Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to use in future works (such as articles or books) all or part of this thesis.

_____

Signature of Student Date

BLOOD METAL CONCENTRATIONS AND TIMING OF PUBERTAL ONSET IN A LONGITUDINAL COHORT OF GIRLS, NORTHERN CALIFORNIA, 2006–2011

BY

Jason Andrew Wilken
Degree to be awarded: M.P.H.
Executive MPH

Lyndsey Darrow PhD, Committee Chair	Date
Dana Barr PhD, Committee Member	Date
Gayle Windham PhD, Committee Member	Date
Laura Gaydos, PhD Associate Chair for Academic Affairs, Executive MPH program	Date

BLOOD METAL CONCENTRATIONS AND TIMING OF PUBERTAL ONSET IN A LONGITUDINAL COHORT OF GIRLS, NORTHERN CALIFORNIA, 2006–2011

BY

Jason Andrew Wilken
M.P.H., Emory University, 2016
Ph.D., University of Nebraska Medical Center, 2004
B.S., University of Nebraska—Lincoln, 1998

Thesis Committee Chair: Dr. Lyndsey Darrow, Ph.D.

An abstract of
A Thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements of the degree of
Master of Public Health in the Executive MPH program
2016

Abstract

BLOOD METAL CONCENTRATIONS AND
TIMING OF PUBERTAL ONSET IN A
LONGITUDINAL COHORT OF GIRLS,
NORTHERN CALIFORNIA, 2006–2011

BY
Jason Andrew Wilken

Background: Endocrine-disruptive toxicants might alter the complex interplay of hormones that regulates timing of pubertal onset. The few studies of metals as potential disruptors of pubertal timing have yielded inconsistent results. We investigated the associations between blood concentrations of arsenic, cadmium, lead, manganese, mercury, and uranium and pubertal onset among girls.

Methods: Study participants included 313 Kaiser Permanente Northern California members followed at annual intervals during 2004–2011, who provided a blood specimen during their first, second, or third annual clinical visit. Metal concentrations were measured in serum from the first blood specimen available for each participant. Pubertal onset was defined as Tanner stage ≥2 for breast (thelarche) or pubic hair (pubarche) development. Associations between blood metals concentrations and pubertal onset were assessed by multivariable logistic regression and Cox proportional hazards modeling, controlling for age at blood draw, race, body mass index, annual family income, and primary caregiver's educational attainment.

Results: At blood draw, participants were age 6.5–10.1 (median 7.6) years, and 10% and 12% had attained thelarche and pubarche, respectively. Participants were followed 0–5 years (median 4). Most (91% and 88%) had attained thelarche and pubarche, respectively, at most recent clinical visit. Median ages of attaining thelarche and pubarche were 10.3 and 10.6 years, respectively. Odds of having achieved thelarche or pubarche at time of blood draw were not associated with metal concentrations after adjusting for covariates. Using time of blood draw to time of exam at which pubertal onset was observed as the follow-up interval, decreased risk of pubarche was associated with blood concentrations of arsenic (adjusted hazard ratio [aHR] 0.84, 95% confidence interval [CI] 0.71, 1.00, P=0.05; per 1-log increase), cadmium (aHR 0.71, 95% CI 0.55, 0.92, P<0.01; cadmium >limit of detection), and manganese (aHR 0.94, 95% CI 0.91, 0.98, P<0.01; per µg/L); similar associations were observed when birth to time of follow-up exam at which pubertal onset was observed was the follow-up interval.

Summary: Higher prepubertal blood concentrations of arsenic, cadmium, and manganese are associated with later pubarche.

BLOOD METAL CONCENTRATIONS AND TIMING OF PUBERTAL ONSET IN A LONGITUDINAL COHORT OF GIRLS, NORTHERN CALIFORNIA, 2006–2011

BY

Jason Andrew Wilken
M.P.H., Emory University, 2016
Ph.D., University of Nebraska Medical Center, 2004
B.S., University of Nebraska—Lincoln, 1998

Thesis Committee Chair: Dr. Lyndsey Darrow, Ph.D.

A Thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements of the degree of
Master of Public Health in the Executive MPH program
2016

Table of Contents

TABLES

CHAPTER I: INTRODUCTION

PUBERTY AND THE MECHANISMS REGULATING PUBERTAL ONSET

Puberty is the process of physical development that marks the transition into sexual maturity. Rather than being a single "endpoint" of sexual maturation (i.e., attainment of fertility), puberty consists of a series of overlapping developmental processes with variable timing. Historically, for females, menarche has been the most commonly described pubertal endpoint for population studies of sexual maturity. Contemporary views of the mechanisms that control pubertal onset are increasingly more refined and complex, accounting for development of secondary sexual characteristics (e.g., development of breasts, pubic hair, axillary hair, body odor, acne, etc.).

Puberty can be described as a complex series of changes in hormone production, resulting in the attainment of fertility and secondary sexual characteristics. Among the major alterations in hormonal production/function during puberty are: 1) increasing hypothalamic pulsatile secretion of gonadotropin releasing hormone (GnRH); 2) GnRH stimulation of the pituitary to produce gonadotropins (luteinizing hormone [LH] and follicle stimulating hormone [FSH]); 3) LH and FSH stimulating ovarian maturation; 4) increasing production of estrogens and, to a lesser extent, androgens by the developing ovary; 5) increasing production of androgens by the adrenal glands when stimulated by adrenocorticotropic hormone produced by the pituitary (i.e., "adrenarche"); 6) androgen-dependent development of sebaceous glands, apocrine glands, and pubic hair development (i.e., "pubarche"); 7) estrogen-dependent breast development (i.e., "thelarche"); and 8) maturation of ovarian follicles, release of competent oocytes, and commencement of menstrual cycles (i.e., "menarche") [1].

No single endpoint fully captures the complex interplay of processes governing sexual development. Increasingly, studies of sexual maturation have focused on earlier developmental outcomes (i.e.,

thelarche and pubarche). The timing of thelarche and pubarche incompletely accounts for variability in the timing of the other; correlations of 0.34–0.74 have been observed in reports spanning decades [2-6]. The incomplete overlap of thelarche and pubarche timing reflects their common upstream, but distinct downstream signaling components, in that thelarche is driven by a hypothalamic-pituitary-gonadal axis and pubarche is driven by a hypothalamic-pituitary-adrenal axis.

STAGES OF PUBERTY

Breast development and pubic hair development are among the major physiological processes defining pubertal onset. Breast development is largely dependent on the actions of estrogen, a hormone synthesized by the ovaries, and pubic hair development is largely dependent on the actions of androgens, synthesized by adrenal glands [1]. Thelarche is typically the first physical sign of pubertal onset in girls, although pubarche sometimes occurs first. A five-stage classification scheme for describing pubertal onset and progression developed by Tanner and colleagues [7], is the contemporary standard for assessing sexual development[a]. These Tanner stages of pubertal development are shown in in Figure 1. Tanner breast stage 1 and Tanner pubic hair stage 1 are considered prepubertal [1]. The first stage of pubertal breast development, Tanner breast stage 2, is defined by the appearance of a subalveolar bud which may not be visible but can be palpated. Tanner breast stage 3 is defined as visible enlargement and elevation of the entire breast. Tanner breast stage 4 includes the initiation of alveolar mounding. Tanner breast stage 5 is represented by the development of the adult breast contour.

[a] The five Tanner stages of breast and pubic hair development are modified from the five-stage classification scheme described by Reynolds and Wines [5]. More recently, Tanner stages have sometimes been renamed "sexual maturity rating." Source: Biro, F., Personal Communication, Feb 24, 2016.

The first stage of pubic hair development, Tanner pubic hair stage 2, is characterized by the development of straight, light hairs that are longer than typical vellus hair. In Tanner pubic hair stage 3, sexual pubic hair (i.e., long, curly, dark hair derived from androgen-regulated synthesis of keratins unique to sexual hair) begins to develop on the labia. In Tanner pubic hair stage 4, sexual hair is expressed in the pubis, and in Tanner pubic hair stage 5, sexual hair is expressed in an inverted triangle pattern on the pelvis and legs.

Tanner stages might coincide or occur so close in time that they cannot be clearly distinguished. Girls also sometimes undergo transient developmental regression, in that on physical examination at a later date a girl might have a lower Tanner stage.

SECULAR TRENDS IN PUBERTAL TIMING

A trend of decreasing age of pubertal onset among girls in the United States has been documented [8-11]. Reynolds and Wines reported a median age of thelarche of 10.8 years and pubarche of 11.0 years in a longitudinal study of girls followed 1930–1948[b] [5]. Foster et al. reported a median age of thelarche of 10.4 years among white girls and 10.2 years among black girls, and a median age of pubarche of 10.9 years among white girls and 10.1 years among black girls in a cross-sectional study of girls examined in 1973 and 1974 [12]. Herman-Giddens et al. reported in the cross-sectional Pediatric Research in Office Settings (PROS) study median ages of thelarche of 10.0 years for white girls and 8.9 for black girls, and median ages of pubarche of 10.5 years for white girls and 8.8 for black girls examined in 1992 and 1993 [13]. And in a cross-sectional analysis of National Health and Nutrition Examination Survey (NHANES)

[b] Stage 2 of both the five-stage classification scheme of Reynolds and Wines and the five-stage Tanner scheme are similar; the median ages presented here represent attainment of stage 2 by the Reynolds and Wines classification scheme.

1988–1994 data, Sun et al. and Wu et al. reported median ages of thelarche of 10.3–10.4 years for white girls and 9.5 for black girls, and median ages of pubarche of 10.6 years for white girls and 9.5 for black girls [14, 15]. These were considered earlier onset than clinical standards at the time.

Biro et al reported median age of thelarche among non-Hispanic white, black, Asian, and Hispanic girls as 9.3, 8.8, 9.7, and 9.7 years, respectively, among girls followed 2004–2011 in the longitudinal Breast Cancer and the Environment Research Program (BCERP) cohort [16]. The authors compared their findings to those of the PROS and NHANES studies, and found that the age of thelarche in their cohort was indeed younger than in earlier cohorts. Biro et al. ascribed these differences more to a decrease in age of thelarche among black participants than among non-Hispanic white participants, but also posited that much of the difference was attributable to differences in body mass index (BMI), with higher BMI more prevalent among black girls.

CONSEQUENCES OF ALTERED PUBERTAL TIMING

Puberty is one of numerous coincident developmental changes including growth and neural development. Endocrine networks activated during puberty have profound effects on physical and neurological development, and dysregulation of pubertal onset can result in serious health consequences. Adolescent risk behaviors and health effects associated with earlier age at puberty include depression, anxiety, early sexual activity, substance abuse, smoking, and suicide [17-28]. Early puberty has also been associated with adverse health outcomes later in life, including breast, endometrial, and ovarian cancer, polycystic ovary syndrome, metabolic syndrome, and depression [24, 27, 29-33]. Only a limited number of studies have evaluated associations between later pubertal onset

and health effects. Late pubertal onset has been associated with increased risk of later-life osteoporosis and risk of fractures, as reviewed by Chevalley [34].

POSITED FACTORS REGULATING PUBERTAL TIMING

A limited number of studies have evaluated anthropometric, demographic, social, and exogenous determinants of pubertal timing. Some have demonstrated an association between black race and/or Hispanic ethnicity and earlier thelarche, pubarche, and/or menarche [15, 16, 35, 36], and that genetics might account for a substantial amount of variability in pubertal timing [37]. However, a cross-sectional analysis of black South African girls in 1988 revealed a mean age of thelarche of 10.4 years [38], similar to that for white U.S. girls reported by cross-sectional analysis of 1988–1994 NHANES data by Sun et al. and Wu et al. (see above), suggesting that the association between race and pubertal timing among U.S. girls are confounded by other factors. Others have demonstrated that overweight/obese girls attain earlier thelarche, pubarche, and/or menarche than normal weight girls [10, 39-44], and differences in thelarche timing observed across racial/ethnic groups are likely attributable, at least in part, to differences in BMI (which is, in turn, influenced by diet and exercise) [16, 43]

Studies addressing socioeconomic status as a predictor of thelarche and menarche have produced inconsistent findings, in some cases finding associations, mixed results based on the tested indicator, or finding no association [43, 45-50]. Deardorff et al. demonstrated that at least some of the disparity of menarchal timing between white vs. Hispanic and black girls might be attributable to socioeconomic factors [51]. Other studies have identified relationships between timing of thelarche and pubarche and neighborhood characteristics (i.e., availability of nearby safe recreation facilities such as parks and walking trails) [52] and father absence [53], and between menarche and infant-mother attachment [54],

early maternal harshness (i.e., hostile and/or controlling parenting styles) [55], and mother's age of pubertal onset [56, 57].

Toxicant exposure: A growing body of literature suggests that some environmental toxicants are endocrine-disruptive in humans, and might alter pubertal timing by inhibiting or mimicking endocrine function. Exposure to polybrominated biphenyls *in utero* and by breast feeding has been associated with earlier menarche and pubarche in a Michigan cohort [58], and exposure to polychlorinated biphenyls and dichlorodiphenyldichloroehylene (a byproduct of DDT) *in utero* has been associated with earlier thelarche and pubarche in a North Carolina cohort [59]. Later thelarche and pubarche have been associated with phthalate exposure in a BCERP study [13]. A meta-analysis identified an association between earlier menarche and prenatal tobacco smoke exposure [20], while later pubertal onset has been associated with prenatal alcohol exposure [60]. Later age of thelarche has been associated with higher blood concentrations of polybrominated diphenyl ethers, polychlorinated bisphenols, and organochlorine pesticides in the BCERP cohort [61].

Metals as Potential Regulators of Pubertal Timing

Metals have received relatively little attention as a disruptor of pubertal timing. Metals exist in numerous forms with differing bioavailability and reactivity (e.g., elemental, organic, free or carrier-bound, etc.), and numerous mechanisms of endocrine disruption by metals have been described [62, 63]. Four metal toxicants, arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) are among the World Health Organization's "ten chemicals of major health concern" due to their myriad health effects, persistence in the environment, and ubiquity

(http://www.who.int/ipcs/assessment/public_health/chemicals_phc/en). These four metals, plus manganese (Mn) and uranium (U) are the primary exposures evaluated in this thesis.

Arsenic: Arsenic plays no known role in normal human physiologic processes. Inorganic arsenic is more toxic than organic arsenic due to differences in the metabolism and bioavailability of these isoforms. Humans can be exposed to inorganic arsenic through inhalation and ingestion, and common sources of arsenic include industrial processes (especially mining, smelting, and the legacies of these processes), tobacco smoke, and drinking water; the major source of human exposure to organic arsenic exposure is diet [64]. Chronic arsenic exposure is associated with adverse effects including neuropathies, gastrointestinal symptoms, cardiovascular disease, and diabetes. Arsenic delays pubertal onset in rodent models (as measured by day of vaginal opening) [65, 66] and delayed menarche among women in the Bengal Delta [67, 68] has been associated with arsenic groundwater contamination.

Cadmium: Cadmium plays no known role in normal human physiologic processes. Humans can be exposed to cadmium through inhalation and ingestion, and common sources of cadmium include industrial processes (especially mining, smelting, and the legacies of these processes), fuel exhaust, tobacco smoke, electronic wastes, and drinking water [69]. Cadmium is a carcinogen, and cadmium exposure is also associated with pneumonitis and renal disease [69]. In one laboratory study, inclusion of cadmium in drinking water resulted in delayed pubertal onset among rat pups [70]. Gollenberg et al. reported that cadmium exposure might also delay pubertal timing in humans (using blood inhibin B concentrations as an endpoint rather than clinical examination) [71]. Interdonato et al. reported an association between urinary cadmium concentrations and delayed pubertal onset in boys [72], and Kim et al. reported delayed menarche and pubarche in girls associated with increased blood cadmium concentrations [73].

Lead: Lead plays no known role in normal human physiologic processes. Humans can be exposed to lead through inhalation and ingestion, and common sources of lead exposure include lead-based paint, industrial processes (especially mining, smelting, and the legacies of these processes), exhaust from leaded fuels (and legacies thereof), electronic wastes, and drinking water [74]. Lead is a well-established neurotoxin, and lead exposure is also associated with cardiovascular and renal disease [74]. Early pubertal onset in mice can be induced with the introduction of low-dose lead into diet [75]. Cross-sectional studies suggest lead is associated with delayed pubic hair development [76] and menarche [15, 16]. A large longitudinal study in the United States found no association between prenatal lead exposure and timing of menarche, thelarche, or pubarche [77], while another large longitudinal study in South Korea identified an association with earlier thelarche [73].

Manganese: Manganese is an essential trace element and enzyme cofactor. Manganese deficiency is associated with birth defects and retarded growth and fertility, and very high exposures can cause manganism, a Parkinson's-like disease [78]. People are most often exposed to manganese through diet and inhalation (especially in and near mining and metalworking). In rat models, manganese has been shown to stimulate gonadotropin secretion and accelerate pubertal onset (i.e., vaginal opening) [79] and mammary gland development [80]. We are not aware of any studies that evaluated the role of manganese in human pubertal timing.

Mercury: Mercury plays no known role in normal human physiologic processes. The elemental, inorganic, and organic forms of mercury have different toxic effects. Humans can be exposed to mercury through inhalation (elemental mercury), diet (especially organic mercury in fish), drinking water, soil contamination, and dermal applications (e.g., mercury-containing creams, especially inorganic mercury) [81]. Mercury is both an acute and chronic neurotoxin, and is also associated with immunodeficiencies

and renal disease. Mercury concentrations in hair were inversely associated with age of menarche in a cross-sectional study of Flemish girls [82].

Uranium: Uranium plays no known role in normal physiologic processes. Humans can be exposed to uranium by ingestion and drinking water, particularly in or near areas where uranium mining or processing occurs [83]. Uranium exposure is associated with kidney and lung disease. Limited data exists on human exposures to uranium, but uranium has estrogenic activities and has been shown to accelerate pubertal onset (i.e., vaginal opening) in mice [84].

SUMMARY OF CURRENT PROBLEM AND STUDY RELEVANCE

The research presented in this thesis explores the relationship between blood metal concentrations and pubertal timing in girls. This information may aid in prioritizing the classification of environmental toxicants as endocrine disruptors and to direct further research of metals as endocrine disruptors. The cohort described here is annotated with extensive longitudinal anthropometric data, and is therefore well-suited to assess onset of the earliest stages of puberty (i.e., thelarche and pubarche), and is amenable to both cross-sectional and longitudinal analyses. Multiple analytic approaches might identify associations between blood metal concentrations and pubertal timing in girls and reconcile differing conclusions drawn from cross-sectional and longitudinal approaches, thereby contributing to our understanding of the determinants of pubertal timing and potentially leading to interventions on health outcomes related to pubertal timing.

THESIS PURPOSE STATEMENT

Test associations between blood metal concentrations and pubertal timing (defining puberty as attaining thelarche or pubarche) by cross-sectional and longitudinal approaches.

The specific research questions and analytic approaches in this thesis are:

1. **Do blood metal concentrations vary by pubertal status at time of blood draw?**

   Analytic approach: Compare mean blood metal concentrations by pubertal status at time of blood draw by Student's T-test. Estimate odds ratios of having achieved puberty at time of blood draw by logistic regression, adjusting for demographic and anthropometric covariates.

   Null hypotheses: Mean blood metal concentrations do not vary by pubertal status time of blood draw. After adjusting for covariates, blood metal concentrations are not associated with odds of having attained puberty at time of blood draw.

2. **Are blood metal concentrations associated with risk of puberty?**

   Analytic approach: Estimate hazard ratios of pubertal onset by longitudinal Cox proportional hazards analysis, adjusting for demographic and anthropometric covariates.

   Null hypothesis**:** After adjusting for covariates, blood metal concentrations are not associated with risk of pubertal onset.

CHAPTER II: METHODOLOGY

INTRODUCTION

This thesis is an analysis of data from a prospective cohort designed to identify determinants of pubertal timing in girls. The BCERP is a multi-site study funded by the National Institute of Environmental Health Sciences and the National Cancer Institute, and one of the core BCERP studies is the Puberty Study (http://www.bcerc.org/granteesPS.htm). The Puberty Study enrolled females aged 6–8 (hereafter referred to as "girls") at three sites (New York, Cincinnati, and the San Francisco Bay Area) beginning in 2005. Participants in the BCERP puberty study underwent a physical examination upon enrollment (i.e., baseline) and subsequent physical examinations annually or semi-annually. The subject's parent or legal guardian completed a questionnaire at the time of the subject's enrollment and annual questionnaires at the time of the subject's physical examination.

Members of Kaiser Permanente Northern California (KPNC) born and still residing in the San Francisco Bay area were included in the BCERP San Francisco Bay cohort, referred to as the Cohort of Young Girls' Nutrition, Environment, and Transitions (CYGNET); the CYGNET cohort is the focus of the analyses presented in this thesis. The author of this thesis did not have access to identifying information for any of the girls enrolled in the CYGNET study, and Emory University Institutional Review Board determined that this research project did not meet the definition of "Research Involving Human Subjects."

POPULATION AND SAMPLE

Prospective CYGNET enrollees were identified from KPNC databases and were born in and currently resided in the catchment area of San Francisco, Marin County, and certain communities in Alameda and Contra Costa County, California. The girls' pediatricians were notified of the CYGNET project's intent to

contact her family with an invitation letter to participate in the CYGNET study; if no objection was raised by the pediatrician, the girls' families were mailed a letter (including a reply post card) inviting them to join the study. If the family replied that they would like to be contacted by a CYGNET representative or if no response had been received after 3 weeks, a CYGNET representative would call the family to describe the study, confirm eligibility, and, if the family agreed, enroll the girl and schedule a baseline clinical visit.

The CYGNET study team identified 2,245 eligible girls; of these, 444 were enrolled in the study. Of these 444, 324 both 1) provided a blood specimen during the baseline clinical visit and/or first or second subsequent clinical visit; and 2) underwent an assessment of breast and pubic hair development during the same clinical visit where a blood specimen was provided (described in "Research Design and Procedures," below). Blood metal concentrations were measured (also described in "Research Design and Procedures," below) in the first collected specimens for 314 of these girls. Of these 314 girls, one was identified with hypothyroidism by laboratory testing [85]; that girl was excluded from this study. The study cohort for this thesis, therefore, consists of 313 girls that were: 1) members of KPNC at birth and enrollment (and therefore had health insurance and access to care); 2) enrolled at age 6–7 in the CYGNET study; 3) provided a blood sample during the baseline clinic visit or first or second annual follow-up visit for which metal concentrations were measured; 4) underwent an assessment of breast and pubic hair at the time of the blood draw. Details on the girls' participation in follow-up visits, anthropometric and demographic characteristics, and blood metal concentrations are provided in Chapter III.

RESEARCH DESIGN AND METHODS

The research design was a primary analysis of longitudinal clinical, anthropometric, demographic, and laboratory data collected for each participant. Pubertal status was assessed at each clinical visit as described in "Tanner Staging," below. Blood specimens were collected from each participant during one of the first three clinical visits (i.e., at baseline, follow-up year 1, or follow-up year 2), and concentrations of arsenic, cadmium, lead, manganese, mercury, and uranium were measured as described in "Measurement of Blood Metals," below. The major research questions were to assess: (1) whether blood metal concentrations vary by pubertal status at the time of blood draw; and (2) whether blood metal concentrations are associated with risk of pubertal onset, longitudinally.

Tanner Staging: Pubertal onset was ascertained by a standardized method based on Tanner staging. The principles of Tanner staging are described in Chapter I. The Tanner scale defines five stages from pre-puberty to adult for breast and pubic hair development. Girls' breast and pubic hair development were assessed by clinic staff trained in standard methods [86]. Breast development was assessed through both observation and palpation, and pubic hair development was assessed by observation. BCERP studies have previously reported high inter-rater agreement of girls' Tanner breast stages [86]; inter-rater agreement of girls' Tanner pubic hair stages has not been reported.

Anthropometric and Demographic Covariates: Previous studies from the BCERP and others have identified BMI [10, 11, 16, 42, 87-90], race/ethnicity [9, 13, 15, 16, 35, 36, 51, 67], and socioeconomic status [43, 46-48, 51, 91] as predictors of pubertal onset, although these studies do not establish causality. Following prior practice [85], girls' body mass index (BMI) at time of blood draw was chosen as

a priori as the anthropometric covariate of interest, and age at time of blood draw, race/ethnicity, primary caregiver's educational attainment, and annual family income as obtained from the first annual questionnaire were chosen as a priori demographic covariates of interest.

Variables were categorized as described below, in accordance with prior BCERP publications [61, 85, 92].

- Age at blood draw was calculated in months. Participant age at blood draw was also categorized as 6.0–6.9, 7.0–7.9, 8.0–8.9, and ≥9.0.
- Participant's race/ethnicity was classified into mutually-exclusive categories in the following priority order: black (regardless of Hispanic ethnicity), Hispanic (including any race other than black), non-Hispanic Asian or Pacific Islander, and non-Hispanic white (referent).
- BMI was calculated as weight/height-squared (kg body weight/m^2) at the time of blood draw, and then classified as <85th (referent) or ≥85th (defining obesity) national percentile, using age and sex-specific CDC growth charts [93].
- Primary caregiver's educational attainment was categorized as ≤high school, some college, college degree (referent).
- Annual family income was categorized as <$50,000, $50,000–$100,000, and >$100,000 (referent).

Race, primary caregiver's educational attainment, and annual family income were compared to publicly-available US Census American FactFinder data (http://factfinder.census.gov). FactFinder-reported American Community Survey data representing the San Francisco-Oakland-Hayward Metropolitan Statistical Area (encompassing San Francisco, Marin, Alameda, Contra Costa, and San Mateo Counties) for 2007 was chosen as a validation dataset, reflecting the geographic distribution of girls and the timing of their blood draws.

Measurement of Blood Metals: Blood arsenic, cadmium, lead, manganese, mercury, and uranium concentrations were measured by the Environmental Health Laboratory Branch of the California Department of Public Health using methods previously described [94, 95]. Briefly, sera were separated from whole blood, and serum samples were injected onto an inductively-coupled mass spectrometer, in duplicate, and concentrations were defined as the mean of the duplicate samples. Metal concentrations are presented in µg/L, except for lead which by convention is presented as µg/dL. The limit of detection (LOD) for these assays is defined by the Environmental Health Laboratory Branch as follows: arsenic, 0.12 µg/L; cadmium, 0.14 µg/L; lead, 0.01 µg/dL; manganese, 1.50 µg/L; mercury, 0.06 µg/L; uranium, 0.02 µg/L [96]. Following prior practice [97], LOD/√2 was substituted as the value when blood metals were <LOD.

Blood metal concentrations were compared, when possible, to publicly-available NHANES laboratory data (http://wwwn.cdc.gov/nchs/nhanes/search/datapage.aspx?Component=Laboratory). Blood arsenic and uranium concentrations are not reported in NHANES. Cadmium, lead, and mercury concentrations were measured and reported from multiple NHANES 2-year cycles. Blood concentrations of manganese were only reported in the 2011–2012 NHANES cycle. For sake of consistency among metals, we chose the 2011–2012 NHANES cycle as a comparison dataset for cadmium, lead, manganese, and mercury. Blood metal concentrations were also compared to the Mayo Medical Laboratories published reference values (http://www.mayomedicallaboratories.com/index.html).

Statistical Methods:

Definition of timing of pubertal onset and longitudinal intervals. For girls who attained puberty after the time of blood draw, age of thelarche and pubarche was defined as the girl's age at clinical examination when first consistent Tanner breast or pubic hair stage ≥2 was observed (i.e., no subsequent stage 1), as

previously described [85]. Therefore, age of puberty was not assigned for girls who attained puberty before the blood draw or that had not attained puberty by the time of the most recent clinical examination.

Age of girls prepubertal vs. pubertal at time of blood draw was compared by Student's T-test. Age at thelarche and age at pubarche, among girls prepubertal at time of blood draw, were compared by Pearson's correlation. To evaluate the degree by which timing of thelarche and pubarche account for each other, time from blood draw to thelarche and to pubarche were compared using both intervals described below by Pearson's correlation.

To evaluate whether timing of thelarche and pubarche were indeed distinct, timing of thelarche and pubarche were also compared by the Kaplan-Meier method. For these analyses (and subsequent Cox proportional hazards analyses, see below), onset of puberty was the analysis endpoint, and we conducted analyses using two intervals, with follow-up time in months and age in months to define survival time. In the first interval, the start point was the time of blood draw, and the end point was the clinical visit at which first consistent Tanner breast or pubic hair stage ≥2 was observed (i.e., no subsequent stage 1), or the most recent clinical visit for girls who had not reached puberty by the end of the study [85]; the time axis for this interval is follow-up time. In the second interval, the start point was birth, and endpoint is the same as the first interval; the time axis for this interval is defined by girls' age. Since pubertal onset occurs between clinical visits, both intervals overestimate age at pubertal onset (except for girls who did not achieve puberty by the time of most recent clinical visit, for whom age of puberty cannot be assigned). We compared product limit estimates by log-rank test for attaining thelarche and pubarche for both intervals described above. In accordance with the methods of Kleinbaum and Klein: 1) girls who had already attained thelarche or pubarche at time of blood draw were left-censored from analyses using the first interval and were excluded from analyses using the

second interval; 2) girls that were lost to follow-up and had not achieved thelarche or pubarche at the time of the last clinical visit were right-censored (i.e., still contributed to the model though an endpoint was not attained); and 3) girls that were followed throughout the study and that had not achieved thelarche or pubarche at the time of their final clinical visit were also right-censored [98].

Associations between anthropometric and demographic variables and pubertal onset: We used three approaches of differing analytic complexity to evaluate the association between anthropometric and demographic variables (age at blood draw, race, BMI, annual family income, and primary caregiver's educational attainment) and pubertal onset. Two of the approaches utilized cross-sectional data to identify factors associated with pubertal status at the time of blood draw. The third approach utilized longitudinal data in a survival analysis to identify predictors of pubertal timing.

- In the first method, anthropometric and demographic variables were compared among girls prepubertal (had not attained thelarche/pubarche) vs. pubertal (had attained thelarche/pubarche) at time of blood draw by chi-square analysis; age was categorized as described above.
- The second method consisted of calculating odds ratios (OR) for having attained thelarche or pubarche at time of blood draw by logistic regression. Crude ORs (cOR) and adjusted ORs (aORs) were calculated for each anthropometric and demographic variable, with aORs calculated adjusting for each anthropometric and demographic variable simultaneously; age was treated as a continuous variable. Girls missing data for any of these covariates were excluded from logistic regression models. When confounding was evident (defined as ≥10% difference between cOR and aOR), the confounding covariate was identified by testing

additional models including only one other covariate at a time; the results of these additional models are presented in the text, not in tables.

- In the third method, we assessed risk of future pubertal onset by calculating hazard ratios (HRs) for each anthropometric and demographic variable using Cox proportional hazards modeling. Models were evaluated using the two intervals defined in "Definition of timing of pubertal onset and longitudinal intervals." When using the first interval (time of blood draw to clinical visit where pubertal onset was first observed), girls that had attained puberty by the time of blood draw were left-censored. When using the second interval (birth to clinical visit where pubertal onset was first observed), girls that had attained puberty by the time of blood draw were excluded from the model. For both intervals, girls that had not attained puberty by the time of most recent clinical visit were right-censored. Crude HRs (cHR) and adjusted HRs (aHRs) were calculated for race, BMI, annual family income, and primary caregiver's educational attainment, with aHRs calculated adjusting for each of these anthropometric and demographic variables simultaneously. For analyses using the first interval (time of blood draw to time of pubertal onset), aHRs were also adjusted for age at blood draw (treated as a continuous variable). Girls that were missing data for any of these covariates were excluded from these models. As described above, when confounding was evident, the confounding covariate was identified by testing additional models including only one other covariate at a time.

Categorization of blood metals: continuous or log-transformed vs. categorical. The distribution of each blood metal was plotted. Blood metals concentrations were treated as follows in subsequent statistical analyses:

- Metals whose distributions were mostly normal (as assessed by visual analysis of concentration distribution and comparing median and mean concentrations) were treated as continuous variables for some analyses, and categorized into quartiles for others, and arithmetic means of these metals are presented.
- Metals whose distribution was highly skewed were log-transformed, and log-transformed concentrations were assessed for normality; normally-distributed log-transformed concentrations were used for some analyses, and categorized into quartiles for other analyses (see below). Geometric means (calculated as the antilog of the arithmetic mean of log-transformed concentrations) of these metals are presented.
- Metals where >25% of measurements were ≤LOD (i.e., could not be meaningfully divided into quartiles) were dichotomized as detected or not (≤ vs. >LOD) for all analyses.

Associations between blood metals. Associations between normally-distributed (or log-normally distributed) blood metals were assessed by Pearson's correlation. Associations between blood metals categorized as ≤ vs. >LOD and blood metals normally-distributed (or log-normally distributed) were assessed by Student's T-test. Blood metals that were associated with each other (i.e., collinear) were considered ineligible for co-inclusion in subsequent multivariable models, as described below.

Associations between anthropometric/demographic variables and blood metals: Blood metal concentrations were compared by covariate categories (i.e., age at blood draw categorized by year, race, BMI, annual family income, primary caregiver's educational attainment). Normally-distributed (or log-normally distributed) blood metals were compared by Student's T-test or by analysis of variance (ANOVA), and metals categorized by ≤ vs. >LOD were compared by chi-square analysis.

Associations between blood metals concentrations and pubertal onset: Similar to our approach to identify associations between anthropometric and demographic variables, we used three approaches of differing analytic complexity to evaluate the association between blood metal concentrations and pubertal onset, as follows:

- In the first method, blood metal concentrations were compared among girls prepubertal (had not attained thelarche/pubarche) vs. pubertal (had attained thelarche/pubarche) at time of blood draw. Metals with normal or log-normal distribution were compared by Student's T-test. Metals categorized as < vs. ≥LOD, were evaluated by chi-square analysis. These analyses are presented below under "Research Question #1."
- In the second method, we calculated cORs and aORs for having attained thelarche or pubarche at time of blood draw for each metal; aORs were adjusted for each covariate as described above. cORs were also calculated for each blood metal categorized into quartiles, and trends by quartile were assessed by the Cochrane-Armitage trend test; aORs were not calculated for each blood metal categorized as quartiles because there were insufficient girls that had attained puberty at the time of blood draw to adjust for covariates. These analyses are presented below under "Research Question #1."
- In the third method, we assessed risk of future pubertal onset by calculating HRs for each metal using the two intervals defined above, and girls were left-censored (or excluded, when using the second interval) or right-censored. cHRs and aHRs (adjusted for race, BMI, annual family income, and primary caregiver's educational attainment) were calculated for each metal; for analyses using time of blood draw to time of pubertal onset as the interval, aHRs were also adjusted for age at blood draw (treated as a continuous variable). cHRs and aHRs for

each metal with normal or log-normal distribution were also calculated categorized into quartiles, and trends by quartile were assessed by the Cochrane-Armitage trend test. If more than one metal was associated with pubertal timing, those metals were also tested simultaneously in an additional model; however, metals whose concentrations were associated with each other by Pearson's correlation were not included in the same model due to concerns about collinearity. If multiple metals were simultaneously associated with risk of thelarche or pubarche, we evaluated their significance together in a backward selection model, with $P<0.05$ as criteria for remaining in the model. These analyses are presented below under "Research Question #2."

P-values <0.05 were considered significant, and *P*-values <0.10 were considered tending towards significance. All statistical analyses were performed by using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina).

CHAPTER III: RESULTS

INTRODUCTION

In this Chapter, demographics and timing of pubertal onset of the sampled cohort are presented, followed by the distribution of blood metal concentrations, followed by the findings for the two research questions; each section is followed by a summary of the results.

PARTICIPANT ANTHROPOMETRICS AND DEMOGRAPHICS

Age at blood draw: Age at blood draw ranged from 6.5–10.1 years (7.8 mean, 7.6 median), with 72% aged 7.0–8.9 years (Table 1). Age at blood draw is treated as a continuous variable (months) for all subsequent analyses, unless otherwise specified.

Race/ethnicity: The sample was racially diverse, with 42% non-Hispanic white, 22% black, 12% Asian, and 23% Hispanic (Table 1). By comparison, 2007 American Community Survey 1-Year Estimate for the San Francisco-Oakland-Hayward Metropolitan Statistical Area reported population distributions of 46% non-Hispanic White, 10% black, 22% Asian, and 21% Hispanic. This oversampling of black girls and undersampling of Asian girls might reflect the large number of CYGNET enrollees that received medical care at Kaiser Permanente medical centers in two communities with high proportions of black residents (Richmond and Oakland) [99]. Age at blood draw did not vary by race as evaluated by ANOVA (mean age in years: non-Hispanic white, 7.8; black 7.7; Asian 8.0; Hispanic 7.8; P=0.28).

BMI: Girls' BMI at time of blood draw ranged from the 1st–100th of the national percentile, using age and sex-specific CDC growth charts (median 71st percentile). Sixty nine percent (69%) had a BMI <85th national percentile and 31% had a BMI ≥85th national percentile (Table 1).

Primary caregiver's educational attainment: The primary caregiver of half of the girls (50%) had a college degree, 31% had completed some college, and 19% had completed ≤high school (Table 1). By comparison, 2007 American Community Survey 1-Year Estimate for the San Francisco-Oakland-Hayward Metropolitan Statistical Area reported that among persons aged 25 years or older, 43% had a college degree, 25% had completed some college, and 32% had completed ≤high school; however, educational attainment estimates specifically for members of this population with children are not available. The high prevalence of primary caregivers having a college degree or some college in our cohort may be reflective of sampling KPNC members, who are all insured and have access to health care, as well as the degree of commitment needed to participate in a longitudinal study.

Annual family income. The girls' annual family income was <$50,000 for 21%, $50,000–$100,000 for 37%, and >$100,000 for 41% (Table 1). By comparison, 2007 American Community Survey 1-Year Estimate for the San Francisco-Oakland-Hayward Metropolitan Statistical Area reported annual family incomes of <$50,000 for 35%, $50,000-$100,000 for 29%, and >$100,000 for 36%. The higher family income among our cohort is likely reflective of sampling KPNC members. Annual family income for 4 girls was missing.

TIMING OF PUBERTAL ONSET

Girls were followed for up to 5 years following their blood draw for this analysis (Table 2). Ten percent (10%) of girls had attained thelarche at time of blood draw, and 91% had attained thelarche at the most recent clinical visit. Most girls (78%) were followed with annual Tanner breast staging for 4–5 years after blood draw. Based on age at examination, age of thelarche among the girls that had attained thelarche by the end of the study was 7.3–14.7 years (mean 10.4 years; median 10.3 years); these ages are overestimates, given that thelarche would have occurred during the interval between clinical visits. Girls that had achieved thelarche at time of blood draw were older than those that had not (9.0 vs. 7.7 years, Student's T-test $P<0.01$).

At the time of blood draw, 12% of participants had attained pubarche, and 88% had attained pubarche at the most recent clinical visit (Table 2). Most girls (73%) were followed with annual Tanner pubic hair staging for 4–5 years after blood draw. Based on age at examination, age of pubarche among the girls that had attained pubarche by the end of the study was 6.7–14.7 years (mean 10.6 years; median 10.7 years). Girls that had achieved pubarche at time of blood draw were older than those that had not (8.2 vs. 7.7 years, Student's T-test $P<0.01$).

By Pearson's correlation, we determined that:

- Age at attainment of thelarche and age of attainment of pubarche were correlated ($r=0.48$, $P<0.01$).
- Time from blood draw to attainment of thelarche and time from blood draw to attainment of pubarche were correlated ($r=0.61$, $P<0.01$).
- Time to puberty from time of blood draw was associated with time to puberty from time of birth for both thelarche ($r=0.85$, $P<0.01$) and pubarche ($r=0.88$, $P<0.01$).

These correlations demonstrate that timing of pubarche and thelarche incompletely account for one another. Indeed, significant differences were noted by Kaplan-Meier survival analysis with thelarche and pubarche as endpoints, whether the interval is time of blood draw to onset of puberty (log-rank P=0.02, Figure 2A) or birth to onset of puberty (log-rank P<0.01, Figure 2B).

These findings demonstrate that timing of thelarche and pubarche are indeed related but distinct for both intervals, justifying the use of (and comparing the results using) both intervals for longitudinal analyses. Therefore, as described later in this thesis, we compared longitudinal models using both intervals.

PUBERTAL STATUS AT TIME OF BLOOD DRAW BY DEMOGRAPHIC AND ANTHROPOMETRIC MEASUREMENTS

Pubertal status of girls at time of blood draw as assessed by Tanner breast staging and Tanner pubic hair staging was compared across age at blood draw categorized by year (6.0–6.9, 7.0–7.9, 8.0–8.9, ≥9.0), race, BMI, primary provider's education, and annual family income by Chi-square analysis. cORs of having attained thelarche or pubarche at time of blood draw were calculated for the individual anthropometric and demographic covariates, and aORs were calculated for each anthropometric and demographic covariate simultaneously; 4 girls were missing data for annual family income and were excluded from logistic regression analyses.

Thelarche:

Age at blood draw:

Chi-square analysis: Girls that were older at age of blood draw were more likely to have achieved thelarche at time of blood draw (ages 6.0–6.9, 0%; 7.0–7.9, 1%; 8.0–8.9, 14%; ≥9.0, 59%) (Table 3).

Logistic regression: A 1-year increase in age at blood draw was associated with increased odds of having attained thelarche at time of blood draw (cOR 16.04, 95% confidence interval [CI]: 6.47, 39.74) (Table 4). When adjusting for other covariates, this association was somewhat stronger (aOR 22.96, 95% CI: 7.38, 71.44) but no single covariate appeared responsible for this confounding.

Race:

Chi-square analysis: Black girls were more likely to have achieved thelarche at time of blood draw than white non-Hispanic girls (17% vs. 5%, *P*=0.05) (Table 3).

Logistic regression: Black girls had increased odds of having attained thelarche at time of blood draw relative to white girls (cOR 3.47, 95% CI 1.28, 9.42) (Table 4). This association increased in magnitude when adjusting for covariates (aOR 4.63, 95% CI 0.98, 21.89). The greater aOR vs. cOR for black race and lower aOR vs. cOR for Hispanic race suggests that other covariates confound these associations, but no single covariate appeared responsible for this confounding. The overall 3-degree of freedom test of race was not significant for either cOR (*P*=0.11) or aOR (*P*=0.17) of having achieved thelarche at time of blood draw.

BMI:

Chi-square analysis: Girls with BMI ≥ vs. <85th percentile were more likely to have attained thelarche at time of blood draw (16% vs. 7%, *P*<0.01) (Table 3).

Logistic regression: Girls with BMI ≥85th percentile had greater odds of having achieved thelarche at time of blood draw (cOR 2.29, 95% CI 1.06, 4.97) (Table 4). When adjusting for other covariates, this association increased in magnitude and tended towards significance (aHR 3.00, 95% CI 0.95, 9.45), but no single covariate appeared responsible for this confounding.

Primary provider's education:

Chi-square analysis: Girls whose primary caregiver's education attainment was ≤high school or some college education tended to be more likely to have achieved thelarche at time of blood draw than girls whose primary caregiver had a college degree (≤high school, 12%; some college, 14%; college degree ≥9.0, 6%, *P*=0.10) (Table 3).

Logistic regression: Girls whose primary caregiver had completed some college had increased odds of having achieved thelarche (cOR 2.62, 95% CI 1.07, 6.40) at time of blood draw relative to girls whose primary caregiver had a college degree (Table 4). This association decreased in magnitude and was not statistically significant when adjusting for covariates (aOR 1.20, 95% CI 0.31, 4.63), but no single covariate appeared responsible for this confounding. The overall 2-degree of freedom test of primary caregiver's educational attainment tended towards significance (*P*=0.09), but was not significant when adjusting for other covariates (*P*=0.96).

Annual family income:

Chi-square analysis: Girls with lower family incomes were more likely to have attained thelarche at time of blood draw (>$100,000, 4%; $50,000–$100,000, 9%; <$50,000, 21%; *P*<0.01) (Table 3).

Logistic regression: Girls with annual family income <$50,000 had increased odds of having attained thelarche (cOR 6.62, 95% CI 2.27, 19.34) at time of blood draw relative to girls with annual family income >$100,000 (Table 4). When adjusting for covariates, the association decreased in magnitude (aOR 5.44, 95% CI 1.00, 29.63), but no single covariate appeared responsible for this confounding. The overall 2-degree of freedom test of annual family income categories was significant when not adjusting for covariates (*P*<0.01), but was not significant when adjusting for covariates (*P*=0.12).

Pubarche:

Age at blood draw:

Chi-square analysis: Girls that were older at age of blood draw were more likely to have achieved pubarche at time of blood draw (ages 6.0–6.9, 7%; 7.0–7.9, 8%; 8.0–8.9, 14%; ≥9.0, 34%, *P*<0.01) (Table 3).

Logistic regression: A 1-year increase in age at blood draw was associated with increased odds of having attained pubarche (cOR 1.99, 95% CI: 1.32, 3.01) at time of blood draw (Table 4). When adjusting for other covariates, this association increased in magnitude (aOR 2.57, 95% CI: 1.56, 4.26), and race confounded this association (1-year increase in age at blood draw, adjusted for race, OR 2.62, 95% CI 1.60, 4.27).

Race:

Chi-square analysis: Black girls were more likely to have achieved pubarche at the time of blood draw than white non-Hispanic, Asian, or Hispanic girls (34% vs. 6%, 0%, and 7%, respectively, *P*<0.01) (Table 3).

Logistic regression: Black girls had greater odds of having attained pubarche at time of blood draw (cOR 7.92, 95% CI 3.31, 18.98) (Table 4). This association decreased in magnitude when adjusting for covariates (aOR 5.86, CI 2.04, 16.86), but no single covariate appeared responsible for this confounding. No Asian girls had attained pubarche at time of blood draw; therefore odds ratios could not be calculated. The overall 3-degree of freedom test of race was significant for both cOR and aOR estimates (*P*<0.01 for both) of having achieved pubarche at time of blood draw.

BMI:

Chi-square analysis: We observed no difference in likelihood of having achieved pubarche at time of blood draw among girls by BMI (Table 3).

Logistic regression: No association was observed between BMI and odds of having attained pubarche at time of blood draw (Table 4).

Primary provider's education:

Chi-square analysis: Girls whose primary caregiver's educational attainment was ≤high school or some college education were more likely to have achieved pubarche at time of blood draw, as compared to college degree (high school or less, 12%; some college, 21%; college degree ≥9.0, 6%, *P*<0.01) (Table 3).

Logistic regression: Girls whose primary caregiver had completed some college had increased odds of having achieved pubarche (cOR 4.48, 95% CI: 1.94, 10.32) at time of blood draw relative to girls whose primary caregiver had a college degree (Table 4). This association decreased in magnitude and was not statistically significant when adjusting for covariates (aOR 2.49, 95% CI 0.87, 7.10), but no single covariate appeared responsible for this confounding. The overall 2-degree of freedom test of primary caregiver's educational attainment was significant for pubarche (*P*<0.01), but was not significant when adjusting for other covariates (*P*=0.24).

Annual family income:

Chi-square analysis: Girls with lower family incomes were also more likely to have attained pubarche at time of blood draw (>$100,000, 7%; $50,000–$100,000, 10%; <$50,000, 24%; *P*<0.01) (Table 3).

Logistic regression: Girls with annual family income <$50,000 had increased odds of having attained pubarche (cOR 4.32, 95% CI 1.79, 10.43) at time of blood draw relative to girls with annual family income >$100,000 (Table 4). When adjusting for covariates, this association decreased in magnitude and was not statistically significant (aOR 1.68, 95% CI 0.49, 5.72), but no single covariate appeared responsible for this confounding. The overall 2-degree of freedom test of annual family income categories was significant when not adjusting for covariates (P<0.01) but was not significant when adjusting for covariates (*P*=0.47).

Summary. These results indicate that, by crude analysis, each demographic and anthropometric variable was associated with odds of either having achieved thelarche or puberty at time of blood draw. The association between each covariate and odds of having achieved thelarche or pubarche at time of blood

draw was confounded by other covariates. After adjusting for covariates, age at blood draw was the only variable associated with odds of having achieved both thelarche and pubarche at time of blood draw, with BMI associated only with odds of having achieved thelarche and race associated with only having achieved pubarche. The greater odds of having achieved thelarche vs. pubarche at time of blood draw associated with increased age at blood draw might reflective the difference in age of girls that had attained thelarche vs. pubarche at time of blood draw (mean 9.0 vs. 8.2 years). These findings support the practice of adjusting for these covariates (and, if confounding is evident, identifying the confounding variable if possible) in later analyses of associations between pubertal status/outcome and blood metal concentrations.

RISK OF PUBERTAL ONSET BY DEMOGRAPHIC AND ANTHROPOMETRIC MEASUREMENTS

As described in "Definition of timing of pubertal onset and longitudinal intervals", either of two intervals (time of blood draw to time of pubertal onset, and birth to time of pubertal onset) are appropriate for survival analyses. We conducted longitudinal analyses using both of these intervals by Cox proportional hazards modeling. cHRs for risk of thelarche and pubarche were calculated for anthropometric and demographic covariates (race, BMI, primary caregiver's education, and annual family income); age at blood draw (treated as a continuous variable) was included as a covariate for models where the interval was time of blood draw to time of puberty. aHRs were also calculated for each anthropometric and demographic covariate simultaneously. Participants who had attained thelarche (n=31) or pubarche (n=37) at the time of blood draw were left-censored from the first interval and were excluded from the

second interval. Four girls were missing data for annual family income and were excluded from this analysis.

Interval—time of blood draw to time of pubertal onset:

Thelarche

Age at blood draw: A 1-year increase in age at blood draw was associated with increased risk of thelarche (cHR 2.07, 95% CI: 1.76, 2.43; aHR 2.24, 95% CI: 1.89, 2.86) (Table 5).

Race: Black girls had increased risk of thelarche (cHR 1.61, 95% CI 1.18, 2.19) (Table 5). This association decreased slightly in magnitude when accounting for other covariates (aHR 1.44, 95% CI 1.02, 2.05), but no single covariate appeared responsible for this confounding. The overall 3-degree of freedom test of race was significant for both cOR and aOR estimates (P=0.04 and P=0.02, respectively).

BMI: We did not identify associations between dichotomized BMI at time of blood draw and risk of thelarche.

Primary provider's educational attainment: We did not identify any associations between primary provider's educational attainment and risk of thelarche (Table 5). Annual family income attenuated the effect estimates between primary caregivers' educational attainment and risk of thelarche (≤high school

adjusted for income, HR 0.82, 95% CI 0.57, 1.17; some college, adjusted for income, HR 1.02, 95% CI 0.76, 1.38).

Annual family income: Girls with annual family income <$50,000 were at increased risk of thelarche (cHR 1.59, 95% CI 1.16, 2.17) (Table 5). This association was more pronounced when adjusting for other covariates (aHR 1.92, 95% CI 1.28, 2.88), but no single covariate appeared responsible for this confounding. The overall 3-degree of freedom test of family income categories was significant for both cOR and aOR estimates (*P*<0.01 and *P*=0.02, respectively).

Pubarche

Age at blood draw: A 1-year increase in age at blood draw was associated with increased risk of pubarche (cHR 1.67, 95% CI: 1.43, 1.95) (Table 5). When accounting for other covariates, this association was more pronounced (aHR 1.90, 95% CI: 1.61, 2.23), and race was the primary confounder of this association (1-year increase in age at blood draw, adjusted for race, HR 1.83, 95% CI 1.56, 2.15).

Race: Black girls had increased risk of pubarche (cHR 2.23, 95% CI 1.63, 3.05) (Table 5). When accounting for other covariates, this association decreased slightly in magnitude (aHR 1.99, 95% CI 1.41, 2.86). In contrast, Asian girls had decreased risk of pubarche when accounting for other covariates (cHR 0.76, 95% CI 0.51, 1.13; aHR 0.62, 95% CI 0.41, 0.94). No single covariate was responsible for this confounding. The overall 3-degree of freedom test of race was significant for both cOR and aOR estimates (*P*<0.01 for each).

BMI: Girls with BMI ≥85th percentile were at increased risk of pubarche (cHR 1.38, 95% CI 1.06, 1.78) (Table 5). This association tended towards statistical significance when adjusting for other covariates (aHR 1.26, 95% CI 0.96, 1.65).

Primary caregiver's education: We did not identify any associations between primary provider's education and risk of pubarche (Table 5). No single covariate appeared responsible for the differences noted in cHRs vs. aHRs.

Annual family income: Girls with annual family income <$50,000 were at increased risk of pubarche (cHR 1.79, 95% CI 1.30, 2.47), and this association was more pronounced when adjusting for other covariates (aHR 2.11, 95% CI 1.39, 3.22) (Table 5). No single covariate appeared responsible for this confounding. Similarly, girls with annual family income $50,000–$100,000 tended towards increased risk of pubarche (cHR 1.29, 95% CI 0.99, 1.69), and also tended towards increased risk when adjusting for other covariates (aHR 1.30, 95% CI 0.97, 1.75). The overall 3-degree of freedom test of family income categories was significant for both cOR and aOR estimates (P<0.01 for each).

Interval—birth to time of pubertal onset:

Here, we present only those findings that differ using the birth to time of pubertal onset interval vs. the time of blood draw to time of pubertal onset interval.

Thelarche

Race: Income was the primary confounder of the association between race and risk of thelarche (black race, adjusted for income, HR 1.51, 95% CI 1.08, 2.10; Asian race, adjusted for income, HR 0.83, 95% CI 0.59, 1.17; Hispanic race, HR 1.04, 95% CI 0.70, 1.55) (Table 6).

Primary provider's education: No single covariate appeared responsible for the difference in cHRs and aHRs for primary caregivers' educational attainment and risk of thelarche (Table 6).

Annual family income: Primary caregiver's educational attainment was the primary confounder of the association between income and risk of thelarche (income <$50,000, adjusted for primary caregiver's educational attainment, HR 2.19, 95% CI 1.49, 3.22; income $50,000–$100,000, adjusted for primary caregiver's educational attainment, HR 1.33, 95% CI 1.01, 1.75) (Table 6).

Pubarche

BMI (<85th percentile as referent): BMI ≥85th percentile was not significantly associated with risk of pubarche when adjusting for other covariates (Table 6). The lower aHR vs. cHR suggests that other covariates confound this association, but no single covariate was responsible for this confounding.

Primary caregiver's education (college degree as referent): Annual family income was the primary confounder of the association between primary caregiver's educational attainment and risk of pubarche

(≤high school adjusted for income, HR 0.65, 95% CI 0.45, 0.94; some college, adjusted for income, HR 1.00, 95% CI 0.75, 1.34) (Table 6).

Annual family income (>$100,000 as referent): There was no evidence of other covariates confounding the association of annual family income <$50,000 and risk of pubarche (Table 6). Annual family income $50,000–$100,000 was not associated with risk of pubarche after adjusting for other covariates, and no single covariate appeared responsible for the difference between cHR and aHR (Table 6).

Summary. These results indicate that, by crude analysis, each demographic and anthropometric variable except for primary caregiver's educational attainment was associated with risk of either thelarche or pubarche, and that, using the second interval, primary caregiver's educational attainment confounds the association between income and risk of thelarche. After adjusting for covariates, race, and annual family income were associated with risk of both thelarche and pubarche when using either interval, and age at blood draw was associated with risk of both thelarche and pubarche when using the first interval; using the first interval, BMI also tended to be associated with risk of thelarche. These findings support inclusion of age, race, and annual family income in all adjusted models of metals and risk of thelarche and pubarche, with more limited justification of including primary caregiver's educational attainment and BMI in these models. For sake of consistency with prior publication, all five covariates are included in adjusted models of metals and risk of thelarche and pubarche; however, a more parsimonious model might be described.

DISTRIBUTION OF BLOOD METALS

Concentrations of blood metals among participants

Arsenic: Blood arsenic concentrations were >LOD for all girls, and ranged from 0.15–5.75 µg/L (Table 7). Mean (0.69 ± 0.69 µg/L) and median (0.43 µg/L) concentrations were substantially different, and the distribution was right-skewed (see Figure 3A). Therefore, we log-transformed arsenic concentrations. The geometric mean arsenic concentration was 0.21 µg/L. The distribution of log-arsenic concentrations was relatively normal (see Figure 3B). Blood arsenic concentrations are not measured in NHANES 2011–2012 and therefore cannot be compared to this cohort. Blood arsenic concentrations in this cohort are within the 0–12 µg/L reference range published by Mayo Medical Laboratories.

Cadmium: Blood cadmium concentrations were >LOD in blood specimens of 50.8% of participants, and ranged from <LOD to 0.56 µg/L (see Table 7 and Figure 4). Mean concentration was not calculated; the median concentration was 0.14 ug/L. Cadmium concentrations were categorized as ≤ vs >LOD, and ≤LOD was chosen as the referent value for later analyses. Median cadmium concentration of NHANES 2011–2012 females age 6–9 years was 0.11 ug/L, which is comparable to this cohort. Blood cadmium concentrations in this cohort are within the 0–4.9 µg/L reference range published by Mayo Medical Laboratories.

Lead: Blood lead concentrations were >LOD in blood specimens of all participants, and ranged from 0.18–3.73 µg/dL. Mean (1.03 ± 0.51 µg/dL) and median (0.92 µg/dL) concentrations were comparable (Table 7), and the distribution was relatively normal (see Figure 5). Therefore, lead concentrations were treated as a continuous variable. Median lead concentration of NHANES 2011–2012 females age 6–9

years was 0.73 ug/dL, which is somewhat less than this cohort. Blood lead concentrations in this cohort are within the 0–4.9 µg/dL reference range published by Mayo Medical Laboratories.

Manganese: Blood manganese concentrations were >LOD for all girls, and ranged from 5.51–26.51 µg/L (Table 7). Mean (11.33 ± 3.19 µg/L) and median (10.85 µg/L) concentrations were comparable, and the distribution was relatively normal (see Figure 6). Therefore, manganese concentrations were treated as a continuous variable. Median manganese concentration of NHANES 2011–2012 females age 6–9 years was 10.22 ug/L, which is comparable to this cohort. Most blood manganese concentrations in the cohort are within the 4.7–18.3 µg/L reference range published by Mayo Medical Laboratories; Mayo Medical Laboratories notes that blood manganese concentrations up to two-times the upper reference limit of normal might be observed due to differences in hematocrit and other normal biological variations.

Mercury: Blood mercury concentrations were >LOD in blood specimens of 99% of participants (Table 7). Concentrations ranged from <LOD to 10.61 µg/L; those 3 samples with concentrations <LOD were assigned a concentration of LOD/√2 (0.06 µg/L /√2 = 0.04 µg/L). Mean (0.97 ± 1.33 µg/L) and median (0.54 µg/L) concentrations differed substantially, and the distribution was right-skewed (see Figure 7A). Therefore, we log-transformed mercury concentrations. The geometric mean mercury concentration was 0.25 µg/L. The distribution of log-mercury concentrations was relatively normal (see Figure 7B). Median mercury concentration of NHANES 2011–2012 females age 6–9 years was 0.36 ug/L, which is greater than the geometric mean and less than the arithmetic mean of this cohort. Most blood mercury concentrations in this cohort are within the 0–4.9 µg/L reference range published by Mayo Medical Laboratories.

Uranium: Uranium was detectable in blood specimens of only 10% of participants (see Table 7 and Figure 8). Because of the low prevalence of detectable blood uranium in this cohort, uranium concentrations were excluded from further analyses.

Associations between blood metal concentrations. Associations were identified between concentrations of log-arsenic and manganese (r=0.18, *P*<0.01), log-arsenic and log-mercury (r=0.62, *P*<0.01), and manganese and log-mercury (r=0.13, *P*=0.02) using Pearson's correlation (Table 8). An inverse association was identified between concentrations of lead and manganese (r=-0.12, *P*=0.03). Log-arsenic, lead, manganese, and log-mercury concentrations did not vary by cadmium ≤ vs >LOD as evaluated by Student's T-test (Table 9).

Associations between blood metals concentrations and demographic and anthropometric measurements

Race: Log-arsenic, lead, manganese, and log-mercury concentrations each varied by race by ANOVA (*P*<0.01 for each) (Table 10). Arsenic concentrations were greater among Asian girls than white non-Hispanic, black, and Hispanic girls (geometric mean 0.71 vs. 0.19, 0.21, and 0.14 µg/L, respectively). Lead concentrations were greater among black girls than non-Hispanic white and Asian girls (mean 1.22 vs. 0.94 and 0.88 µg/dL, respectively). Manganese concentrations were greater among Asian girls than black girls (mean 13.34 vs. 9.75 µg/L, respectively). Mercury concentrations were greater among Asian girls than white non-Hispanic, black, and Hispanic girls (geometric mean 1.55 vs. 0.19, 0.22, and 0.19 µg/L, respectively).

Cadmium concentrations, categorized ≤ vs >LOD were examined by race by chi-square analysis. Asian participants were more likely to have blood cadmium concentrations ≥LOD (78.4%) than white (48.9%), black (50.0%), or Hispanic (42.5%) participants (*P*<0.01).

Age at blood draw: Log-arsenic, lead, manganese, and log-mercury concentrations were examined by age of blood draw (categorized by year) using ANOVA; no associations were noted. Age at blood draw did not vary by blood cadmium concentrations ≤ vs >LOD as assessed by Student's T-test (7.9 vs. 7.7 years, *P*=0.10) (Table 10).

BMI: Log-arsenic, lead, manganese, and log-mercury did not vary by BMI. Likelihood of having cadmium concentrations >LOD did not vary between girls with BMI < vs. ≥85th percentile (54.2% vs. 44.3%, *P*=0.11) (Table 10).

Primary caregiver's education: Arsenic and manganese concentrations varied by primary caregivers' educational attainment by ANOVA (*P*<0.01 and *P*=0.03, respectively). Arsenic concentrations were lower among girls whose primary caregiver's educational attainment was ≤high school vs. some college or college degree (geometric mean 0.11 vs. 0.23 and 0.27 μg/L, respectively), and manganese concentrations were lower among girls whose primary caregiver's educational attainment was some college vs. ≤high school (10.75 vs. 12.15 μg/L, respectively). Lead, and mercury concentrations did not vary by primary caregiver's educational attainment. Cadmium concentrations were less likely to be >LOD among girls whose primary caregiver had an education ≤high school vs. some college or college degree (33.9% vs. 49.5% and 58.6%, respectively, chi-square *P*<0.01) (Table 10).

Annual family income: Lead concentrations varied by annual family income by ANOVA (P<0.01), and were greater among girls with annual family income <$50,000 than $50000–$100,000 or >$100,000 (1.20 vs. 1.09 and 0.87 µg/dL, respectively) (Table 10). Arsenic, manganese, and mercury concentrations did not vary by primary caregiver's education. Likelihood of having cadmium concentrations >LOD did not vary by annual family income of <$50,000, $50,000–$100,000, and >100,000 (47.0%, 51.3%, and 51.6%, respectively, P=0.81).

Summary: These results indicate that each metal concentrations varied by either race, primary caregiver's educational attainment, and/or family income. These findings support the decision to consistently adjust for all these covariates in later analyses of associations between metals and pubertal status/outcome.

RESEARCH QUESTION #1: DO BLOOD METAL CONCENTRATIONS VARY BY PUBERTAL STATUS AT TIME OF BLOOD DRAW?

Blood metal concentrations were compared by pubertal status at time of blood draw. cORs of having attained thelarche or pubarche at time of blood draw were calculated for each blood metal by logistic regression, and adjusted models included age at blood draw (treated as a continuous variable), race, BMI, primary caregiver's educational attainment, and annual family income. Four girls were missing data for annual family income and were excluded from these analyses.

Blood metal concentrations by pubertal status at time of blood draw. As shown in Table 11, blood concentrations of arsenic, lead, manganese, and mercury did not vary by pubertal status as assessed by Tanner breast staging by Student's T-test. Likelihood of having detectable blood cadmium did not vary between prepubertal vs. pubertal girls by chi-square analysis (51.8% vs. 45.2%, *P*=0.48).

Assessed by Tanner pubic hair staging, girls pubertal at time of blood draw had lower manganese concentrations than girls prepubertal (9.81 vs 11.54 µg/L). Blood lead concentrations tended to be greater among girls pubertal vs. prepubertal at time of blood draw (1.22 vs 1.01 µg/dL). Arsenic and mercury concentrations did not vary by pubertal status, and likelihood of having detectable blood cadmium did not vary between prepubertal vs. pubertal girls (52.6% vs. 40.5%, *P*=0.17).

Logistic regression of pubertal status by metal concentrations

Thelarche

As shown in Tables 12 and 13, none of the blood metals were significantly associated with having attained thelarche at time of blood draw. The greater aOR vs. cOR for each blood metal suggests that covariates confound the association between these blood metals and having attained thelarche at time of blood draw; income was the primary confounder of this association for arsenic (1-log increase in arsenic, adjusted for income, OR 1.23, 95% CI 0.71, 2.12), but no single covariate was responsible for confounding these associations for cadmium, lead, manganese, or mercury.

Pubarche

Higher lead concentrations, treated as continuous variable, were associated with greater odds of having attained pubarche at time of blood draw (cOR 1.96, 95% CI 1.09, 3.53, per µg/dL increase of lead) (Table 12); this association was not observed when categorizing lead by quartiles (Table 13). This association decreased in magnitude and was not significant when adjusting for covariates (aOR 1.56, 95% CI 0.75, 3.24), but no single covariate appeared responsible for this confounding.

Higher manganese concentrations were associated with lower odds of having attained pubarche at time of blood draw when treated as a continuous variable (cOR 0.81, 95% CI 0.70, 0.93, per µg/L increase of manganese) (Table 12). This association decreased in magnitude and was not significant when adjusting for covariates (aOR 0.93, 95% CI 0.78, 1.10), and race was the primary confounder of this association (1 µg/L increase in manganese concentrations, adjusted for race, OR 0.90, 95% CI 0.77, 1.04). When categorized by quartiles, a significant trend was identified for manganese cOR (P<0.01); however, point estimates for quartiles suggest that the association between manganese concentrations and crude odds of having attained pubarche at time of blood draw might be non-linear (Table 13).

Arsenic, cadmium, and mercury concentrations were not associated with having attained pubarche at time of blood draw (Tables 12 and 13). The increased aORs vs. cORs for each of these metals suggests that other covariates confound these associations. Race was the primary confounder of this association for mercury (1-log increase in mercury concentrations, adjusted for race, OR 1.14, 95% CI 0.78, 1.67), but no single covariate confounded this association for arsenic or cadmium.

Summary. As assessed by Student's t-test, mean blood metals vary by pubertal status assessed by Tanner pubic hair staging, but not by Tanner breast staging; specifically, pubertal girls had lower blood concentrations of manganese and trended towards higher blood concentrations of lead.

As assessed by logistic regression, none of the tested metals were associated with odds of having attained thelarche at time of blood draw. Lead and manganese were associated with increased and decreased odds of achieving pubarche at time of blood draw, respectively, paralleling the results of Student's T-test; however, when adjusting for covariates, these associations were not statistically significant. These results indicate that the association between blood metal concentrations and having attained thelarche or pubarche at time of blood draw are confounded by demographic and anthropometric variables. As noted previously, we did not attempt adjusted logistic regression with blood metals characterized as quartiles due to limited power.

Unlike Student's T-test, logistic regression allows for accounting for covariates (i.e., potential confounders) when evaluating the association between blood metal concentrations and pubertal status at time of blood draw. However, both Student's T-test and logistic regression suffer from limited power considering that only a small minority of girls were pubertal at time of blood draw. In the next section, we analyze the risk of achieving thelarche or pubarche by blood metals using a Cox proportional hazards modeling approach, which takes advantage of this study's longitudinal observations and overcomes the limitation of few girls having achieved puberty at time of blood draw.

RESEARCH QUESTION #2: ARE BLOOD METAL CONCENTRATIONS ASSOCAITED WITH RISK OF PUBERTY?

As described in "Risk of Pubertal Onset by Demographic and Anthropometric Measurements," two intervals (time of blood draw to time of pubertal onset, or birth to time of pubertal onset) are used for longitudinal analyses. For both analyses, cHRs for risk of thelarche and pubarche were calculated for blood metal concentrations by Cox proportional hazards modeling. aHRs were also calculated for each blood metal separately, adjusting for the anthropometric and demographic covariates; age at blood draw was included as a covariate for models where the interval was age at blood draw to time of puberty. Blood concentrations of log-arsenic, lead, manganese, and log-mercury were treated as continuous variables, and cadmium was dichotomized as ≤ vs >LOD. cHRs and aHRs for arsenic, lead, manganese, and manganese categorized into quartiles were also calculated.

Interval—time of blood draw to time of pubertal onset:

Thelarche

As shown in Table 14 and 15, none of the measured metals, treated either as continuous variables or categorized by quartiles, were associated with risk of thelarche. Annual family income adjusted the effect estimates between lead concentrations and risk of thelarche (1 µg/dL increase in lead concentrations, adjusted for income, HR 0.91, 95% CI 0.71, 1.16).

Pubarche

Arsenic was associated with decreased risk of pubarche when treated as a continuous variable and adjusting for covariates (aHR 0.84, 95% CI 0.71, 1.00, per 1-log increase of arsenic) (Table 14). Arsenic concentrations by quartiles were not associated with risk of pubarche (Table 15).

Cadmium concentrations >LOD was associated with decreased risk of pubarche (cHR 0.78, 95% CI 0.61, 0.98) (Table 14). This association was more pronounced when adjusting for covariates (aHR 0.71, 95% CI 0.55, 0.92), but no single covariate was responsible for this confounding.

Increased manganese concentrations were also associated with decreased risk of pubarche whether treated as a continuous variable (cHR 0.94, 95% CI 0.91, 0.98, per µg/L increase of manganese) (Table 14) or categorized as quartiles (*P*<0.01) (Table 15). This association remained significant when adjusting for covariates whether manganese was treated as a continuous variable (aHR 0.94, 95% CI 0.91, 0.98, per µg/L increase of manganese) (Table 14) or categorized as quartiles (*P*<0.01) (Table 15).

We did not identify any associations between blood lead and mercury concentrations and risk of pubarche (Tables 14 and 15). The lower aHR vs. cHR for lead suggests that other covariates confound this association, but no single covariate was responsible for this confounding.

Interval—birth to time of pubertal onset:

Here, we present only those findings that differ using the birth to time of pubertal onset interval vs. the time of blood draw to time of pubertal onset interval

Thelarche

The difference in cOR vs. aOR suggests confounding by covariates (cOR 1.09, 95% CI 0.86, 1.38; aOR 0.91, 95% CI 0.71, 1.16) (Table 16), but we did not identify a single covariate apparently responsible for this confounding.

Pubarche

Higher concentrations of lead tended towards increased risk of pubarche when lead was treated as a continuous variable (cHR 1.28, 95% CI 1.02, 1.60) or categorized into quartiles (*P*=0.08) (Table 17). Similar to the other interval, this association decreased in magnitude and was not statistically significant when adjusting for other covariates (aHR 1.04, 95% CI 0.81, 1.34) (Table 16), and no single covariate was responsible for this confounding.

Modeling of risk of multiple metals simultaneously

Higher concentrations of arsenic, cadmium, and manganese were each individually associated with decreased risk of pubarche in Cox proportional models adjusting for covariates. As noted in "Correlations between blood metal concentrations," correlations were identified between arsenic and manganese concentrations; because of concerns about collinearity, we chose not to include arsenic and manganese together in models. Therefore, we tested the associations of cadmium & arsenic and cadmium & manganese, adjusted for each other, with risk of pubarche, in crude models (no covariates included) and adjusted models (age, race, BMI, primary caregiver's education, and annual family income).

To determine whether arsenic or manganese might be better predictors of pubarche, we also tested a Cox proportional hazards model with cadmium, arsenic, and manganese included with backwards selection for these three metals, with *P*<0.05 as a criteria to remain in the model.

Interval—time of blood draw to time of pubertal onset:

As shown in Table 18, in a model simultaneously testing the association of log-arsenic and cadmium with risk of pubarche, cadmium concentration >LOD was associated with decreased risk of pubarche (HR 0.77, 95% CI 0.60, 0.97). When adjusting for covariates, both log-arsenic (HR 0.83, 95% CI 0.70, 0.98, per 1-log increase of arsenic) and cadmium concentration >LOD (HR 0.83, 95% CI 0.70, 0.98) were associated with decreased risk of pubarche.

In a model simultaneously testing the association of cadmium and manganese with risk of pubarche, cadmium >LOD (HR 0.76, 95% CI 0.60, 0.97) and manganese (HR 0.94, 95% CI 0.91, 0.98, per μg/L increase of manganese) were associated with decreased risk of pubarche. When adjusting for covariates, both cadmium concentration >LOD (HR 0.70, 95% CI 0.54, 0.91) and manganese (HR 0.94, 95% CI 0.91, 0.98, per μg/L increase of manganese) and were associated with decreased risk of pubarche.

When evaluating log-arsenic, cadmium, and manganese together in backward selection models where $P<0.05$ was the criteria for metals to remain in the model, log-arsenic was dropped from both models including no covariates ($P=0.33$) and models adjusting for anthropometric and demographic covariates ($P=0.08$). Cadmium and manganese remained in these models.

Interval—birth to time of pubertal onset:

Here, we present only those findings that differ using the birth to time of pubertal onset interval vs. the time of blood draw to time of pubertal onset interval.

Unlike the other interval, when evaluating log-arsenic, cadmium, and manganese together in backward selection models where $P<0.05$ was the criteria for metals to remain in the model, both log-arsenic and

cadmium were dropped from models using this interval and including no covariates (*P*=0.13 and *P*=0.10, respectively). Similar to the other interval, log-arsenic was dropped from both models in this interval adjusting for anthropometric and demographic covariates (*P*=0.12).

Summary

Blood metal concentrations were not associated with risk of thelarche. When adjusting for anthropometric and demographic variables, increased blood arsenic, cadmium, and manganese concentrations were similarly associated with decreased risk of pubarche using either interval. Models including multiple metals suggest that cadmium and manganese each independently decrease risk of pubarche, and cadmium and arsenic each independently decrease risk of pubarche. Because arsenic and manganese concentrations are correlated, it is not clear whether manganese and arsenic independently decrease risk of pubarche; backwards selection modeling suggests (but does not establish) that manganese might more powerfully predict decrease risk of pubarche than arsenic. However, because manganese and log-arsenic concentrations are correlated, we cannot discount the role of arsenic in predicting risk of pubarche.

RESULTS SUMMARY

The girls in this study comprised a racially-diverse cohort, of somewhat higher socioeconomic status and BMI than the population of the region; these differences might be a result of the strategy of sampling KPNC members. At time of blood draw, most girls were age 7.0–8.9 years and most were prepubertal by Tanner breast and pubic hair staging. A majority of girls were followed until at least the time they

attained thelarche and pubarche, which provided a rich dataset amenable to both cross-sectional and longitudinal studies of predictors of pubertal onset.

Timing of thelarche and pubarche incompletely accounted for one another by Pearson's correlation and Kaplan-Meier survival analysis (using either interval). In adjusted cross-sectional logistic regression analyses, age, race, and BMI were associated with having attained either thelarche or pubarche at time of blood draw. By adjusted Cox proportional hazards analyses, age, race, and annual family income were associated with risk of both thelarche and pubarche, with some evidence of primary caregiver's educational attainment confounding the association between annual family income and risk of thelarche, and also BMI tending to be associated with risk of pubarche when using the first interval. Each of these covariates was included in adjusted logistic regression and Cox proportional hazards of the association of metals and pubertal onset for consistency with prior publications, however more parsimonious models might also be described.

Lead and manganese concentrations were relatively normally distributed. Arsenic and mercury concentrations were right-skewed, but log-transformed arsenic and mercury concentrations were relatively normally distributed. Approximately half of cadmium concentrations were ≤LOD. The vast majority of uranium concentrations were ≤LOD, and uranium was therefore not considered for future analyses. Blood metal concentrations were comparable with those reported from NHANES 2011–2012, and nearly all blood metal concentrations were within the reference range published by Mayo Medical Laboratories. Pearson's test identified positive correlations between log-arsenic & manganese, log-arsenic & log-mercury, and manganese & log-mercury, and a negative correlation between lead & manganese.

Assessment of metals by pubertal status at time of blood draw using Student T-test and chi-square analyses identified lower manganese concentrations and a tendency towards higher lead concentrations

among girls who had attained pubarche at time of blood draw. However, we observed no consistent associations between concentrations of any blood metals and odds of having attained thelarche or pubarche at time of blood draw when adjusting for covariates.

We observed no consistent associations between concentrations of any blood metals and risk of thelarche. Higher concentrations of arsenic, cadmium, and manganese were associated with decreased risk of pubarche when adjusting for covariates. Both cadmium & manganese and cadmium & arsenic are independently associated with decreased risk of pubarche in models containing both metals simultaneously. A backward selection model indicates (but does not establish) that manganese might better predict pubarche than arsenic.

CHAPTER IV: DISCUSSION

In this thesis, we report on the associations between blood metal concentrations and pubertal onset by multiple analytic methods in a cohort of young girls. Comparison of the results of the two research questions reflects the strengths and weaknesses of our approaches.

- Lead and manganese were associated with having achieved pubarche at time of blood draw, as assessed by both Student's T-Test and unadjusted logistic regression. However, both of these approaches were limited by not knowing at what age girls attained puberty; because pubertal girls were older than prepubertal girls, it is possible that these approaches spuriously identified increased lead as associated with pubertal status at blood draw. These approaches were also somewhat limited in that only 31 and 34 girls had attained thelarche and pubarche at time of blood draw, respectively, and this limited number of observations might have spuriously identified (or failed to identify) associations between metal concentrations and odds of having attained thelarche or pubarche.
- The longitudinal design either left-censored (when using the first interval) or excluded (when using the second interval) girls that were pubertal at time of blood draw. While this approach excluded ~10% of the cohort, it also assured that only prepubertal girls were included in the analysis for prospective follow-up. This approach allowed us to estimate age of pubertal onset for all girls (except those that were right- or left-censored); therefore, though this longitudinal approach is a more resource-intensive and complex analysis, it also had much greater power than the cross-sectional approaches, increasing our confidence in any identified associations between blood metals and risk of pubertal onset. The comparable results of analyses using either interval increases our confidence in this analytic approach. However, the second interval

(birth to pubertal onset) would be most appropriate if all girls had provided a blood specimen before pubertal onset so that some were not excluded.

Though we observed an association between lead and increased odds of having attained pubarche at time of blood draw by cross-sectional analysis, we did not observe an association between lead and increased risk of pubarche. The association identified between lead and increased odds of having attained pubarche at time of blood draw might also be spurious resulting from multiple comparisons or from differences in methodologies; a recent analysis of association between thyroid hormone concentrations and pubertal timing in the CYGNET cohort similarly identified disparate results of cross-sectional vs. longitudinal analyses [85].

Cross-sectional approaches such as those presented here are also subject to reverse causality bias, that is, it is not possible to determine whether the exposure (e.g., increased blood metals) preceded the outcome (thelarche or pubarche at time of blood draw). While blood metals might disrupt endocrine function and thus influence pubertal timing, it is also possible that the changing patterns of hormone production and function during pubertal onset changes metal concentrations in circulation. For example, lead deposits in bones, and estrogens and androgens are well-known regulators of bone resorption; changes in circulating estrogen and androgen concentrations as a result of pubertal onset could modulate deposition (or perhaps liberation) of blood lead, thereby changing blood lead concentrations. We have identified no studies addressing whether changes in circulating hormone concentrations precede changes in blood metal concentrations, but also cannot discount this possibility. Our longitudinal approach avoids the problem of reverse causation bias in that only girls prepubertal at the time of blood draw were included in the analyses.

We identified associations between blood metals and risk of pubarche, but not thelarche. It is possible that both breast and pubic hair development are influenced by metal toxicants, and that effects specific

to thelarche might be observed in a larger cohort. It is also possible that the biological effects of metal toxicants influence the pathways to thelarche and pubarche differently, given the differences in signaling pathways regulating breast and pubic hair development (hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes, respectively). Pubarche is preceded by adrenarche, a mid-childhood developmental process involving a dramatic increase in the production of androgens, including dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S), but not cortisol or other adrenal steroids [1]. Toxic effects upon androgen synthesis or androgen signaling might explain delayed pubarche associated with increased arsenic, cadmium, and manganese. Arsenic inhibits androgen receptor-dependent gene transcription at non-cytotoxic doses in cell culture studies, [100-103] and adrenal development in an animal model [104]. Rotter et al. reported an inverse association between blood cadmium and DHEA-S concentrations in men [105], and in vitro studies have demonstrated that cadmium binds to androgen receptors [106]. We have found no reports demonstrating a direct action of manganese on the androgen receptor, but Rotter et al. reported that free blood androgen concentrations are inversely associated with manganese concentrations in men [105].

Few published studies compared arsenic, cadmium, or manganese to pubertal timing in humans. Blood arsenic concentrations are associated with delayed menarche among women in the Bengal Delta [67, 68], but thelarche or pubarche were not specifically assessed. Kim et al. and reported an association between increased cadmium and delayed pubarche in a longitudinal cohort of Korean girls, which is in agreement with the findings in this thesis [73]. We have found no reports evaluating an association of manganese and pubertal timing in girls.

Anthropometric and demographic factors (i.e., race and annual family income) were similarly associated with risk of both thelarche and pubarche in this cohort. Given that thelarche and pubarche are regulated

by distinct but overlapping axes, we hypothesize that race and family income either exert their influence upstream of the divergence of these axes, or (perhaps less likely) influence both axes by distinct but indirect means. Given that others studies have demonstrated that associations between race and pubertal timing are confounded by other factors and that socioeconomic status is not consistently associated with pubertal timing (see "Posited factors regulating pubertal timing"), it is also possible that in this cohort the contribution of race and annual family income to risk of pubertal onset might in fact be the result of confounding by other variables not tested here.

The findings presented in this thesis are among the first to relate metal toxicants to pubertal onset in girls. However, certain limitations must be acknowledged when considering the conclusions presented here. This cohort varied from the population in some aspects, including annual family income, primary caregiver's educational attainment, race, and BMI. While this is among the first longitudinal studies to examine this subject with multiple years of anthropometric and demographic data, we must highlight that only a single blood specimen from each girl was analyzed for blood metal concentrations. Blood metal concentrations might fluctuate based on frequency and magnitude of exposure, and of clearance times, and a single measurement might not characterize the true relative concentrations of blood metals adequately in the cohort or the most critical timing window. Blood is not the only specimen amenable to metal measurement (e.g., arsenic is most commonly measured in urine), and it is possible that metal concentrations measured from other biospecimen types might yield different associations with pubertal status and pubertal risk. Despite training of clinical staff on Tanner stage assessment, measurement error by staff is a possibility. Furthermore, although we adjust for age in our models, we must also acknowledge that pubertal onset occurring between clinical visits can result in misclassification of estimated age at onset. And finally, our models only account for a limited number of explanatory covariates, and others covariates including genetics, nutritional status, exercise, and other

environmental exposures could be considered; the CYGNET study collected data on many such variables that could be included in future analyses.

Some sources of metal exposure are ubiquitous (e.g., automobile exhaust, environmental tobacco smoke), while others are region and even household-specific (e.g., house paints, industrial wastes, drinking water, consumption of bioaccumulated metals in fish), and dosage might depend on route of exposure (e.g., cadmium is absorbed through tobacco smoke far more efficiently than by ingestion). The girls in this study had little evidence of excess metal exposure, in that blood metal concentrations were near the NHANES median and mostly within the Mayo Medical Laboratories reference ranges. While our studies suggest that cadmium, manganese, and arsenic may delay pubarche, we must stress the difficulties in attributing effects on human sexual development to a single environmental contaminant. Few published studies are available examining combinations of different metals or other toxicants on endocrine signaling, including those related to pubertal development. It is unknown whether these toxicants have additive, synergistic, or antagonistic effects in humans. Additional studies carefully controlling for not only raw body burdens of environmental toxicants, but also likely discriminating between bioactive forms and in the context of other toxicants and subject behaviors and genetic backgrounds will likely be necessary to identify which endocrine-disruptive toxicants and under what circumstances these toxicants are relevant to human sexual maturation. If metals indeed disrupt pubertal timing in humans, these findings might provide justification to further limit human exposure and to evaluate and possibly modify existing blood concentration standards (e.g., similar to the evolving standards for blood lead concentration and concomitant changes in targeting efforts to prevent lead exposures [e.g., discontinuation of leaded gasoline, discontinuation and abatement of lead-containing paint, remediation of contaminated sites, etc.]).

In conclusion, higher concentrations of arsenic, manganese, and cadmium are associated with later pubarche among the CYGNET cohort of girls. Few studies have evaluated the association between blood metals and pubertal timing, and our findings suggest that additional studies of metals as endocrine disruptors are warranted. Longitudinal cohort studies with multiple years of anthropometric data are better suited to the study of the effect of toxicants and pubertal timing than cross-sectional studies, and studies that include both earlier pubertal events (e.g., thelarche and pubarche) and later pubertal events (e.g., menarche) might provide insight into the determinants of pubertal timing.

REFERENCES

1. Rosenfeld, R.L., R.W. Cooke, and S. Radovick, *Puberty and its disorders in the female*, in *Pediatric endocrinology, fourth edition*, M. Sperling, Editor. 2014, Saunders/Elsevier: Philadelphia, PA. p. 563-663.
2. Christensen, K.Y., et al., *Characterization of the correlation between ages at entry into breast and pubic hair development.* Ann Epidemiol, 2010. **20**(5): p. 405-8.
3. Largo, R.H. and A. Prader, *Pubertal development in Swiss girls.* Helv Paediatr Acta, 1983. **38**(3): p. 229-43.
4. Nicolson, A.B. and C. Hanley, *Indices of physiological maturity: derivation and interrelationships.* Child Dev, 1953. **24**(1): p. 3-38.
5. Reynolds, E.L. and J.V. Wines, *Individual differences in physical changes associated with adolescence in girls.* Am J Dis Child, 1948. **75**(3): p. 329-50.
6. Taranger, J., et al., *VI. Somatic pubertal development.* Acta Paediatr Scand Suppl, 1976(258): p. 121-35.
7. Marshall, W.A. and J.M. Tanner, *Variations in pattern of pubertal changes in girls.* Arch Dis Child, 1969. **44**(235): p. 291-303.
8. Euling, S.Y., et al., *Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings.* Pediatrics, 2008. **121 Suppl 3**: p. S172-91.
9. Herman-Giddens, M.E., *Recent data on pubertal milestones in United States children: the secular trend toward earlier development.* Int J Androl, 2006. **29**(1): p. 241-6; discussion 286-90.
10. Kaplowitz, P.B., et al., *Earlier onset of puberty in girls: relation to increased body mass index and race.* Pediatrics, 2001. **108**(2): p. 347-53.

11. Rosenfield, R.L., R.B. Lipton, and M.L. Drum, *Thelarche, pubarche, and menarche attainment in children with normal and elevated body mass index.* Pediatrics, 2009. **123**(1): p. 84-8.

12. Foster, T.A., et al., *Anthropometric and maturation measurements of children, ages 5 to 14 years, in a biracial community--the Bogalusa Heart Study.* Am J Clin Nutr, 1977. **30**(4): p. 582-91.

13. Herman-Giddens, M.E., 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**(4): p. 505-12.

14. Sun, S.S., et al., *National estimates of the timing of sexual maturation and racial differences among US children.* Pediatrics, 2002. **110**(5): p. 911-9.

15. Wu, T., P. Mendola, and G.M. Buck, *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**(4): p. 752-7.

16. Biro, F.M., et al., *Onset of breast development in a longitudinal cohort.* Pediatrics, 2013. **132**(6): p. 1019-27.

17. Dunbar, J., et al., *Age at menarche and first pregnancy among psychosocially at-risk adolescents.* Am J Public Health, 2008. **98**(10): p. 1822-4.

18. Golub, M.S., et al., *Public health implications of altered puberty timing.* Pediatrics, 2008. **121 Suppl 3**: p. S218-30.

19. Hayatbakhsh, M.R., et al., *Early pubertal maturation in the prediction of early adult substance use: a prospective study.* Addiction, 2009. **104**(1): p. 59-66.

20. Kaltiala-Heino, R., E. Kosunen, and M. Rimpela, *Pubertal timing, sexual behaviour and self-reported depression in middle adolescence.* J Adolesc, 2003. **26**(5): p. 531-45.

21. Kaltiala-Heino, R., et al., *Early puberty is associated with mental health problems in middle adolescence.* Soc Sci Med, 2003. **57**(6): p. 1055-64.

22. Kaltiala-Heino, R., et al., *Early puberty and early sexual activity are associated with bulimic-type eating pathology in middle adolescence.* J Adolesc Health, 2001. **28**(4): p. 346-52.

23. Wichstrom, L., *Social, psychological and physical correlates of eating problems. A study of the general adolescent population in Norway.* Psychol Med, 1995. **25**(3): p. 567-79.

24. Copeland, W., et al., *Outcomes of early pubertal timing in young women: a prospective population-based study.* Am J Psychiatry, 2010. **167**(10): p. 1218-25.

25. Deardorff, J., et al., *Early puberty and adolescent pregnancy: the influence of alcohol use.* Pediatrics, 2005. **116**(6): p. 1451-6.

26. Ge, X., R.D. Conger, and G.H. Elder, Jr., *Coming of age too early: pubertal influences on girls' vulnerability to psychological distress.* Child Dev, 1996. **67**(6): p. 3386-400.

27. Graber, J.A., et al., *Is pubertal timing associated with psychopathology in young adulthood.* J Am Acad Child Adolesc Psychiatry, 2004. **43**(6): p. 718-26.

28. Johansson, T. and E.M. Ritzen, *Very long-term follow-up of girls with early and late menarche.* Endocr Dev, 2005. **8**: p. 126-36.

29. Ibanez, L., et al., *Premature adrenarche--normal variant or forerunner of adult disease?* Endocr Rev, 2000. **21**(6): p. 671-96.

30. Clavel-Chapelon, F. and E.N. Group, *Cumulative number of menstrual cycles and breast cancer risk: results from the E3N cohort study of French women.* Cancer Causes Control, 2002. **13**(9): p. 831-8.

31. Frontini, M.G., S.R. Srinivasan, and G.S. Berenson, *Longitudinal changes in risk variables underlying metabolic Syndrome X from childhood to young adulthood in female subjects with a history of early menarche: the Bogalusa Heart Study.* Int J Obes Relat Metab Disord, 2003. **27**(11): p. 1398-404.

32. McPherson, C.P., et al., *Reproductive factors and risk of endometrial cancer. The Iowa Women's Health Study.* Am J Epidemiol, 1996. **143**(12): p. 1195-202.

33. Moorman, P.G., et al., *Ovarian cancer risk factors in African-American and white women.* Am J Epidemiol, 2009. **170**(5): p. 598-606.

34. Chevalley, T., et al., *The influence of pubertal timing on bone mass acquisition: a predetermined trajectory detectable five years before menarche.* J Clin Endocrinol Metab, 2009. **94**(9): p. 3424-31.

35. Chumlea, W.C., et al., *Age at menarche and racial comparisons in US girls.* Pediatrics, 2003. **111**(1): p. 110-3.

36. Freedman, D.S., et al., *Relation of age at menarche to race, time period, and anthropometric dimensions: the Bogalusa Heart Study.* Pediatrics, 2002. **110**(4): p. e43.

37. Ellis, B.J., *Timing of pubertal maturation in girls: an integrated life history approach.* Psychol Bull, 2004. **130**(6): p. 920-58.

38. Cameron, N. and C.A. Wright, *The start of breast development and age at menarche in South African black females.* S Afr Med J, 1990. **78**(9): p. 536-9.

39. Adair, L.S. and P. Gordon-Larsen, *Maturational timing and overweight prevalence in US adolescent girls.* Am J Public Health, 2001. **91**(4): p. 642-4.

40. Frisch, R.E. and R. Revelle, *Height and weight at menarche and a hypothesis of critical body weights and adolescent events.* Science, 1970. **169**(3943): p. 397-9.

41. Frisch, R.E. and R. Revelle, *Height and weight at menarche and a hypothesis of menarche.* Arch Dis Child, 1971. **46**(249): p. 695-701.

42. Kaplowitz, P.B., *Link between body fat and the timing of puberty.* Pediatrics, 2008. **121 Suppl 3**: p. S208-17.

43. Lee, J.M., et al., *Weight status in young girls and the onset of puberty.* Pediatrics, 2007. **119**(3): p. e624-30.

44. Biro, F.M., et al., *Pubertal correlates in black and white girls.* J Pediatr, 2006. **148**(2): p. 234-40.

45. Buttke, D.E., K. Sircar, and C. Martin, *Exposures to endocrine-disrupting chemicals and age of menarche in adolescent girls in NHANES (2003-2008).* Environ Health Perspect, 2012. **120**(11): p. 1613-8.

46. James-Todd, T., et al., *The impact of socioeconomic status across early life on age at menarche among a racially diverse population of girls.* Ann Epidemiol, 2010. **20**(11): p. 836-42.

47. Windham, G.C., et al., *Age at menarche in relation to maternal use of tobacco, alcohol, coffee, and tea during pregnancy.* Am J Epidemiol, 2004. **159**(9): p. 862-71.

48. Braithwaite, D., et al., *Socioeconomic status in relation to early menarche among black and white girls.* Cancer Causes Control, 2009. **20**(5): p. 713-20.

49. Jean, R.T., et al., *Psychosocial risk and correlates of early menarche in Mexican-American girls.* Am J Epidemiol, 2011. **173**(10): p. 1203-10.

50. Windham, G.C., et al., *Maternal smoking, demographic and lifestyle factors in relation to daughter's age at menarche.* Paediatr Perinat Epidemiol, 2008. **22**(6): p. 551-61.

51. Deardorff, J., et al., *Socioeconomic status and age at menarche: an examination of multiple indicators in an ethnically diverse cohort.* Ann Epidemiol, 2014. **24**(10): p. 727-33.

52. Deardorff, J., et al., *Does neighborhood environment influence girls' pubertal onset? findings from a cohort study.* BMC Pediatr, 2012. **12**: p. 27.

53. Deardorff, J., et al., *Father absence, body mass index, and pubertal timing in girls: differential effects by family income and ethnicity.* J Adolesc Health, 2011. **48**(5): p. 441-7.

54. Belsky, J., R.M. Houts, and R.M. Fearon, *Infant attachment security and the timing of puberty: testing an evolutionary hypothesis.* Psychol Sci, 2010. **21**(9): p. 1195-201.

55. Belsky, J., et al., *The development of reproductive strategy in females: early maternal harshness --> earlier menarche --> increased sexual risk taking.* Dev Psychol, 2010. **46**(1): p. 120-8.

56. Morris, D.H., et al., *Familial concordance for age at menarche: analyses from the Breakthrough Generations Study.* Paediatr Perinat Epidemiol, 2011. **25**(3): p. 306-11.

57. Karapanou, O. and A. Papadimitriou, *Determinants of menarche.* Reprod Biol Endocrinol, 2010. **8**: p. 115.

58. Blanck, H.M., et al., *Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl.* Epidemiology, 2000. **11**(6): p. 641-7.

59. Gladen, B.C., N.B. Ragan, and W.J. Rogan, *Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene.* J Pediatr, 2000. **136**(4): p. 490-6.

60. Robe, L.B., R.S. Robe, and P.A. Wilson, *Maternal heavy drinking related to delayed onset of daughters menstruation.* Curr Alcohol, 1979. **7**: p. 515-20.

61. Windham, G.C., et al., *Brominated Flame Retardants and Other Persistent Organohalogenated Compounds in Relation to Timing of Puberty in a Longitudinal Study of Girls.* Environ Health Perspect, 2015. **123**(10): p. 1046-52.

62. Henson, C.H., et al., */ edited by J. Charles Eldridge, James T. Stevens*, in *Endocrine toxicology* J.C. Eldridge and J.T. Stevens, Editors. 2010, Informa Healthcare: New York. p. xiii, 408 p.

63. Iavicoli, I., L. Fontana, and A. Bergamaschi, *The effects of metals as endocrine disruptors.* J Toxicol Environ Health B Crit Rev, 2009. **12**(3): p. 206-23.

64. WHO, *Exposure to arsenic: a major public health concern*. 2010: Geneva, Switzerland.

65. Davila-Esqueda, M.E., et al., *Effects of arsenic exposure during the pre- and postnatal development on the puberty of female offspring.* Exp Toxicol Pathol, 2012. **64**(1-2): p. 25-30.

66. Reilly, M.P., et al., *Prepubertal exposure to arsenic(III) suppresses circulating insulin-like growth factor-1 (IGF-1) delaying sexual maturation in female rats.* Reprod Toxicol, 2013.

67. Sen, J. and A.B. Chaudhuri, *Effect of arsenic on the onset of menarcheal age.* Bull Environ Contam Toxicol, 2007. **79**(3): p. 293-6.

68. Sengupta, M., *Does arsenic consumption influence the age at menarche of woman.* Indian Pediatr, 2004. **41**(9): p. 960-1.

69. WHO, *Exposure to cadmium: a major public health concern*. 2010: Geneva, Switzerland.

70. Samuel, J.B., et al., *Gestational cadmium exposure-induced ovotoxicity delays puberty through oxidative stress and impaired steroid hormone levels.* J Med Toxicol, 2011. **7**(3): p. 195-204.

71. Gollenberg, A.L., et al., *Association between lead and cadmium and reproductive hormones in peripubertal U.S. girls.* Environ Health Perspect, 2010. **118**(12): p. 1782-7.

72. Interdonato, M., et al., *Cadmium delays puberty onset and testis growth in adolescents.* Clin Endocrinol (Oxf), 2015. **83**(3): p. 357-62.

73. Kim, K., et al., *Impacts of heavy metal exposure on adiposity and pubertal development in Koran children and adolescents*, in *Endocrine Society's 97th Annual Meeting and Expo*. 2015: San Diego. p. THR-297.

74. WHO, *Exposure to lead: a major public health concern*. 2010: Geneva, Switzerland.

75. Iavicoli, I., et al., *Low doses of dietary lead are associated with a profound reduction in the time to the onset of puberty in female mice.* Reprod Toxicol, 2006. **22**(4): p. 586-90.

76. Feingold, D., *Pediatric Endocrinology*, in *Atlas of Pediatric Physical Diagnosis*. 1992, W.B. Saunders. p. 9.16-19.

77. Maisonet, M., et al., *Prenatal lead exposure and puberty timing in girls.* Epidemiology, 2014. **25**(1): p. 153-5.

78. Aschner, M., B. Lukey, and A. Tremblay, *The Manganese Health Research Program (MHRP): status report and future research needs and directions.* Neurotoxicology, 2006. **27**(5): p. 733-6.

79. Dees, W.L., J.K. Hiney, and V.K. Srivastava, *Actions of manganese on pubertal development*, in *Developmental neurotoxicology research : principles, models, techniques, strategies, and mechanisms*, C. Wang and W. Slikker, Editors. 2011, Wiley: Hoboken, N.J. p. 195-209.

80. Dearth, R.K., et al., *Prepubertal exposure to elevated manganese results in estradiol regulated mammary gland ductal differentiation and hyperplasia in female rats.* Exp Biol Med (Maywood), 2014. **239**(7): p. 871-882.

81. WHO, *Exposure to mercury: a major public health concern*. 2010: Geneva, Switzerland.

82. Croes, K., et al., *Health effects in the Flemish population in relation to low levels of mercury exposure: from organ to transcriptome level.* Int J Hyg Environ Health, 2014. **217**(2-3): p. 239-47.

83. ATSDR, *Toxicological profile for uranium*. 2013.

84. Raymond-Whish, S., et al., *Drinking water with uranium below the U.S. EPA water standard causes estrogen receptor-dependent responses in female mice.* Environ Health Perspect, 2007. **115**(12): p. 1711-6.

85. Wilken, J.A., et al., *Thyroid hormones and timing of pubertal onset in a longitudinal cohort of females, Northern California, 2006-2011.* Perinatal and Paedeatric Epidemiology, 2016. **In press**.

86. Biro, F.M., et al., *Pubertal assessment method and baseline characteristics in a mixed longitudinal study of girls.* Pediatrics, 2010. **126**(3): p. e583-90.

87. Ahmed, M.L., K.K. Ong, and D.B. Dunger, *Childhood obesity and the timing of puberty.* Trends Endocrinol Metab, 2009. **20**(5): p. 237-42.

88. Aksglaede, L., et al., *Recent decline in age at breast development: the Copenhagen Puberty Study.* Pediatrics, 2009. **123**(5): p. e932-9.

89. Burt Solorzano, C.M. and C.R. McCartney, *Obesity and the pubertal transition in girls and boys.* Reproduction, 2010. **140**(3): p. 399-410.

90. Jasik, C.B. and R.H. Lustig, *Adolescent obesity and puberty: the "perfect storm".* Ann N Y Acad Sci, 2008. **1135**: p. 265-79.

91. Shackleton, N., D. Hale, and R.M. Viner, *Trends and socioeconomic disparities in preadolescent's health in the UK: evidence from two birth cohorts 32 years apart.* J Epidemiol Community Health, 2015.

92. Windham, G.C., et al., *Body burdens of brominated flame retardants and other persistent organo-halogenated compounds and their descriptors in US girls.* Environ Res, 2010. **110**(3): p. 251-7.

93. CDC. *CDC Growth Charts: United States. 2000.* 2000; Available from: http://www.cdc.gov/growthcharts/

94. Dobraca, D., et al., *Biomonitoring in California firefighters: metals and perfluorinated chemicals.* J Occup Environ Med, 2015. **57**(1): p. 88-97.

95. Sen, I., et al., *Development and validation of a simple and robust method for arsenic speciation in human urine using HPLC/ICP-MS.* J AOAC Int, 2015. **98**(2): p. 517-23.

96. Gajek, R., *Personal communication*.

97. Wolff, M.S., et al., *Exposures among pregnant women near the World Trade Center site on 11 September 2001.* Environ Health Perspect, 2005. **113**(6): p. 739-48.

98. Kleinbaum, D.G. and M. Klein, *Parametric survival models*, in *Survival analysis: a self-learning text*. 2005, Springer: New York, NY. p. 257-329.

99. Kushi, L.H., *Personal communication*. 2015.

100. Bodwell, J.E., et al., *Arsenic disruption of steroid receptor gene activation: Complex dose-response effects are shared by several steroid receptors.* Chem Res Toxicol, 2006. **19**(12): p. 1619-29.

101. Bodwell, J.E., L.A. Kingsley, and J.W. Hamilton, *Arsenic at very low concentrations alters glucocorticoid receptor (GR)-mediated gene activation but not GR-mediated gene repression: complex dose-response effects are closely correlated with levels of activated GR and require a functional GR DNA binding domain.* Chem Res Toxicol, 2004. **17**(8): p. 1064-76.

102. Kaltreider, R.C., et al., *Arsenic alters the function of the glucocorticoid receptor as a transcription factor.* Environ Health Perspect, 2001. **109**(3): p. 245-51.

103. Rosenblatt, A.E. and K.L. Burnstein, *Inhibition of androgen receptor transcriptional activity as a novel mechanism of action of arsenic.* Mol Endocrinol, 2009. **23**(3): p. 412-21.

104. Goggin, S.L., M.T. Labrecque, and A.M. Allan, *Perinatal exposure to 50 ppb sodium arsenate induces hypothalamic-pituitary-adrenal axis dysregulation in male C57BL/6 mice.* Neurotoxicology, 2012. **33**(5): p. 1338-45.

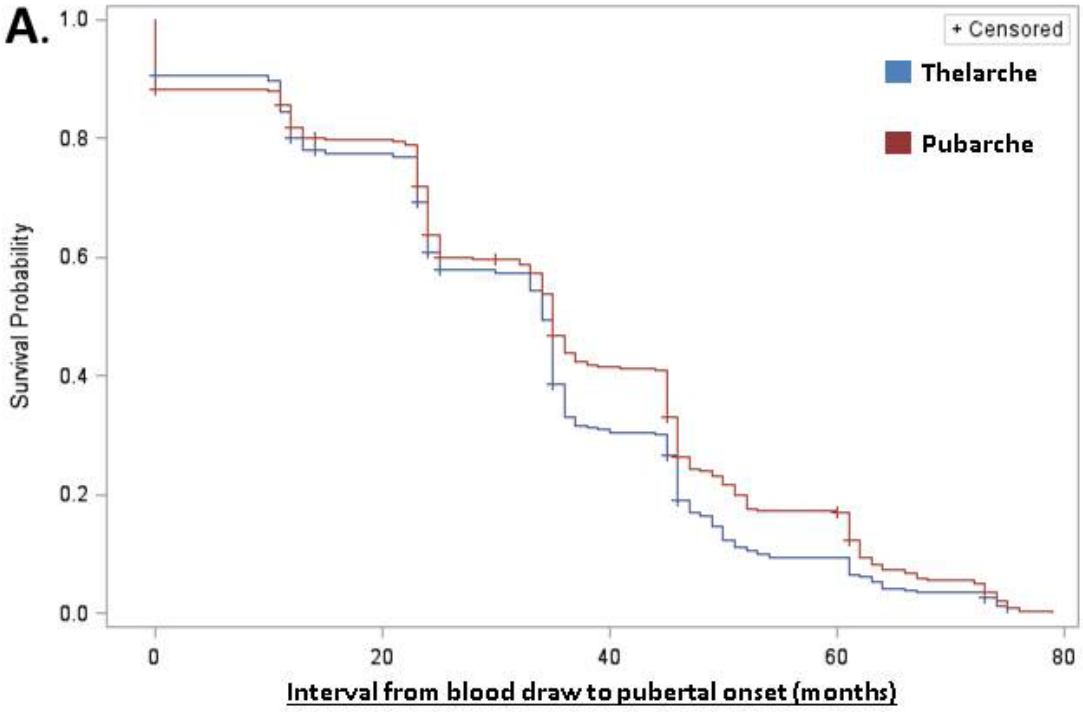
105. Rotter, I., et al., *Analysis of the relationship between the blood concentration of several metals, macro- and micronutrients and endocrine disorders associated with male aging.* Environ Geochem Health, 2015.

106. Byrne, C., et al., *Cadmium--a metallohormone?* Toxicol Appl Pharmacol, 2009. **238**(3): p. 266-71.

Figure 1. Tanner stages of breast and pubic hair development. Stage 1 is considered prepubertal, and stage ≥2 indicates pubertal onset (thelarche for breast development, and pubarche for pubic hair development). Adapted from D. Feingold [76].

Figure redacted due to copyright restrictions

Figure 2. Kaplan-Meier curves of attainment of thelarche (blue line) and pubarche (red line) as the outcome. Panel A, the interval is time from blood draw to time at pubertal onset in months. Panel B, the interval is birth to time at pubertal onset in months.



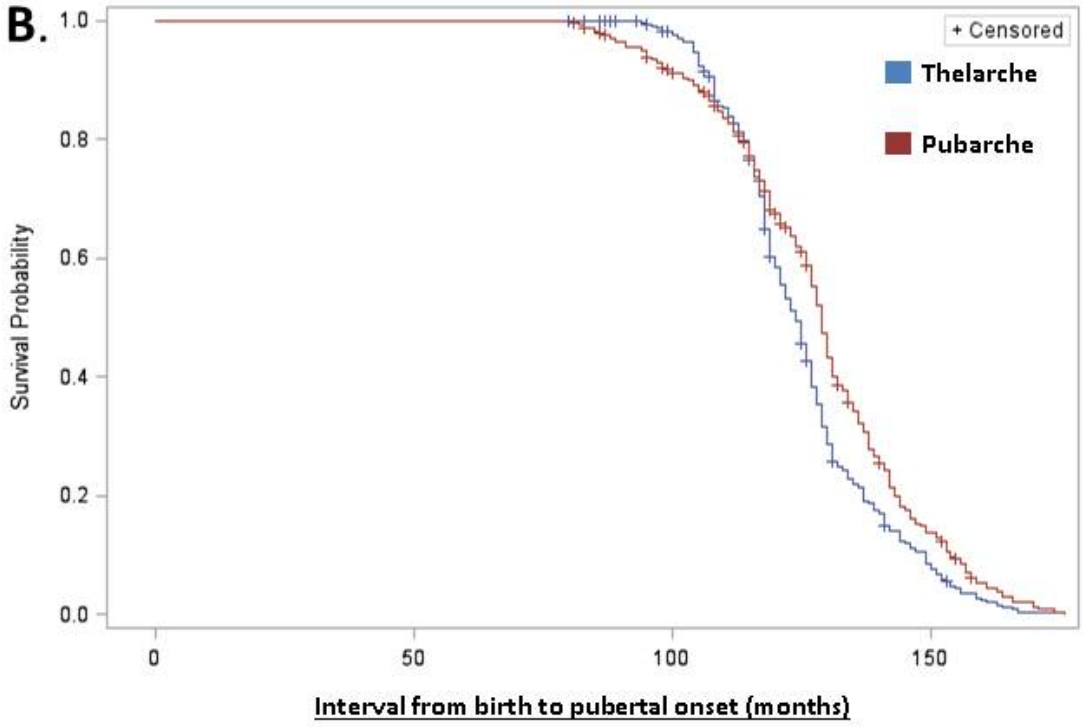


Figure 3. Distribution of blood arsenic concentrations among girls. A hypothetical normal curve is superimposed on the histogram. The LOD is represented as a solid vertical line. Panel A, arsenic concentrations are right skewed. Panel B, log-transformed arsenic concentrations are relatively normal.

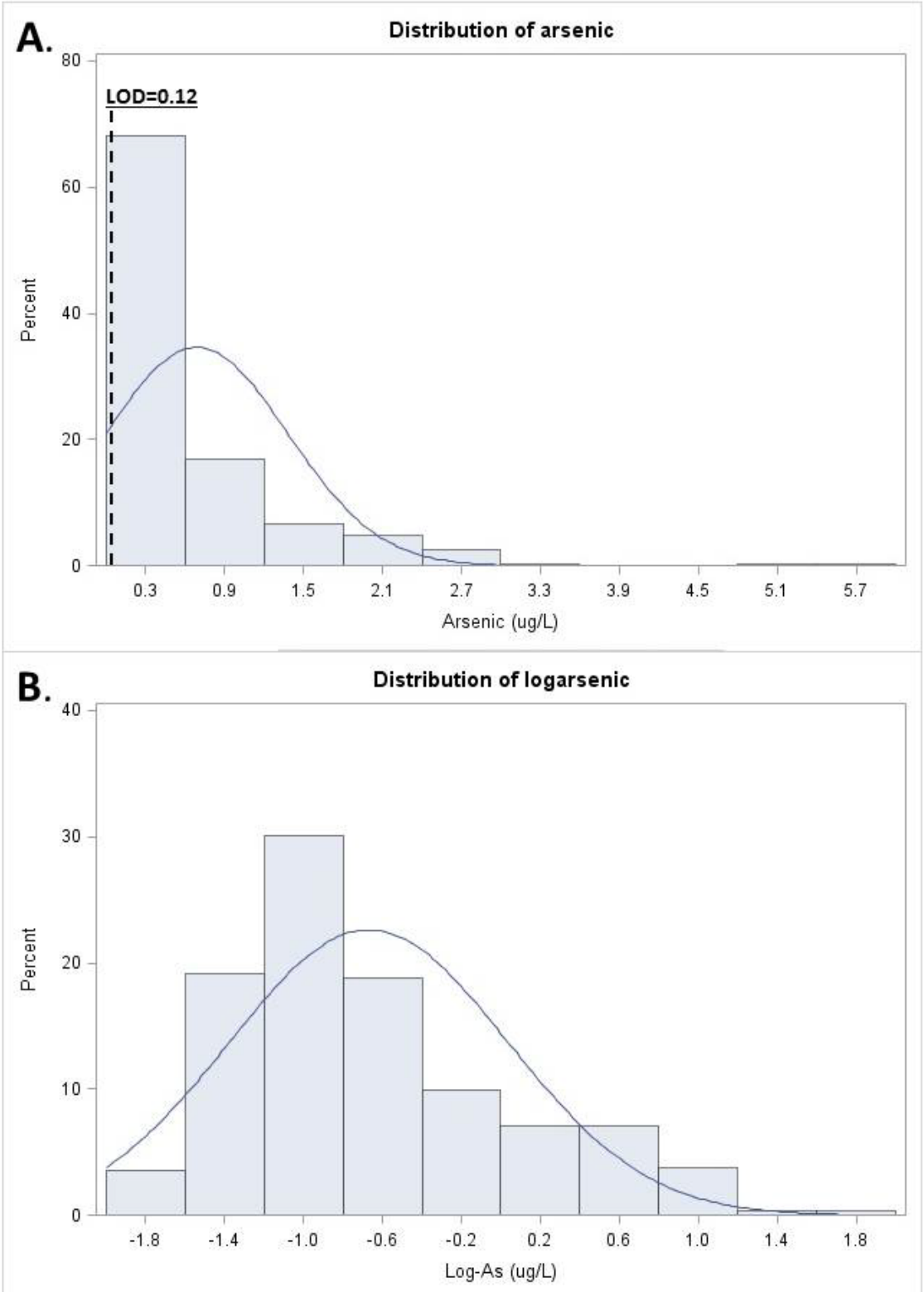


Figure 4. Distribution of blood cadmium concentrations among girls. A hypothetical normal curve is superimposed on the histogram. The LOD is represented as a solid vertical line. Panel A, nearly half of cadmium concentrations are ≤LOD; cadmium concentrations were therefore dichotomized as ≤ vs. >LOD.

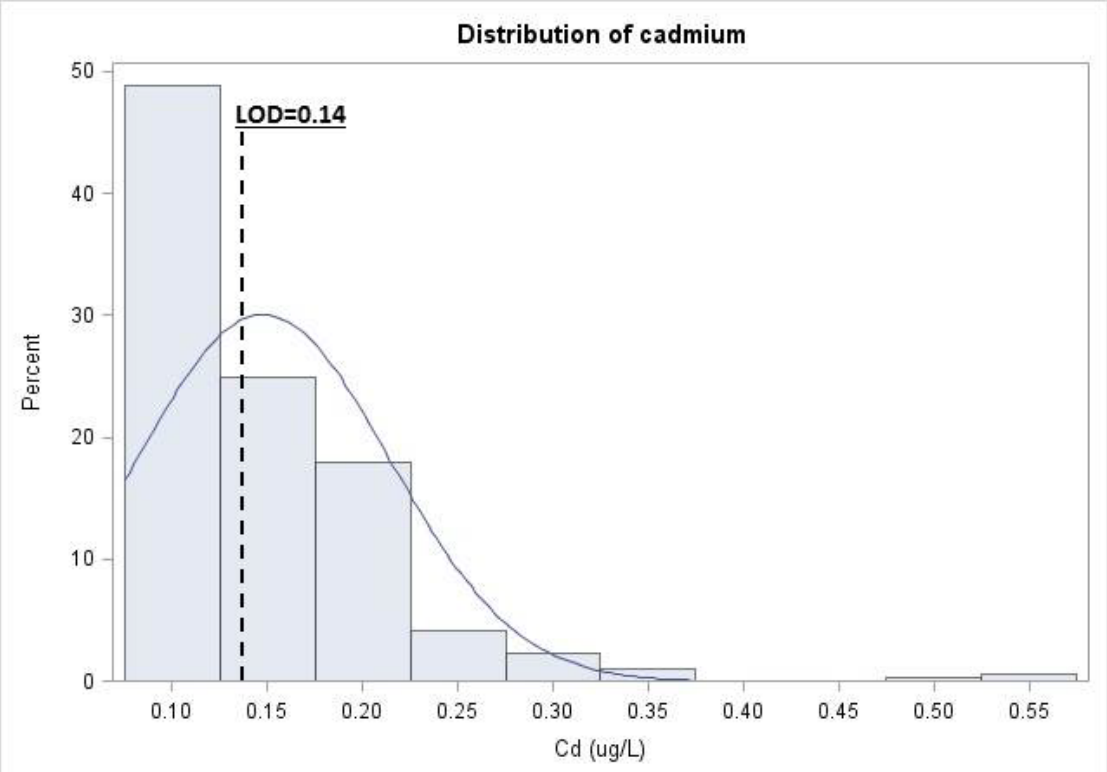


Figure 5. Distribution of blood lead concentrations among girls. A hypothetical normal curve is superimposed on the histogram. The LOD is represented as a solid vertical line. Lead concentrations are relatively normal.

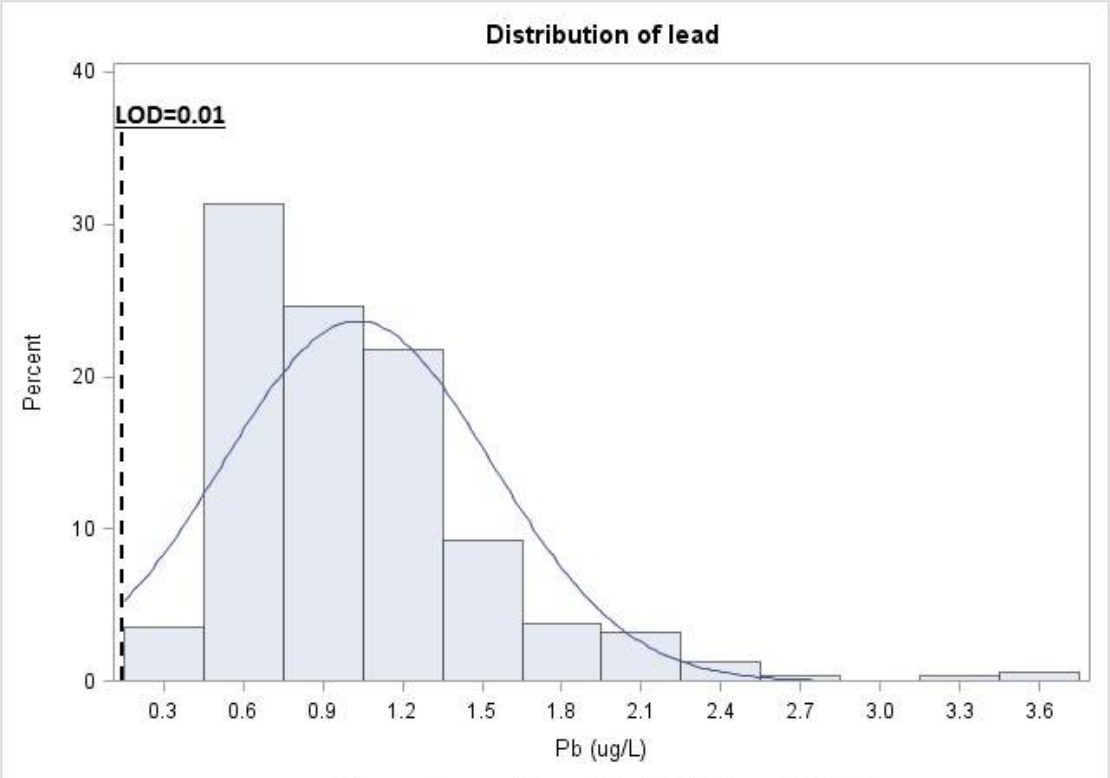


Figure 6. Distribution of blood manganese concentrations among girls. A hypothetical normal curve is superimposed on the histogram. The LOD is represented as a solid vertical line. Manganese concentrations are relatively normal.

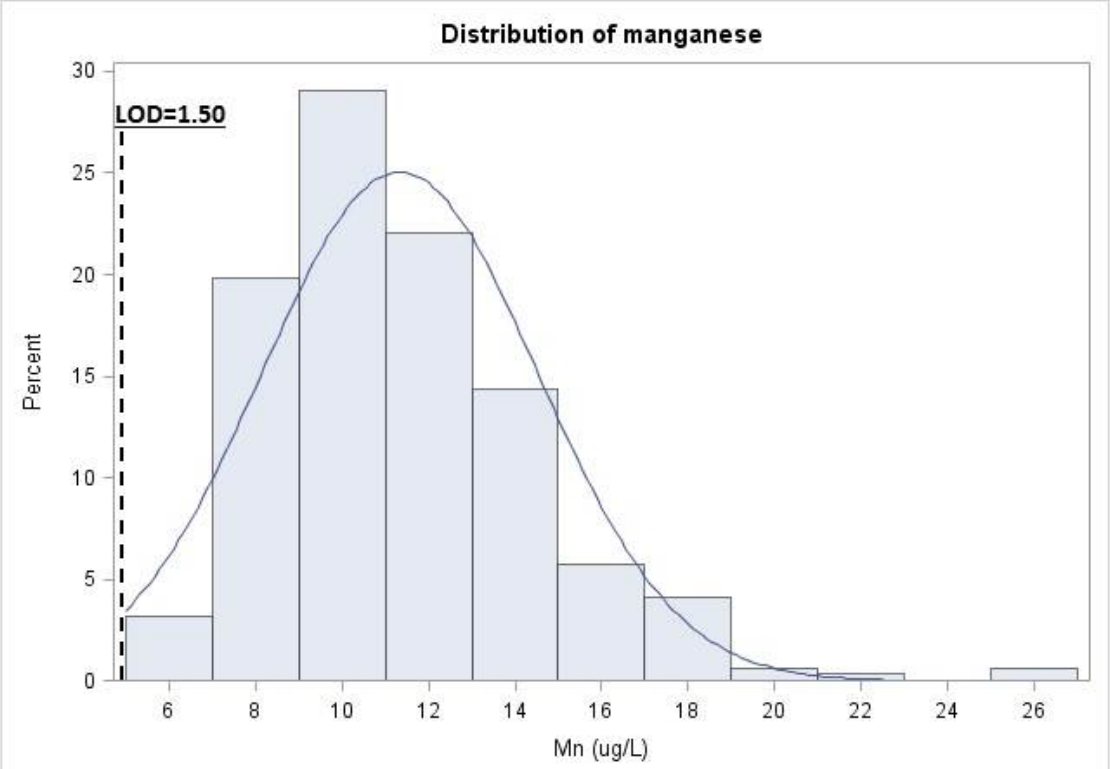


Figure 7. Distribution of blood mercury concentrations among girls. A hypothetical normal curve is superimposed on the histogram. The LOD is represented as a solid vertical line. Panel A, mercury concentrations are right skewed. Panel B, log-transformed mercury concentrations are relatively normal.

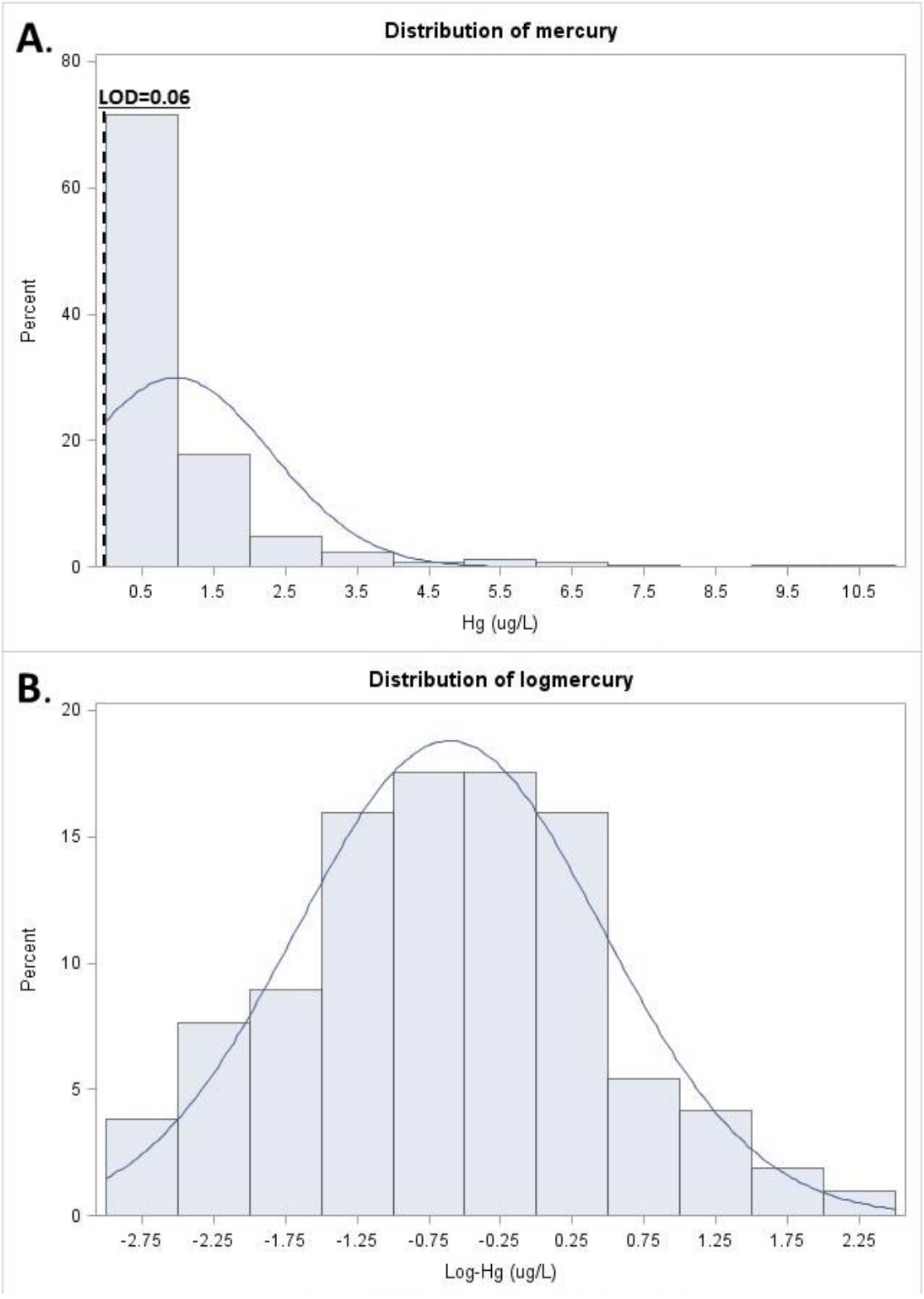


Figure 8. Distribution of blood uranium concentrations among girls. A hypothetical normal curve is superimposed on the histogram. The LOD is represented as a solid vertical line. Panel A, most uranium concentrations are <LOD.

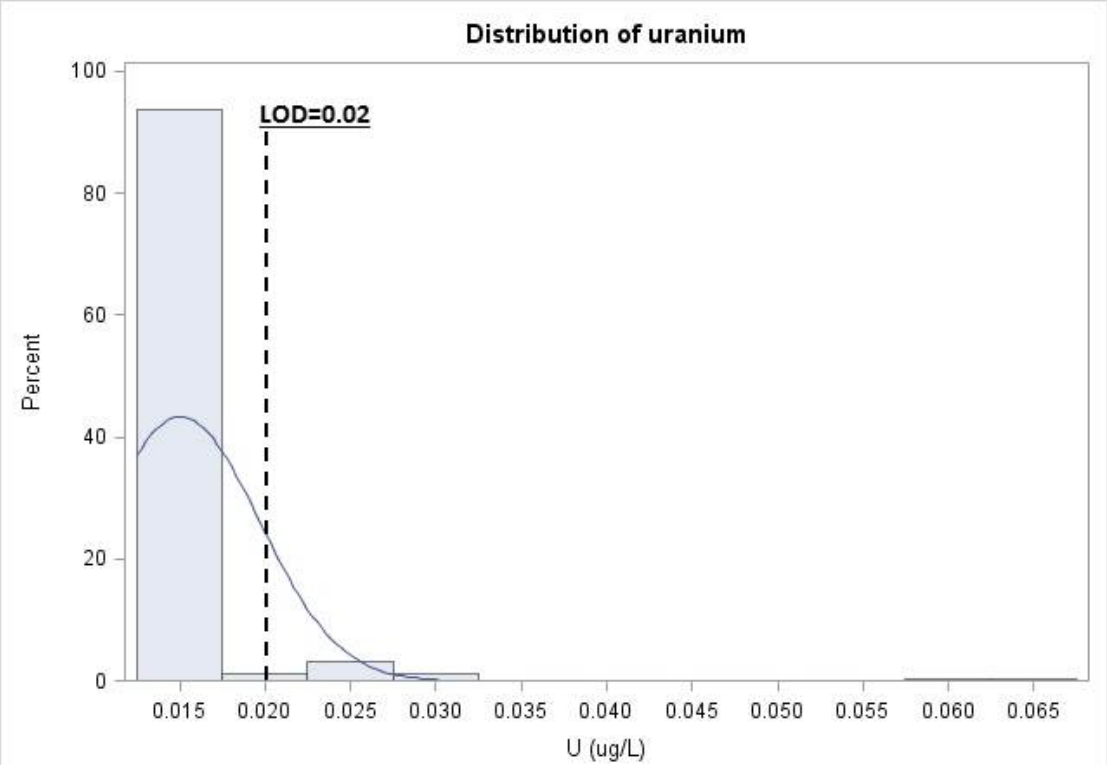


Table 1. Demographic and anthropometric characteristics of girls at time of blood draw (N=313).

Characteristic	No.	%
Age at blood draw (yrs)		
6–6.9	59	19
7–7.9	139	44
8–8.9	86	27
≥9.0	29	9
Race/ethnicity		
White non-Hispanic	133	42
Black	70	22
Asian	37	12
Hispanic	73	23
Body mass index (percentile)		
<85th	216	69
≥85th	97	31
Primary caregiver's educational attainment		
≤High school	59	19
Some college	97	31
College degree	157	50
Annual family income		
<$50,000	66	21
$50,000–$100,000	115	37
>$100,000	128	41
Missing	4	1

Table 2. Timing of pubertal onset and participation in CYGNET study over time (N=313).

Characteristic	No.	%
Tanner breast stage at blood draw		
1	282	90
≥2	31	10
Tanner breast stage at most recent clinical visit		
1	27	9
≥2	286	91
Interval between blood draw and most recent Tanner breast staging (years)		
0[a]	13	4
1	5	2
2	16	5
3	34	11
4	124	40
5	121	39
Tanner pubic hair stage at blood draw		
1	274	88
≥2	37	12
Missing	2	1
Tanner pubic hair stage at most recent clinical visit		
1	34	11
≥2	277	88
Missing	2	1
Interval between blood draw and most recent Tanner pubic hair staging		
0[a]	16	5
1	6	2
2	20	6
3	43	14
4	115	37
5	113	36

[a]—An interval of 0 years indicates that no clinical data is available for years following the year of blood draw where the blood specimen was used to measure blood metal concentrations.

Table 3. Pubertal status at time of blood draw by covariates.

Characteristic	Tanner breast stage				Tanner pubic hair stage[1]		
	n	1 (%)	≥2 (%)		n	1 (%)	≥2 (%)
Age at blood draw (yrs)							
6–6.9	313	59 (100)	0 (0)		311	55 (93)	4 (7)
7–7.9	313	137 (99)	2 (1)		311	126 (92)	11 (8)
8–8.9	313	74 (86)	12 (14)		311	74 (86)	12 (14)
≥9.0	313	12 (41)	17 (59)		311	19 (66)	10 (34)
χ^2 *P-value*			<0.01				<0.01
Race/ethnicity							
White non-Hispanic	313	126 (95)	7 (5)		311	124 (94)	8 (6)
Black	313	58 (83)	12 (17)		311	46 (66)	24 (34)
Asian	313	32 (86)	5 (14)		311	37 (100)	0 (0)
Hispanic	313	66 (90)	7 (10)		311	67 (93)	5 (7)
χ^2 *P-value*			0.05				<0.01
Body mass index (percentile)							
<85th	313	201 (93)	15 (7)		311	191 (89)	24 (11)
≥85th	313	81 (84)	16 (16)		311	83 (86)	13 (13)
χ^2 *P-value*			<0.01				0.56
Primary caregiver's educational attainment							
≤High school	313	52 (88)	7 (12)		311	51 (88)	7 (12)
Some college	313	83 (86)	14 (14)		311	76 (79)	20 (21)
College degree	313	147 (94)	10 (6)		311	147 (94)	10 (6)
χ^2 *P-value*			0.10				<0.01
Annual family income [2]							
<$50,000	309	52 (79)	14 (21)		307	49 (75)	16 (24)
$50,000–$100,000	309	105 (91)	10 (9)		307	103 (90)	11 (10)
>$100,000	309	123 (96)	5 (4)		307	119 (93)	9 (7)
χ^2 *P-value*			<0.01				<0.01

[1]Data missing for 2 girls; [2]data missing for 4 girls;

Table 4. Crude odds ratios (cORs) and adjusted[1] odds ratios (aORs), 95% confidence intervals (CIs) and *P*-values of having achieved thelarche and pubarche at time of blood draw by covariates.

	Thelarche (Tanner breast stage ≥2; n=309)[2]		**Pubarche (Tanner pubic hair stage ≥2; n=307)[2,3]**	
Characteristic	**cOR (95% CI) *P*-value**	**aOR (95% CI) *P*-value**	**cOR (95% CI) *P*-value**	**aOR (95% CI) *P*-value**
One year increase in age at blood draw	16.04 (6.47, 39.74)	22.96 (7.38, 71.44)	1.99 (1.32, 3.01)	2.57 (1.56, 4.26)
P-value	<0.01	<0.01	<0.01	<0.01
Race/ethnicity				
White non-Hispanic	Ref	Ref	Ref	Ref
Black	3.47 (1.28, 9.42)	4.63 (0.98, 21.89)	7.92 (3.31, 18.98)	5.86 (2.04, 16.86)
Asian	2.32 (0.64, 8.44)	1.44 (0.18, 11.47)	N.A.[4]	N.A.[4]
Hispanic	1.91 (0.64, 5.67)	0.79 (0.11, 5.59)	1.16 (0.36, 3.68)	0.60 (0.14, 2.56)
P-value[5]	0.11	0.17	<0.01	<0.01
Body mass index (percentile)				
<85th	Ref	Ref	Ref	Ref
≥85th	2.29 (1.06, 4.97)	3.00 (0.95, 9.45)	1.15 (0.55, 2.42)	0.82 (0.35, 1.94)
P-value	0.04	0.06	0.71	0.65
Primary caregiver's educational attainment				
≤High school	2.20 (0.78, 6.20)	1.03 (0.13, 8.09)	2.24 (0.79, 6.33)	1.92 (0.47, 7.96)
Some college	2.62 (1.07, 6.40)	1.20 (0.31, 4.63)	4.48 (1.94, 10.32)	2.49 (0.87, 7.10)
College degree	Ref	Ref	Ref	Ref
P-value[6]	0.09	0.96	<0.01	0.24
Annual family income				
<$50,000	6.62 (2.27, 19.34)	5.44 (1.00, 29.63)	4.32 (1.79, 10.43)	1.68 (0.49, 5.72)
$50,000–$100,000	2.34 (0.78, 7.07)	3.50 (0.82, 14.86)	1.41 (0.56, 3.54)	0.88 (0.30, 2.55)
>$100,000	Ref	Ref	Ref	Ref
P-value[6]	<0.01	0.12	<0.01	0.47

[1]Adjusted for BMI and age at blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by logistic regression; [2]annual family income missing for 4 girls; [3]Tanner pubic hair staging missing for 2 girls; [4]at time of blood draw, no Asian girls had achieved pubarche; [5]overall 3-degree of freedom test; [6]overall 2-degree of freedom test.

Table 5. Crude hazard ratios (cHRs) and adjusted[1] hazard ratios (aHRs), 95% confidence intervals (CIs) and *P*-values predicting thelarche and pubarche by covariates. Interval—time of blood draw to pubertal onset.

	Thelarche (Tanner breast stage ≥2; n=309)[2]		**Pubarche (Tanner pubic hair stage ≥2; n=307)[2,3]**	
Characteristic	**cHR (95% CI) *P*-value**	**aHR (95% CI) *P*-value**	**cHR (95% CI) *P*-value**	**aHR (95% CI) *P*-value**
One year increase in age at blood draw	2.07 (1.76, 2.43)	2.24 (1.89, 2.66)	1.67 (1.43, 1.95)	1.90 (1.61, 2.23)
P-value	<0.01	<0.01	<0.01	<0.01
Race/ethnicity				
White non-Hispanic	Ref	Ref	Ref	Ref
Black	1.61 (1.18, 2.19)	1.44 (1.02, 2.05)	2.23 (1.63, 3.05)	1.99 (1.41, 2.86)
Asian	1.22 (0.83, 1.80)	0.95 (0.64, 1.43)	0.76 (0.51, 1.13)	0.62 (0.41, 0.94)
Hispanic	1.06 (0.79, 1.44)	0.85 (0.58, 1.24)	1.05 (0.77, 1.42)	0.89 (0.61, 1.36)
P-value[4]	0.02	0.04	<0.01	<0.01
Body mass index (percentile)				
<85th	Ref	Ref	Ref	Ref
≥85th	1.15 (0.90, 1.49)	1.24 (0.95, 1.62)	1.38 (1.06, 1.78)	1.26 (0.96, 1.65)
P-value	0.27	0.11	0.02	0.09
Primary caregiver's educational attainment				
≤High school	1.05 (0.77, 1.43)	0.78 (0.51, 1.17)	1.04 (0.75, 1.43)	0.70 (0.46, 1.06)
Some college	1.21 (0.92, 1.58)	0.94 (0.69, 1.29)	1.18 (0.89, 1.55)	0.83 (0.62, 1.13)
College degree	Ref	Ref	Ref	Ref
P-value[5]	0.40	0.47	0.51	0.22
Annual family income				
<$50,000	1.59 (1.16, 2.17)	1.92 (1.28, 2.88)	1.79 (1.30, 2.47)	2.11 (1.39, 3.22)
$50,000–$100,000	1.19 (0.92, 1.55)	1.24 (0.94, 1.65)	1.29 (0.99, 1.69)	1.30 (0.97, 1.75)
>$100,000	Ref	Ref	Ref	Ref
P-value[5]	0.02	<0.01	<0.01	<0.01

[1]Adjusted for BMI and age at blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by Cox proportional hazards models; [2]annual family income missing for 4 girls; [3]Tanner pubic hair staging missing for 2 girls; [4]overall 3-degree of freedom test; [5]overall 2-degree of freedom test.

Table 6. Crude hazard ratios (cHRs) and adjusted[1] hazard ratios (aHRs), 95% confidence intervals (CIs) and *P*-values predicting thelarche and pubarche by covariates. Interval—birth to pubertal onset.

	Thelarche (Tanner breast stage ≥2; n=309)[2]		**Pubarche (Tanner pubic hair stage ≥2; n=307)[2,3]**	
Characteristic	**cHR (95% CI) *P*-value**	**aHR (95% CI) *P*-value**	**cHR (95% CI) *P*-value**	**aHR (95% CI) *P*-value**
Race/ethnicity				
White non-Hispanic	Ref	Ref	Ref	Ref
Black	1.80 (1.33, 2.45)	1.53 (1.09, 2.16)	2.81 (2.06, 3.84)	2.31 (1.64, 3.25)
Asian	1.00 (0.68, 1.49)	1.09 (0.73, 1.63)	0.60 (0.40, 0.91)	0.60 (0.40, 0.91)
Hispanic	1.05 (0.77, 1.42)	0.92 (0.63, 1.35)	1.09 (0.80, 1.48)	0.94 (0.64, 1.37)
P-value[4]	<0.01	0.03	<0.01	<0.01
Body mass index (percentile)				
<85th	Ref	Ref	Ref	Ref
≥85th	1.20 (0.93, 1.54)	1.19 (0.91, 1.55)	1.42 (1.10, 1.84)	1.23 (0.94, 1.61)
P-value	0.16	0.20	<0.01	0.13
Primary caregiver's educational attainment				
≤High school	0.97 (0.71, 1.33)	0.72 (0.48, 1.09)	1.00 (0.73, 1.38)	0.69 (0.45, 1.05)
Some college	1.25 (0.95, 1.64)	0.92 (0.67, 1.25)	1.26 (0.96, 1.66)	0.94 (0.70, 1.28)
College degree	Ref	Ref	Ref	Ref
P-value[5]	0.21	0.29	0.21	0.20
Annual family income				
<$50,000	1.80 (1.31, 2.46)	1.99 (1.32, 2.99)	2.07 (1.50, 2.85)	2.03 (1.34, 3.08)
$50,000–$100,000	1.28 (0.98, 1.66)	1.32 (1.00, 1.75)	1.40 (1.07, 1.84)	1.24 (0.93, 1.67)
>$100,000	Ref	Ref	Ref	Ref
P-value[5]	<0.01	<0.01	<0.01	<0.01

[1]Adjusted for BMI, race/ethnicity, annual family income, and primary caregiver's educational attainment by Cox proportional hazards models; [2]annual family income missing for 4 girls; [3]Tanner pubic hair staging missing for 2 girls; [4]overall 3-degree of freedom test; [5]overall 2-degree of freedom test.

Table 7. Distribution of blood metals (N=313).

	Limit of Detection (LOD)[a]	% of girls >LOD	Min	25th percentile	Median	75th percentile	Max	Mean (standard deviation)	NHANES 2011–2012 median[b]	Mayo Medical Laboratories reference interval[c]
Metal (µg/L)										
Arsenic (µg/L)	0.12	100.0	0.15	0.31	0.43	0.75	5.75	0.69 (0.69)	N/A[d]	0–12
Cadmium (µg/L)	0.14	51.1	<LOD	<LOD	0.14	0.18	0.56	N/A[e]	0.14	0–4.9
Lead (µg/dL)	0.01	100.0	0.18	0.68	0.92	1.25	3.73	1.03 (0.51)	0.74	0–4.9
Manganese (µg/L)	1.50	100.0	5.51	9.08	10.85	13.05	26.51	11.33 (3.19)	10.62	4.7–18.3
Mercury (µg/L)	0.06	99.0	<LOD	0.27	0.54	1.13	10.61	0.97 (1.33)	0.34	0–4.9
Uranium (µg/L)	0.02	6.4	<LOD	<LOD	<LOD	<LOD	0.06	N/A[e]	N/A[d]	N/A[f]

LOD, limit of detection.

[a]LOD as defined by the Environmental Health Laboratory Branch of the California Department of Public Health [96].

[b]Reference group is NHANES 2011–2012 laboratory data for girls age 6–9 (N=698).

[c]Reference group is Mayo Medical Laboratories published reference ranges (http://www.mayomedicallaboratories.com/index.html).

[d]Blood arsenic and uranium concentrations not available in NHANES 2011–2012 publicly-available dataset.

[e]Mean concentration was not calculated because of the number of girls for whom concentrations were below the LOD.

[f]Blood uranium concentrations not available in Mayo Medical Laboratories published reference ranges.

Table 8. Pearson coefficients (and *P*-values) of metal concentrations (N=313).

	Log-arsenic	**Lead**	**Manganese**	**Log-mercury**
	Pearson's r *P*-value	Pearson's r *P*-value	Pearson's r *P*-value	Pearson's r *P*-value
Log-arsenic	1.00 -			
Lead	0.07 0.23	1.00 -		
Manganese	0.18 <0.01	-0.12 0.03	1.00 -	
Log-mercury	0.62 <0.01	0.07 0.25	0.13 0.02	1.00 -

Table 9. Mean blood metals by cadmium < vs. ≥ LOD (N=313). Geometric means are presented for arsenic and mercury, and arithmetic means are presented for lead and manganese.

	Arsenic (μg/L)	**Lead** (μg/dL)	**Manganese** (μg/L)	**Mercury** (μg/L)
	Concentration (95% confidence interval)			
Cadmium concentration				
≤LOD	0.23 (0.17, 0.30)	0.99 (0.91, 1.08)	11.16 (10.66, 11.00)	0.29 (0.20, 0.42)
>LOD	0.20 (0.16, 0.26)	1.06 (0.99, 1.14)	11.50 (11.00, 12.00)	0.21 (0.14, 0.32)
T-test *P*-value	0.52	0.23	0.35	0.27

Table 10. Mean blood metal concentration by covariate category. Geometric means are presented for arsenic and mercury, and arithmetic means are presented for lead and manganese.

	Arsenic (μg/L)	**Lead** (μg/dL)	**Manganese** (μg/L)	**Mercury** (μg/L)
	Concentration (95% confidence interval)			
Race/ethnicity (n=313)				
Asian	0.71 (0.39, 1.29)	0.88 (0.75, 1.01)	13.34 (12.05, 14.63)	1.55 (0.72, 3.39)
White non-Hispanic	0.19 (0.15, 0.26)	0.94 (0.86, 1.01)	11.03 (10.55, 11.52)	0.19 (0.12, 0.29)
Black	0.21 (0.14, 0.31)	1.22 (1.08, 1.36)	9.75 (9.14, 10.36)	0.22 (0.13, 0.37)
Hispanic	0.14 (0.10, 0.19)	1.09 (0.97, 1.22)	12.34 (11.64, 13.11)	0.19 (0.12, 0.30)
ANOVA P-value	<0.01	<0.01	<0.01	<0.01
Body mass index (percentile) (n=313)				
<85th	0.23 (0.18, 0.29)	1.03 (0.96, 1.10)	11.35 (10.91, 11.79)	0.27 (0.19, 0.38)
≥85th	0.19 (0.14, 0.25)	1.04 (0.94, 1.13)	11.29 (10.68, 11.89)	0.20 (0.13, 0.32)
T-test P-value	0.28	0.89	0.86	0.35
Primary caregiver's educational attainment (n=313)				
≤High school	0.11 (0.08, 0.14)	1.07 (0.93, 1.22)	12.15 (11.31, 12.99)	0.16 (0.09, 0.28)
Some college	0.23 (0.16, 0.34)	1.08 (0.97, 1.19)	10.75 (10.06, 11.43)	0.22 (0.14, 0.35)
College degree	0.27 (0.21, 0.34)	0.98 (0.91, 1.06)	11.39 (10.92, 11.86)	0.32 (0.21, 0.48)
ANOVA P-value	<0.01	0.24	0.03	0.17
Annual family income (n=309)[1]				
<$50,000	0.17 (0.11, 0.25)	1.20 (1.06, 1.35)	11.41 (10.65, 12.16)	0.25 (0.14, 0.42)
$50,000–$100,000	0.21 (0.15, 0.29)	1.09 (0.99, 1.19)	10.91 (10.32, 11.51)	0.20 (0.13, 0.31)
>$100,000	0.24 (0.18, 0.32)	0.87 (0.81, 0.93)	11.67 (11.19, 12.22)	0.30 (0.19, 0.48)
ANOVA P-value	0.36	<0.01	0.18	0.39
Age at blood draw (yrs) (n=313)				
6–6.9	0.15 (0.10, 0.24)	1.04 (0.91, 1.16)	11.89 (10.91, 12.86)	0.14 (0.07, 0.26)
7–7.9	0.23 (0.18, 0.30)	1.09 (1.00, 1.18)	11.12 (10.58, 11.65)	0.26 (0.17, 0.40)
8–8.9	0.24 (0.17, 0.35)	0.96 (0.85, 1.07)	11.30 (10.69, 11.92)	0.31 (0.19, 0.51)
≥9.0	0.21 (0.10, 0.44)	0.95 (0.81, 1.10)	11.34 (10.26, 12.41)	0.32 (0.13, 0.83)
ANOVA P-value	0.38	0.27	0.49	0.23

[1]Data missing for 4 girls.

Table 11. Mean blood metal concentrations by pubertal status at time of blood draw (N=313). Geometric means are presented for arsenic and mercury, and arithmetic means are presented for lead and manganese.

	Arsenic (μg/L)	**Lead** (μg/dL)	**Manganese** (μg/L)	**Mercury** (μg/L)
	Concentration (95% confidence interval)			
Tanner breast stage				
1	0.21 (0.17, 0.26)	1.02 (0.97, 1.08)	11.38 (11.01, 11.76)	0.23 (0.18, 0.32)
≥2	0.23 (0.13, 0.42)	1.09 (0.84, 1.34)	10.89 (9.79, 11.99)	0.40 (0.17, 0.91)
T-test P-value	0.73	0.61	0.41	0.25
Tanner pubic hair stage				
1	0.22 (0.18, 0.26)	1.01 (0.95, 1.06)	11.54 (11.16, 11.92)	0.25 (0.18, 0.33)
≥2	0.19 (0.11, 0.30)	1.22 (0.99, 1.45)	9.81 (9.01, 10.62)	0.26 (0.13, 0.51)
T-test P-value	0.55	0.08	<0.01	0.87

Table 12. Crude odds ratios (cORs) and adjusted[1] odds ratios (aORs), 95% confidence intervals (CIs) and *P*-values of having achieved thelarche and pubarche at time of blood draw by metals.

	Thelarche (Tanner breast stage ≥2; n=309)[2]		**Pubarche (Tanner pubic hair stage ≥2; n=307)[2,3]**	
Metal	**cOR (95% CI)** ***P*-value**	**aOR (95% CI)** ***P*-value**	**cOR (95% CI)** ***P*-value**	**aOR (95% CI)** ***P*-value**
Arsenic (1-log increase)	1.12 (0.66, 1.90) 0.69	1.25 (0.55, 2.88) 0.59	0.84 (0.50, 1.41) 0.50	0.98 (0.53, 1.80) 0.94
Cadmium				
≤LOD	Ref	Ref	Ref	Ref
>LOD	0.67 (0.31, 1.45) 0.31	1.59 (0.47, 5.33) 0.45	0.59 (0.29, 1.20) 0.14	0.91 (0.40, 2.10) 0.82
Lead (1 µg/dL increase)	1.02 (0.48, 2.17) 0.97	1.30 (0.47, 3.62) 0.62	1.96 (1.09, 3.53) 0.02	1.56 (0.75, 3.24) 0.23
Manganese (1 µg/L increase)	0.96 (0.84, 1.09) 0.51	1.05 (0.84, 1.31) 0.65	0.81 (0.70, 0.93) <0.01	0.93 (0.78, 1.10) 0.38
Mercury (1-log increase)	1.28 (0.89, 1.83) 0.19	1.63 (0.88, 3.03) 0.12	1.03 (0.75, 1.44) 0.84	1.19 (0.78, 1.80) 0.42

[1]Adjusted for BMI and age at blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by logistic regression; [2.] annual family income missing for 4 girls; [3.] Tanner pubic hair staging missing for 2 girls.

Table 13. Crude odds ratios (cORs)[1] and 95% confidence intervals (CIs) of having achieved thelarche and pubarche at time of blood draw, by metals categorized into quartiles.

	Thelarche (Tanner breast stage ≥2; n=309)[2]			Pubarche (Tanner pubic hair stage ≥2; n=307)[2,3]		
Metal	**n**	**cOR (95% CI)**	**Trend *P***	**n**	**cOR (95% CI)**	**Trend *P***
Arsenic (µg/L)			0.58			0.61
≤0.31	80	Ref		79	Ref	
>0.31–≤0.43	78	0.56 (0.16, 2.01)		78	1.14 (0.44, 2.99)	
>0.43–≤0.75	75	1.99 (0.74, 5.35)		74	1.36 (0.53, 3.59)	
>0.75	76	0.89 (0.29, 2.79)		76	0.67 (0.23, 1.97)	
Lead (µg/dL)			0.25			0.33
≤0.68	81	Ref		81	Ref	
>0.68–≤0.92	78	1.04 (0.39, 2.78)		77	1.58 (0.57, 4.38)	
>0.92–≤1.25	74	0.71 (0.24, 2.09)		74	1.28 (0.44, 3.73)	
>1.25	76	0.56 (0.18, 1.76)		75	1.82 (0.67, 4.96)	
Manganese (µg/L)			0.80			<0.01
≤9.08	77	Ref		76	Ref	
>9.08–≤10.85	78	0.27 (0.07, 1.02)		78	0.40 (0.16, 0.98)	
>10.85–≤13.05	77	0.89 (0.34, 2.32)		76	0.41 (0.16, 1.01)	
>13.05	77	0.67 (0.24, 1.86)		77	0.14 (0.04, 0.50)	
Mercury (µg/L)			0.25			0.70
≤0.27	77	Ref		77	Ref	
>0.27–≤0.54	79	2.06 (0.59, 7.13)		79	2.84 (0.96, 8.39)	
>0.54–≤1.13	76	2.45 (0.72, 8.33)		75	2.48 (0.82, 7.51)	
>1.13	77	2.12 (0.61, 7.35)		76	1.46 (0.44, 4.82)	

[1]Adjusted odds ratios for metals categorized as quartiles were not calculated due to limited power; [2]annual family income missing for 4 girls; [3]Tanner pubic hair staging missing for 2 girls.

Table 14. Crude hazard ratios (cHRs) and adjusted[1] hazard ratios (aHRs), 95% confidence intervals (CIs) and *P*-values predicting thelarche and pubarche by metals. Interval—time of blood draw to pubertal onset.

	Thelarche (Tanner breast stage ≥2; n=309)[2]		**Pubarche (Tanner pubic hair stage ≥2; n=307)[2,3]**	
Metal	**cHR (95% CI) *P*-value**	**aHR (95% CI) *P*-value**	**cHR (95% CI) *P*-value**	**aHR (95% CI) *P*-value**
Arsenic (1-log increase)	1.00 (0.85, 1.17) 0.98	0.92 (0.77, 1.10) 0.37	0.89 (0.75, 1.04) 0.14	0.84 (0.71, 1.00) 0.05
Cadmium				
≤LOD	Ref	Ref	Ref	Ref
>LOD	0.96 (0.76, 1.21) 0.73	0.95 (0.73, 1.22) 0.67	0.78 (0.61, 0.98) 0.04	0.71 (0.55, 0.92) 0.01
Lead (1 µg/dL increase)	1.00 (0.79, 1.27) 0.98	0.90 (0.70, 1.15) 0.39	1.12 (0.89, 1.40) 0.34	1.01 (0.78, 1.30) 0.94
Manganese (1 µg/L increase)	0.99 (0.95, 1.02) 0.44	1.01 (0.97, 1.05) 0.71	0.94 (0.91, 0.98) <0.01	0.94 (0.91, 0.98) <0.01
Mercury (1-log increase)	1.04 (0.93, 1.16) 0.51	0.97 (0.86, 1.10) 0.62	1.03 (0.92, 1.14) 0.62	1.02 (0.90, 1.14) 0.80

[1]Adjusted for BMI and age at blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by Cox proportional hazards models; [2]annual family income missing for 4 girls; [3]Tanner pubic hair staging missing for 2 girls.

Table 15. Crude hazard ratios (cHRs) and adjusted[1] hazard ratios (aHRs) and 95% confidence intervals (CIs) predicting thelarche and pubarche, by metals categorized into quartiles. Interval—time of blood draw to pubertal onset.

	Thelarche (Tanner breast stage ≥2; n=309)[2]				
Metal	**n**	**cHR (95% CI)**	**Trend *P***	**aHR (95% CI)**	**Trend *P***
Arsenic (µg/L)			0.96		0.56
≤0.31	80	Ref		Ref	
>0.31–≤0.43	78	0.81 (0.59, 1.13)		0.77 (0.55, 1.08)	
>0.43–≤0.75	75	1.09 (0.78, 1.52)		0.99 (0.70, 1.39)	
>0.75	76	0.92 (0.66, 1.27)		0.83 (0.58, 1.18)	
Lead (µg/dL)			0.96		0.53
≤0.68	81	Ref		Ref	
>0.68–≤0.92	78	1.07 (0.77, 1.49)		1.06 (0.76, 1.49)	
>0.92–≤1.25	74	1.03 (0.74, 1.42)		0.89 (0.64, 1.24)	
>1.25	76	1.02 (0.74, 1.41)		0.95 (0.67, 1.33)	
Manganese (µg/L)			0.98		0.84
≤9.08	77	Ref		Ref	
>9.08–≤10.85	78	0.91 (0.65, 1.27)		0.79 (0.56, 1.11)	
>10.85–≤13.05	77	0.91 (0.66, 1.26)		0.83 (0.59, 1.18)	
>13.05	77	0.93 (0.67, 1.30)		1.03 (0.73, 1.44)	
Mercury (µg/L)			0.45		0.97
≤0.27	77	Ref		Ref	
>0.27–≤0.54	79	0.95 (0.68, 1.33)		0.92 (0.65, 1.30)	
>0.54–≤1.13	76	1.07 (0.77, 1.49)		0.95 (0.68, 1.33)	
>1.13	77	1.10 (0.79, 1.54)		0.99 (0.68, 1.43)	

[1]Adjusted for BMI and age at blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by Cox proportional hazards models; [2]annual family income missing for 4 girls; [3]Tanner pubic hair staging missing for 2 girls.

Table 15 (continued). Crude hazard ratios (cHRs) and adjusted[1] hazard ratios (aHRs) and 95% confidence intervals (CIs) predicting thelarche and pubarche, by metals categorized into quartiles. Interval—time of blood draw to pubertal onset.

	Pubarche (Tanner breast stage ≥2; n=307)[2,3]				
Metal	**n**	**cHR (95% CI)**	**Trend *P***	**aHR (95% CI)**	**Trend *P***
Arsenic (µg/L)			0.44		0.29
≤0.31	79	Ref		Ref	
>0.31–≤0.43	78	0.95 (0.68, 1.33)		1.02 (0.73, 1.43)	
>0.43–≤0.75	74	1.26 (0.90, 1.77)		1.22 (0.86, 1.74)	
>0.75	76	0.81 (0.58, 1.13)		0.78 (0.55, 1.10)	
Lead (µ/dL)			0.34		0.99
≤0.68	81	Ref		Ref	
>0.68–≤0.92	77	1.24 (0.89, 1.74)		1.28 (0.90, 1.82)	
>0.92–≤1.25	74	1.10 (0.79, 1.53)		0.88 (0.61, 1.26)	
>1.25	75	1.22 (0.88, 1.69)		1.13 (0.78, 1.62)	
Manganese (µg/L)			<0.01		<0.01
≤9.08	76	Ref		Ref	
>9.08–≤10.85	78	0.73 (0.53, 1.03)		0.70 (0.50, 0.98)	
>10.85–≤13.05	76	0.60 (0.43, 0.85)		0.63 (0.44, 0.91)	
>13.05	77	0.59 (0.42, 0.83)		0.56 (0.40, 0.79)	
Mercury (µg/L)			0.95		0.84
≤0.27	77	Ref		Ref	
>0.27–≤0.54	79	1.19 (0.85, 1.65)		1.10 (0.78, 1.54)	
>0.54–≤1.13	75	1.13 (0.80, 1.59)		0.94 (0.66, 1.34)	
>1.13	76	1.02 (0.73, 1.43)		1.01 (0.71, 1.44)	

Table 16. Crude hazard ratios (cHRs) and adjusted[1] hazard ratios (aHRs), 95% confidence intervals (CIs) and *P*-values predicting thelarche and pubarche by metals. Interval—birth to pubertal onset.

	Thelarche (Tanner breast stage ≥2; n=309)[2]		**Pubarche (Tanner pubic hair stage ≥2; n=307)[2,3]**	
Metal	**cHR (95% CI) *P*-value**	**aHR (95% CI) *P*-value**	**cHR (95% CI) *P*-value**	**aHR (95% CI) *P*-value**
Arsenic (1-log increase)	0.97 (0.83, 1.14) 0.73	0.94 (0.79, 1.13) 0.51	0.85 (0.72, 0.99) 0.04	0.85 (0.71, 1.01) 0.07
Cadmium				
≤LOD	Ref	Ref	Ref	Ref
>LOD	1.03 (0.81, 1.30) 0.83	0.97 (0.76, 1.25) 0.83	0.81 (0.64, 1.03) 0.09	0.71 (0.55, 0.92) <0.01
Lead (1 µg/dL increase)	1.09 (0.86, 1.38) 0.47	0.91 (0.71, 1.16) 0.44	1.28 (1.02, 1.60) 0.03	1.04 (0.81, 1.34) 0.75
Manganese (1 µg/L increase)	0.99 (0.96, 1.03) 0.75	1.01 (0.97, 1.05) 0.55	0.94 (0.91, 0.98) <0.01	0.94 (0.91, 0.98) <0.01
Mercury (1-log increase)	0.99 (0.89, 1.11) 0.90	1.00 (0.89, 1.13) 0.98	0.99 (0.89, 1.10) 0.80	1.02 (0.90, 1.14) 0.81

[1]Adjusted for BMI and blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by Cox proportional hazards models; [2]annual family income missing for 4 girls; [3]Tanner pubic hair staging missing for 2 girls.

Table 17. Crude hazard ratios (cHRs) and adjusted[1] hazard ratios (aHRs) and 95% confidence intervals (CIs) predicting thelarche and pubarche, by metals categorized into quartiles. Interval—birth to pubertal onset.

	Thelarche (Tanner breast stage ≥2; n=309)[2]				
Metal	**n**	**cHR (95% CI)**	**Trend *P***	**aHR (95% CI)**	**Trend *P***
Arsenic (µg/L)			0.93		0.71
≤0.31	80	Ref		Ref	
>0.31–≤0.43	78	0.85 (0.61, 1.18)		0.88 (0.63, 1.23)	
>0.43–≤0.75	75	1.24 (0.89, 1.73)		1.12 (0.80, 1.58)	
>0.75	76	0.91 (0.65, 1.26)		0.86 (0.61, 1.23)	
Lead (µ/dL)			0.49		0.74
≤0.68	81	Ref		Ref	
>0.68–≤0.92	78	1.16 (0.84, 1.61)		1.06 (0.75, 1.48)	
>0.92–≤1.25	74	1.05 (0.76, 1.45)		0.92 (0.65, 1.29)	
>1.25	76	1.16 (0.84, 1.61)		0.98 (0.70, 1.38)	
Manganese (µg/L)			0.70		0.75
≤9.08	77	Ref		Ref	
>9.08–≤10.85	78	0.78 (0.56, 1.08)		0.74 (0.52, 1.04)	
>10.85–≤13.05	77	0.80 (0.58, 1.11)		0.84 (0.60, 1.19)	
>13.05	77	0.93 (0.66, 1.29)		1.03 (0.73, 1.45)	
Mercury (µg/L)			0.72		0.81
≤0.27	77	Ref		Ref	
>0.27–≤0.54	79	1.01 (0.73, 1.41)		0.91 (0.65, 1.27)	
>0.54–≤1.13	76	1.00 (0.72, 1.40)		0.96 (0.68, 1.34)	
>1.13	77	1.07 (0.77, 1.50)		1.04 (0.72, 1.49)	

[1]Adjusted for BMI at blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by Cox proportional hazards models; [2]annual family income missing for 4 girls; [3]Tanner pubic hair staging missing for 2 girls.

Table 17 (continued). Crude hazard ratios (cHRs) and adjusted[1] hazard ratios (aHRs) and 95% confidence intervals (CIs) predicting thelarche and pubarche, by metals categorized into quartiles. Interval—birth to pubertal onset.

	Pubarche (Tanner breast stage ≥2; n=307)[2,3]				
Metal	**n**	**cHR (95% CI)**	**Trend *P***	**aHR (95% CI)**	**Trend *P***
Arsenic (µg/L)			0.27		0.27
≤0.31	79				
>0.31–≤0.43	78	1.06 (0.76, 1.47)		1.04 (0.75, 1.45)	
>0.43–≤0.75	74	1.28 (0.91, 1.80)		1.19 (0.83, 1.69)	
>0.75	76	0.79 (0.56, 1.1)		0.78 (0.55, 1.11)	
Lead (µ/dL)			0.05		0.93
≤0.68	81				
>0.68–≤0.92	77	1.43 (1.03, 2.01)		1.28 (0.90, 1.82)	
>0.92–≤1.25	74	1.21 (0.97, 1.69)		0.87 (0.61, 1.26)	
>1.25	75	1.48 (1.06, 2.06)		1.15 (0.80, 1.65)	
Manganese (µg/L)					
≤9.08	76		<0.01		<0.01
>9.08–≤10.85	78	0.68 (0.48, 0.94)		0.68 (0.49, 0.96)	
>10.85–≤13.05	76	0.52 (0.37, 0.74)		0.63 (0.44, 0.91)	
>13.05	77	0.57 (0.41, 0.80)		0.55 (0.39, 0.77)	
Mercury (µg/L)			0.73		0.74
≤0.27	77				
>0.27–≤0.54	79	1.18 (0.84, 1.64)		1.13 (0.81, 1.59)	
>0.54–≤1.13	75	1.12 (0.80, 1.57)		0.93 (0.65, 1.33)	
>1.13	76	0.95 (0.68, 1.33)		1.01 (0.71, 1.43)	

Table 18. Hazard ratios (HR) modeling for multiple metals simultaneously and adjusted[1] hazard ratios (aHRs) modeling for covariates and multiple metals simultaneously, 95% confidence intervals (CIs) and *P*-values predicting pubarche. n=307[2]. Interval—time of blood draw to pubertal onset.

	Cadmium/Arsenic		Cadmium/Manganese	
Metal	**HR (95% CI)** ***P*-value**	**HR (95% CI)** ***P*-value**	**HR (95% CI)** ***P*-value**	**aHR (95% CI)** ***P*-value**
Arsenic (1-log increase)	0.88 (0.75, 0.98) 0.11	0.83 (0.70, 0.98) 0.03	-	-
Cadmium				
≤LOD	Ref	Ref	Ref	Ref
>LOD	0.77 (0.60, 0.97) 0.03	0.69 (0.53, 0.90) <0.01	0.76 (0.60, 0.97) 0.03	0.70 (0.54, 0.91) <0.01
Manganese (1 μg/L increase)	-	-	0.94 (0.91, 0.98) <0.01	0.94 (0.91, 0.98) <0.01

[1]Adjusted for BMI at blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by Cox proportional hazards models; [2]Tanner pubic hair staging missing for 2 girls, and annual family income missing for 4 girls.

Table 19. Hazard ratios (HR) modeling for multiple metals simultaneously and adjusted[1] hazard ratios (aHRs) modeling for covariates and multiple metals simultaneously, 95% confidence intervals (CIs) and *P*-values predicting pubarche. n=307[2]. Interval—birth to pubertal onset.

	Cadmium/Arsenic		Cadmium/Manganese	
Metal	**cHR (95% CI)** ***P*-value**	**aHR (95% CI)** ***P*-value**	**cHR (95% CI)** ***P*-value**	**aHR (95% CI)** ***P*-value**
Arsenic (1-log increase)	0.82 (0.64, 1.04) 0.10	0.83 (0.70, 0.99) 0.04	-	-
Cadmium				
≤LOD	Ref	Ref	Ref	Ref
>LOD	0.85 (0.72, 1.00) 0.05	0.69 (0.53, 0.90) <0.01	0.82 (0.64, 1.04) 0.10	0.71 (0.55, 0.92) <0.01
Manganese (1 μg/L increase)	-	-	0.94 (0.91, 0.98) <0.01	0.94 (0.91, 0.98) <0.01

[1]Adjusted for BMI at blood draw, race/ethnicity, annual family income, and primary caregiver's educational attainment by Cox proportional hazards models; [2]Tanner pubic hair staging missing for 2 girls, and annual family income missing for 4 girls.