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Blood Lead Levels and Delayed Onset of Puberty in a Longitudinal Study of Russian Boys

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Abstract

Background—World-wide trends towards earlier pubertal onset have raised concerns regarding the potential role of environmental exposures, including lead. Previous animal studies and studies in girls have revealed associations of lead with perturbations in pubertal onset. We evaluated the association of blood lead levels (BLLs) with pubertal onset in a longitudinal cohort of Russian boys.

Methods—489 Russian boys were enrolled in 2003–2005 at ages 8–9 years and followed annually through May 2008. Cox proportional hazards models were used to evaluate the association of BLLs at enrollment with time to pubertal onset during follow-up based on testicular volume (TV) and pubertal staging, adjusting for birth characteristics, nutritional status, maternal exposures during pregnancy, height and BMI, and socioeconomic status.

Results—481 boys had BLLs, with median=3 µg/dL and 28% ≥ 5 µg/dL. The percentage of pubertal boys increased with age, from 12% at age 8 to 83% at age 12 as defined by TV>3ml, from 22% to 90% as defined by genitalia stage 2 or higher, and from 4% to 40% as defined by pubic hair stage 2 or higher. After adjustment for potential confounders including BMI and height, boys with high BLLs (≥ 5 µg/dL) had a 24%–31% reduced risk of pubertal onset based on

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Conflicts of Interest:

The author(s) declare that they have no conflicts of interest.

TV>3ml (Hazard Ratio (HR)= 0.73; 95% CI: 0.55, 0.97, p=0.03), genital staging (HR=0.76, 95% CI: 0.59, 0.98, p=0.04), and pubic hair staging (HR=0.69, 95% CI: 0.44, 1.07, p=0.10) as compared to those with lower lead levels. Pubertal onset occurred 6–8 months later on average for boys with high BLLs compared to those with BLLs < 5 µg/dL.

Conclusions—Higher blood lead was associated with later pubertal onset in this prospective study of peri-pubertal Russian boys. Given the large numbers of children with BLLs ≥ 5 µg/dL worldwide, this shift has important public health implications and supports review of current lead policies.

Keywords

Children; Environment; Epidemiology; Lead; Puberty

Introduction

Puberty is a period of rapid physiological changes including maturation of gonads and secondary sexual characteristics, accelerated growth, and brain development. The timing and duration of this critical period of development are influenced by a number of factors, including genetics, race, body composition, activity, and diet.^{1–4} Several studies have reported a temporal trend of earlier pubertal onset^{5–7}, particularly in girls.^{3,8,9} While these trends may be partly attributable to better nutrition and improved living conditions, they have raised concerns as to whether exposures to persistent organic pollutants -- including endocrine disrupting chemicals such as dioxins, polychlorinated biphenyls (PCBs), pesticides, and heavy metals such as mercury, lead, or arsenic -- may play a role in influencing these shifts.^{1,10–15}

Animal models have demonstrated lead-related alterations in growth and sexual development and suggest an endocrine mechanism for such effects^{16–19}, but epidemiologic studies are limited. In girls, analysis of data from the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994) indicated cross-sectional associations of blood lead levels with delayed breast and pubic hair development and menarche.^{20,21} A cross-sectional analysis of 138 Akwesasne Mohawk girls also found an older age at menarche for those with lead levels of 1.2–4.4 µg/dL compared with < 1.2 µg/dL, despite relatively low lead concentrations in this population.¹⁴ In contrast, blood lead concentrations measured among 192 inner-city girls were similar regardless of pubertal status.¹³ In boys, studies have identified an association between blood lead and diminished early childhood growth,^{22–25} but none evaluated the impact of lead on pubertal onset.

We previously published a cross-sectional analysis of pubertal onset among Russian boys enrolled at ages 8 to 9 years.²⁶ We found a 43% reduction in odds of pubertal onset (defined by genitalia staging) for boys with blood lead ≥ 5 µg/dL; however, the low rate of pubertal onset measured by pubic hair staging or testicular volume at that age provided little power for detecting associations with these measures.²⁶

We now extend our Russian Children's Study by conducting longitudinal analyses of the association of blood lead and pubertal onset. Our study was conducted among boys from Chapaevsk, a town in the Samara region of Russia where several large industries previously manufactured chemical warfare agents and industrial and agricultural chemicals, resulting in environmental contamination with dioxins, PCBs, and metals including lead.²⁷ Lead exposure may have also resulted from use of leaded gasoline, lead solder in plumbing, car batteries, paints, and food grown in lead-contaminated soil.^{28,29}

Methods

Study Population

Our Russian Children's Study is a prospective cohort study of 489 peri-pubertal boys in Chapaevsk, Russia, as described in more detail elsewhere.²⁶ All boys aged 8 to 9 years were identified using the town-wide health insurance information system; of the 550 within the eligible age range, 516 (94%) agreed to participate and were enrolled between May 2003 and March 2005. The primary reasons for non-participation included refusal due to lack of time or interest. At study entry, 27 boys were found to be ineligible (17 residing in orphanages lacked maternal and birth information and 10 had chronic health conditions which could impact childhood growth and development). This left 489 eligible boys, of whom 481 had lead measurements available. The study was approved by the Human Studies Institutional Review Boards of the Chapaevsk Medical Association, Harvard School of Public Health, University of Massachusetts Medical School, and Brigham and Women's Hospital. The parent or guardian signed an informed consent and the boy signed an assent form prior to participation.

At study entry, a physical examination was conducted and each boy provided blood samples for the analyses of lead and other environmental exposures (dioxins and PCBs). A health, lifestyle, and diet questionnaire developed with Russian collaborators^{30,31} was administered by a nurse to each boy's mother or guardian. Information was collected on birth and neonatal history, the child's medical history and physical activity; maternal and household smoking and alcohol use during the pregnancy with the child; family medical, occupational, and residential history; and socioeconomic measures such as household income and parental education. Birth weight and gestational age were also obtained from medical records and were preferentially used when available. A validated Russian Institute of Nutrition semi-quantitative food frequency questionnaire (FFQ) was modified to ascertain the child's typical dietary intake over the previous year,^{32,33} and to estimate total daily caloric intake and distribution of fat, protein and carbohydrate calories.

Physical examination

At study entry and at annual follow-up visits, a standardized anthropometric examination and pubertal assessment was performed by a single study investigator (OS) according to a written protocol and without knowledge of the boys' lead levels. Height was measured to the nearest 0.1 cm using a stadiometer. Weight was measured to the nearest 100 grams with a metric scale. Body mass index (BMI; kg/m^2) was calculated from the weight and height measurements. Pubertal status was staged from 1 to 5 by visual inspection and comparison with published photographs according to internationally accepted criteria.³⁴ Pubarche (pubic hair stage, P) was determined by extent of terminal hair growth. Genital staging (G) was assessed by genital size and maturity. Testicular volume (TV) was measured using Prader beads (orchidometer). Three different measures of pubertal onset were considered: $\text{TV} > 3$ ml of either testis, stage G2 or greater, and stage P2 or greater.

Blood Lead Analysis

A 3.0 ml venous blood sample was collected in a trace metal-free vacutainer tube (Becton-Dickinson, Franklin Lakes, NJ) after cleaning the site with alcohol. Whole blood samples were diluted with a matrix modifier solution and analyzed by Zeeman background-corrected flameless graphite furnace atomic absorption (ESA Laboratories, Chelmsford, MA). The detection limit was 1.0 $\mu\text{g}/\text{dl}$; measured blood lead levels (BLLs) below the limit of detection were set to 0.5 for 14 of 481 boys (2.9%).

Statistical Analysis

We considered longitudinal data based on initial entry visits and up to three annual follow-up visits conducted through May 2008. BLLs were logarithmically-transformed to approximate a normal distribution, and were also grouped as ‘high’ ($\geq 5 \mu\text{g/dL}$) versus ‘low’ ($< 5 \mu\text{g/dL}$) lead levels. We evaluated associations using Cox proportional hazards models for time to pubertal onset defined by TV > 3 ml (either testis), stage G2 or greater, or stage P2 or greater, based on the midpoint between the first visit at which onset was observed and the prior visit. Pubertal onset prior to enrollment was assumed to occur 6 months before enrollment, and those who were still prepubertal at their last study visit were censored at that visit. Cox regression models were first fit to evaluate univariate associations of log-transformed lead or high lead on “risk” of pubertal onset, then adjusted for birth weight, gestational age, nutritional status (caloric intake and percent energy from protein and fat), maternal alcohol consumption during pregnancy, height and BMI at study entry, and socioeconomic measures (household income level and maximum parental education). Because height and BMI are themselves influenced by lead exposure and may be considered in the causal pathway of pubertal onset, Cox models were fit first adjusting for covariates other than BMI and height, and then “fully adjusting” for all covariates including BMI and height at study entry. Models including BMI percentile and BMI Z-score were also considered, but provided results almost identical to those using BMI, and are not presented. Other covariates considered but not associated with pubertal onset included parity, maternal or household smoking during pregnancy, and mother’s age at birth of son.

Several sensitivity analyses were conducted. Because pubertal onset was only observed annually, approaches for interval censored outcomes were also applied. In addition, repeated measures models using generalized estimating equations (GEEs) were applied to pubertal onset at each annual visit adjusting for correlation among multiple visits using an autoregressive structure. GEE approaches were also used to evaluate the impact of clustering within household for twins and siblings included in the study. Finally, a sensitivity analysis of the influence of high blood levels was conducted by excluding 16 subjects with BLLs $> 10 \mu\text{g/dL}$ (3%), and by refitting models adjusting for maternal age at menarche (unavailable for 39 boys).

Results

Demographic Characteristics and Blood Lead Levels

Among 489 eligible boys enrolled at age 8 ($n=306$) or at age 9 ($n=183$), 481 (98%) had blood lead levels determined. Birth, maternal, and household characteristics are presented in Table 1. The boys had a mean BMI of 15.9 at study entry, and a range of growth measures consistent with WHO Child Growth Standards (<http://www.who.int/growthref/en/>).³⁵ The median BLL was $3 \mu\text{g/dL}$, with 28% $\geq 5 \mu\text{g/dL}$ and 3% $\geq 10 \mu\text{g/dL}$; the skewed distribution necessitated log transformation for statistical analysis (Figure 1). Retention rates were high; only 12% discontinued study participation over 3 years of follow-up.

Pubertal Onset at Entry and During Follow-Up

Pubertal onset defined as TV > 3 ml increased during study follow-up, from 12% at age 8 years to 83% at 12 years (Table 2). Similar increases were observed based on genital staging (22% to 90%) and to a lesser extent for pubic hair (4% to 40%). Although these trends with age were apparent among all boys, the prevalence of pubertal onset was lower at each age for those with high BLLs (see Table 2 and Figure 2). At age 11, 22% of 427 boys were not yet pubertal by any of the three measures; however, twice the percentage remained pre-pubertal among boys with high versus low BLLs (34% vs 17%).

Association of Demographic, Body Size, and Socioeconomic Characteristics with Pubertal onset

In multivariable models with adjustment for all other covariates, there was a significant increase in likelihood of pubertal onset with higher birth weight (G2, TV) and lower gestational age (G2). Significantly earlier pubertal onset was also observed for boys with higher caloric intake (P2) and higher percentage dietary fat (TV). Boys whose mothers reported alcohol consumption during pregnancy had significantly later onset (TV); associations for G2 and P2 onset were in the same direction but not significant. Later pubertal onset was also associated with low household income (< \$175/month, TV) and low parental education (G2). While these associations varied according to pubertal onset outcome, the effects of height and BMI were similar across different measures of pubertal onset, indicating significantly earlier onset for taller boys and those with higher BMI. For consistency, the same set of covariates was included in all adjusted models.

Association of Blood Lead Levels with Pubertal onset

Cox models for time to pubertal onset demonstrated significantly later onset for boys with high BLLs (≥ 5 $\mu\text{g/dL}$) as compared to those with lower levels, which was consistent across all three measures of pubertal onset (Table 3). After adjustment for covariates (other than BMI and height), the likelihood of pubertal onset was reduced by 30–40% for those with high BLL as compared to those with lower levels (TV: Hazard Ratio (HR)=0.69; G2: HR=0.70; P2: HR=0.60). Further adjustment for BMI and height at entry attenuated estimated hazard ratios, suggesting delay by 25–30%, but associations remained statistically significant for TV $>3\text{ml}$ and stage G2 or higher, and marginally significant for P2.

The association of pubertal onset with log-transformed blood lead concentrations did not demonstrate as striking a relationship as observed for high versus low lead. However, after adjustment for other covariates, each log unit increase in lead concentration was associated with a significantly later pubertal onset defined as TV $>3\text{ml}$ (HR=0.84, 95% CI: 0.71, 1.00, $p=0.05$), and a marginally significant delay for stage P2 or higher (HR=0.77, 95% CI: 0.57, 1.04, $p=0.09$). Figure 3 shows Kaplan-Meier estimates for the percentage of boys without pubertal onset as defined by TV for low (0–2 $\mu\text{g/dL}$), moderate (3–4 $\mu\text{g/dL}$) and high (≥ 5 $\mu\text{g/dL}$) lead levels. This figure suggests a non-linear trend in dose response, with the median age at pubertal onset estimated at 11.5 years for boys with BLLs ≥ 5 $\mu\text{g/dL}$, but 10.5 years for boys with both low and moderate lead levels.

Sensitivity analyses showed consistency with the above results, both in terms of robustness to inclusion of specific covariates in the model, exclusion of those with BLL ≥ 10 $\mu\text{g/dL}$, and in use of alternative statistical approaches for assessing delay. Older maternal age at menarche was associated with a significant delay in pubertal onset defined by TV $> 3\text{ml}$ (HR=0.83, $p<0.001$), but effects of high lead on pubertal onset were similar after adjustment for maternal age at menarche (TV: HR=0.76, $p=0.06$; G2: HR=0.74, $p=0.03$; P2: HR=0.65, $p=0.08$).

An analysis using repeated measures GEE models for pubertal onset at each visit provided similar results to the Cox proportional hazards models in Table 3. In fully-adjusted GEE models, boys with high lead had significant delays in pubertal onset (G2: Odds Ratio (OR)=0.61, 95% CI: 0.41, 0.91, $p=0.01$; P2: OR=0.57, 95% CI: 0.32, 1.02, $p=0.06$); the estimated OR for TV $> 3\text{ml}$ was in the same direction but not significant (OR=0.75, $p=0.16$). GEE models also confirmed that results were not sensitive to the inclusion of 4 sets of twins and 3 sets of siblings; results were similar excluding all such subjects and adjusting for correlation within household. When interval censored models were applied under the assumption of a normal distribution for age at pubertal onset, the estimated mean age at

pubertal onset was 10.5 years based on TV (95% CI 10.3, 10.7), but a full year earlier based on G2 (mean=9.5 years, 95% CI: 9.3, 9.6) and almost 3 years later based on P2 (mean=13.0, 95% CI: 12.5, 13.6). In adjusted models, pubertal onset occurred 6–8 months later on average for boys with high BLLs (≥ 5 $\mu\text{g/dL}$) compared to those with BLLs < 5 $\mu\text{g/dL}$.

Discussion

In this prospective cohort study of Russian boys, we observed significantly later pubertal onset for boys with BLLs ≥ 5 $\mu\text{g/dL}$ as compared to those with BLLs < 5 $\mu\text{g/dL}$; this finding was consistent across measures of pubertal onset based on testicular volume as well as genital and pubic hair staging, and persisted after adjustment for potential confounders. These findings are consistent with previous cross-sectional studies in US girls,^{20–21} which found delayed pubertal onset for girls with higher lead levels, and with our initial cross-sectional data on Russian boys.²⁶ BLLs in the US have declined over time; however, 6% of US children under age 6 still had BLLs of 5–10 $\mu\text{g/dL}$ in 1999–2004, and rates in some subgroups remain even higher (17% above 5 $\mu\text{g/dL}$ among non-Hispanic blacks).³⁶ BLLs in other countries may be similar or higher, particularly where leaded gasoline is still used.

Changes in genital staging and testicular volume are generally thought to occur in parallel, but few epidemiologic studies have simultaneously assessed both measures of pubertal maturation. We observed a median age at pubertal onset of 10.5 years based on TV $> 3\text{ml}$, but a full year earlier for onset defined by genital staging. Despite these shifts across different pubertal onset measures, BLLs ≥ 5 $\mu\text{g/dL}$ were consistently associated with a 6–8 month delay in pubertal onset relative to those with BLLs < 5 $\mu\text{g/dL}$. This consistency is reassuring and has practical implications, given the relative ease in assessing pubertal staging in clinical settings and the lack of TV measurements in most epidemiologic studies.

It is unclear whether delays in pubertal onset occur at the level of the hypothalamic-pituitary gonadal axis by altering the activation of the GnRH pulse generator, or by altering other hormonal pathways that intersect with the reproductive hormones. Animal models suggest that lead exposure decreases concentrations of growth hormone, insulin-like growth factor 1 (IGF-1), testosterone and other hormones responsible for growth and pubertal development.^{16–19} The implications of altered pubertal timing have received more attention for early maturation, which has been associated with increased incidence of antisocial behaviors, substance use, and depression.^{37,38} However, late maturation in boys has also been associated with risk for psychosocial problems including lower self-esteem, depressive symptoms and eating disorders.³⁹

It should be emphasized that the delays in pubertal onset we observed did not represent clinical delays for individual boys, but rather a shift in the mean age at pubertal onset for those with higher lead levels as compared to lower levels. However, given the large numbers of children with BLLs ≥ 5 $\mu\text{g/dL}$ in the US and worldwide, such a population shift has important implications from a public health perspective.⁴¹ BLLs above 10 $\mu\text{g/dL}$ have been long recognized as having a strong association with neurocognitive and motor deficits in young children, leading to identification of this level as indicative of lead poisoning by the US Centers for Disease Control and Prevention.⁴² Yet BLLs well below 10 $\mu\text{g/dL}$ are increasingly identified as being associated with mild neurologic impairment and diminished growth.⁴³ Our results indicate that timing of pubertal onset can also be affected at BLLs in the 5–10 $\mu\text{g/dL}$ range; these findings add to concern regarding BLLs in this lower range and support review of current policies.

We report an association of high BLLs with later pubertal onset even after adjusting for BMI and height at study entry. These anthropometric measures, however, may be on the causal

pathway between lead exposure and pubertal onset. Measured BLLs may reflect chronic lead exposure or exposures during earlier periods, which in turn may result in diminished growth by age 8 to 9 years. In addition, it is known that bone mass increases during pubertal growth⁴⁰ which leads to an increased distribution volume for lead and thus lower BLLs. Thus, including these growth measures at study entry in our models may have resulted in over-adjustment and attenuation of estimated effects.

Our study's strengths include its size, prospective design, and consistent assessment of pubertal status by a single trained physician. Longitudinal pubertal onset assessments over this age range provided greater power and precision for estimating exposure effects than our previous cross-sectional analysis. It is also one of the few large-scale epidemiologic studies to include both physician-assessed pubertal staging and measured testicular volume. Limitations of our study include the lack of pre-natal and early childhood lead measurements, and the possibility of residual confounding by socioeconomic status.

In conclusion, this is the first prospective epidemiologic study to demonstrate a relationship between lead and later pubertal onset in boys. These associations occurred at levels which remain relevant for US, Russian, and other populations, raising concerns regarding the potential consequences for population-wide alterations in male pubertal timing.

List of Abbreviations

BLL	Blood Lead Level
BMI	Body Mass Index
CI	Confidence Interval
FFQ	Food Frequency Questionnaire
G2	Genitalia Stage 2 or higher
GEEs	Generalized Estimating Equations
GnRH	Gonadotropin-releasing Hormone
HR	Hazard Ratio
IGF-1	Insulin-like growth factor 1
LOD	Limit of Detection
ml	milliliter
NE	Not Evaluated
NHANES	National Health and Nutrition Examination Survey
OR	Odds Ratio
P2	Pubic Hair Stage 2 or higher
PCBs	Polychlorinated Biphenyls
SD	Standard Deviation
TV	Testicular Volume
US	United States
WHO	World Health Organization
µg/dL	micrograms per deciliter

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Figure 1.
Distribution of blood lead levels ($\mu\text{g}/\text{dl}$) among 481 eight to nine year-old Russian boys
($<\text{LOD}$ =below limit of detection of $1.0 \mu\text{g}/\text{dl}$).

Figure 2.
a–c. Percentage of Russian boys with pubertal onset by age at study visit as measured by testicular volume (TV) (panel a), pubertal stage for genitalia (G) (panel b) and pubic hair (P) (panel c) stratified by blood lead categories (<5, ≥5 µg/dl).

Figure 3.

Kaplan-Meier estimates for distributions of age at pubertal onset defined as testicular volume (TV) >3ml, by blood lead category (0–2, 3–4, or ≥ 5 $\mu\text{g/dl}$). Log rank test p-value based on test of any difference in distributions of age at pubertal onset defined by TV>3ml across the three blood lead categories.

Table 1

Demographic, maternal, and body size characteristics among 481 Russian Boys

Characteristic	Overall (N=481)	High Blood Lead ($\geq 5\mu\text{g/dL}$) (N=134)	Low Blood Lead ($<5\mu\text{g/dL}$) (N=347)
Growth Measurements at Study Entry			
Age (years) (mean, (SD)) ^I	8.4 (0.5)	8.5 (0.5)	8.3 (0.5)
Height (cm) (mean, (SD))	130.2 (6.3)	128.5 (6.9)	130.8 (5.9)
Weight (kg) (mean, (SD))	27.2 (5.5)	25.9 (5.2)	27.6 (5.6)
Body mass index (mean, (SD))	15.9 (2.3)	15.6 (2.1)	16.1 (2.3)
Percent calories from fat	34.0 (5.9)	33.4 (6.1)	34.3 (5.8)
Percent calories from protein	11.6 (1.6)	11.5 (1.6)	11.6 (1.6)
Birth and Neonatal History			
Birth weight (kg) (mean, SD)	3.34 (0.53)	3.25 (0.55)	3.38 (0.51)
Gestational age (wks) (mean, (SD))	39.0 (1.8)	38.9 (2.0)	39.1 (1.6)
Preterm (gestational age<37 wks) (N, (%))	37 (8%)	14 (10%)	23 (7%)
Breastfeeding duration (wks) (mean, (SD))	27.7 (34.5)	35.4 (45.1)	24.6 (28.9)
Household Characteristics			
Low income (< 5000 rubles/month) (N, (%))	168 (35%)	64 (48%)	104 (30%)
Low parental education (secondary school or less) (N, (%))	38 (8%)	18 (14%)	20 (6%)
Maternal Characteristics and Exposures During Pregnancy			
Mother's age at son's birth (mean, (SD))	23.9 (5.1)	24.3 (5.2)	23.7 (5.0)
Maternal alcohol consumption (N, (%))	59 (13%)	24 (18%)	35 (10%)
Maternal tobacco smoking (N, (%))	36 (8%)	15 (12%)	21 (6%)
Any smoking in household (N, (%))	228 (48%)	73 (55%)	155 (46%)

SD=standard deviation

Summary statistics including percentages calculated among those with non-missing data for that characteristic; 3 missing dietary information, 3 missing birth weight, 4 missing gestational age, 11 missing breastfeeding duration, 1 missing family income level, 4 missing parental education level, 5 missing mother's age at son's birth, 16 missing maternal alcohol consumption, 13 missing maternal tobacco smoking, 9 missing household smoking during pregnancy.

^I – 301 enrolled at age 8, 180 enrolled at age 9.

Table 2
Testicular Volume and Pubertal Staging by Age at Visit, and Pubertal onset Overall and by Blood Lead Levels.

		Age of Boy at Visit					
		8 years (N=301)	9 years (N=458)	10 years (N=435)	11 years (N=430)	12 years (N=166)	
Testicular volume (TV)	1-2ml	159 (53%)	200 (44%)	130 (30%)	73 (17%)	11 (7%)	
	3	105 (35%)	152 (33%)	142 (33%)	113 (26%)	15 (9%)	
	4	28 (9%)	64 (14%)	96 (22%)	76 (18%)	33 (20%)	
	5	5 (2%)	31 (7%)	32 (7%)	53 (12%)	18 (11%)	
	6	3 (1%)	6 (1%)	18 (4%)	44 (10%)	21 (13%)	
	8	0 (0%)	0 (0%)	12 (3%)	33 (8%)	24 (14%)	
	10+ ml	0 (0%)	1 (0%)	4 (1%)	35 (8%)	41 (25%)	
	NE	1 (0%)	4 (1%)	1 (0%)	3 (1%)	3 (2%)	
	Overall	36 (12%)	102 (22%)	162 (37%)	241 (56%)	137 (83%)	
Pubertal onset (TV>3ml)	High Lead ¹	5 (7%)	21 (16%)	37 (31%)	54 (44%)	44 (80%)	
	Low Lead ²	31 (13%)	81 (25%)	125 (40%)	187 (61%)	93 (84%)	
	Overall	36 (12%)	102 (22%)	162 (37%)	241 (56%)	137 (83%)	
Genitalia Stage	1	236 (78%)	257 (56%)	171 (39%)	108 (25%)	13 (8%)	
	2	62 (21%)	189 (41%)	238 (55%)	245 (57%)	81 (49%)	
	3	3 (1%)	11 (2%)	25 (6%)	72 (17%)	55 (33%)	
	4	0 (0%)	0 (0%)	0 (0%)	1 (0%)	9 (5%)	
	5	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (2%)	
	NE	0 (0%)	1 (0%)	1 (0%)	4 (1%)	4 (2%)	
Stage G2 or higher	Overall	65 (22%)	200 (44%)	263 (60%)	318 (74%)	149 (90%)	
	High Lead ¹	8 (11%)	44 (33%)	65 (54%)	79 (64%)	48 (87%)	
	Low Lead ²	57 (25%)	156 (48%)	198 (63%)	239 (78%)	101 (91%)	
Pubic Hair Stage	1	290 (96%)	412 (90%)	372 (86%)	346 (80%)	96 (58%)	
	2	11 (4%)	44 (10%)	61 (14%)	76 (18%)	51 (31%)	
	3	0 (0%)	1 (0%)	1 (0%)	5 (1%)	12 (7%)	
	4	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (2%)	
	NE	0 (0%)	1 (0%)	1 (0%)	3 (1%)	4 (2%)	
	Overall	11 (4%)	45 (10%)	62 (14%)	81 (19%)	66 (40%)	

		Age of Boy at Visit				
		8 years (N=301)	9 years (N=458)	10 years (N=435)	11 years (N=430)	12 years (N=166)
High Lead ¹		3 (4%)	11 (8%)	13 (11%)	18 (15%)	15 (27%)
Low Lead ²		8 (3%)	34 (10%)	49 (16%)	63 (21%)	51 (46%)

NE= not evaluated

¹ – Percent with onset at each age calculated among those with high lead levels ($\geq 5\mu\text{g/dL}$), n=71, 133, 121, 124, and 55 at ages 8, 9, 10, 11, and 12, respectively

² – Percent with onset at each age calculated among those with low lead levels ($< 5\mu\text{g/dL}$), n=230, 325, 314, 306, and 111 at ages 8, 9, 10, 11, and 12, respectively

Table 3
Effects of Blood Lead Levels on Pubertal onset in Unadjusted and Adjusted Cox Proportional Hazards Models

Exposure	Unadjusted Model (N=481)		Adjusted Model ¹ (N=461)		Fully Adjusted Model ² (N=461)	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Testicular Volume > 3ml						
Blood Lead >=5 ug/dL	0.65 (0.50–0.84)	0.001	0.69 (0.52–0.91)	0.01	0.73 (0.55–0.97)	0.03
Log(lead level)	0.84 (0.71–1.00)	0.05	0.90 (0.74–1.08)	0.25	0.90 (0.75–1.09)	0.27
Genitalia Stage 2 or Higher						
Blood Lead >=5 ug/dL	0.68 (0.54–0.85)	0.001	0.70 (0.54–0.90)	0.005	0.76 (0.59–0.98)	0.04
Log(lead level)	0.90 (0.77–1.05)	0.17	0.93 (0.79–1.10)	0.40	0.95 (0.81–1.12)	0.57
Pubic Hair Stage 2 or Higher						
Blood Lead >=5 ug/dL	0.60 (0.40–0.91)	0.02	0.60 (0.39–0.94)	0.03	0.69 (0.44–1.07)	0.10
Log(lead level)	0.83 (0.64–1.09)	0.19	0.77 (0.57–1.04)	0.09	0.80 (0.59–1.05)	0.14

HR = Hazard Ratio, CI=confidence interval.

¹ Adjusted model includes birth weight, gestational age, caloric intake, percent fat, percent protein, low income, low parental education, and maternal alcohol during pregnancy.

² The fully-adjusted models include above covariates plus BMI and height at study entry.