |
| 10         | 90            | 60.0          | 82            | 51.2    | 36 | 44.4 |
| 11         | 94            | 85.1          | 101           | 74.3    | 25 | 80.0 |
| 12         | 108           | 97.2          | 74            | 96.0    | 14 | 85.7 |
| 13         | 112           | 98.2          | 56            | 100.0   | 0  | 100.0 |
| 14         | 111           | 99.1          | 48            | 100.0   | 0  | 100.0 |
| 15         | 125           | 100.0         | 27            | 100.0   | 0  | 100.0 |
| 16         | 123           | 100.0         | 42            | 100.0   | 0  | 100.0 |
| Breast development |               |               |               |         |
| 8          | 75            | 26.7          | 70            | 22.9    | 43 | 11.6 |
| 9          | 88            | 36.4          | 102           | 35.3    | 41 | 34.2 |
| 10         | 90            | 61.1          | 82            | 72.0    | 36 | 61.1 |
| 11         | 94            | 90.4          | 101           | 87.1    | 25 | 84.0 |
| 12         | 108           | 98.2          | 74            | 96.0    | 14 | 100.0 |
| 13         | 112           | 99.1          | 56            | 98.2    | 7  | 100.0 |
| 14         | 111           | 99.1          | 48            | 100.0   | 0  | 100.0 |
| 15         | 125           | 100.0         | 27            | 100.0   | 0  | 100.0 |
| 16         | 123           | 100.0         | 42            | 100.0   | 0  | 100.0 |
| Attainment of menarche |               |               |               |         |
| 10         | 85            | 5.9           | 74            | 2.7     | 33 | 0.0 |
| 11         | 90            | 70.0          | 99            | 6.2     | 22 | 9.1 |
| 12         | 103           | 68.0          | 70            | 44.3    | 13 | 38.5 |
| 13         | 110           | 87.3          | 53            | 79.3    | 6  | 50.0 |
| 14         | 110           | 97.3          | 46            | 87.0    | 6  | 83.3 |
| 15         | 123           | 100.0         | 26            | 100.0   | 5  | 100.0 |
| 16         | 122           | 99.2          | 38            | 100.0   | 2  | 100.0 |

p-Values are from Cochran-Mantel Haenszel chi-square test of the percentage differences across blood lead levels controlling for age and after weighting and adjusting for NHANES III complex sampling using SUDAAN software.
Extreme delays in the onset of puberty (>18 years) in girls have been reported to be a risk factor for infertility (Komura et al. 1992), and more modest pubertal delays (13 years or older) have been associated with other gynecologic conditions such as endometriosis, which may be in the pathway for female infertility (Berube et al. 1998). On the other hand, early age at menarche increases the risk for breast and endometrial cancer (Garfinkel et al. 1998; McPherson et al. 1996). Hence, alterations in puberty may have life-long implications for human health and disease.

The cross-sectional design used in the NHANES III does not allow us to assess the temporal relation between blood lead and puberty. Therefore, we have considered other explanations for the observed relation between higher blood lead levels and the delay in the pubertal milestones. First, lower blood lead levels among girls who attained sexual maturation may be the consequence rather than antecedent of the pubertal development. Rapid body growth along with sexual maturation is the hallmark of puberty. The fast bone formation during pubertal development induces more rapid deposition of calcium, along with lead, from the blood into the bone, and may cause redistribution of lead in the body—albeit growing bone is turning over rapidly and bone lead is continuously returned to the circulation. A second explanation may be that menstruation is an important pathway for elimination of lead albeit modest in the absence of pathologic menstrual bleeding, which is unlikely in most young girls. If the explanations were true, menstruating girls would be expected to have lower lead levels than nonmenstruating girls. However, the kinetics of lead in relation to puberty milestones other than age is largely unstudied, and empirical data to support these explanations are currently lacking (O’Flaherty 1995; U.S. EPA 1994). Therefore, the results of this study underscore the need for research focusing on lead mobilization in relation to puberty milestones and a greater appreciation for the pathways of exposures for older children. Finally, we did not model dietary and nutritional factors, which may affect blood lead levels, nor did we consider concomitant environmental exposures (Lanphear et al. 1996; Six and Goyer 1972; Watson et al. 1980). The extent to which residual confounding explains our findings remains to be established.

In summary, we found a significant negative association between blood lead at relatively low levels and markers of sexual maturation suggesting that such exposure may delay pubertal development in girls. Judicious interpretation of this finding is needed given the cross-sectional study sample and limited attention to other nutritional or genetic factors that may impact the findings. These findings underscore the importance of considering sensitive markers of human fecundity such as puberty in relation to environmental lead exposures.

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### Table 3. Unweighted mean blood lead levels (µg/dL) by age and presence/absence of Tanner stage 2 pubertal measure and menarche: NHANES III, 1988–1994.

| Age (years) | Pubic hair Absence of pubertal measure: No. Mean ± SD | Presence of pubertal measure: No. Mean ± SD | p-Value |
|------------|------------------------------------------------------|---------------------------------------------|---------|
| 8          | 160 3.5 ± 2.6                                        | 28 2.4 ± 1.4                                |         |
| 9          | 154 3.5 ± 2.6                                        | 77 3.1 ± 2.9                                |         |
| 10         | 96 3.7 ± 2.6                                         | 112 2.9 ± 2.1                               |         |
| 11         | 45 3.2 ± 2.7                                         | 175 2.8 ± 2.1                               |         |
| 12         | 8 4.1 ± 4.7                                          | 188 2.2 ± 1.7                               |         |
| 13         | 2 1.3 ± 0.8                                          | 173 2.0 ± 1.5                               | 0.013   |
| 14         | 1 0.7                                               | 164 1.9 ± 1.5                               |         |
| 15         | 28 2.8 ± 2.4                                         | 156 1.5 ± 1.2                               |         |
| 16         | 167 1.6 ± 1.0                                        |                                            |         |

### Table 4. Likelihood* of having attained pubertal markers by blood lead levels: NHANES III, 1988–1994.

| Blood lead (µg/dL) | Pubic hair OR 95% CI | Breast development OR 95% CI | Attainment of menarche OR 95% CI |
|-------------------|----------------------|-------------------------------|---------------------------------|
| 0.7–2.0           | 1.00 Reference       | 1.00 Reference                | 1.00 Reference                  |
| 2.1–4.9           | 0.48 0.23–0.92       | 1.51 0.90–2.53                | 0.42 0.19–0.97                  |
| 5.0–21.7          | 0.27 0.08–0.93       | 1.20 0.53–2.85                | 0.19 0.09–0.43                  |
| Log transformed*  | 0.54 0.32–0.91       | 1.20 0.76–1.92                | 0.52 0.28–0.97                  |

Abbreviations: 95% CI, 95% confidence interval; OR, odds ratio.

*Logistic regression with adjustment for age, race/ethnicity, poverty income ratio, metro residence, family size, and BMI using SUDAAN software and weights established for the NHANES III exam sample. Natural log transformed, treated as a continuous variable. The categorical and continuous variables of blood lead were analyzed in separate models.
