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Association between maternal urinary arsenic species and infant cord blood leptin levels in a New Hampshire Pregnancy Cohort ☆

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ABSTRACT

Leptin is an important pleiotropic hormone involved in the regulation of nutrient intake and energy expenditure, and is known to influence body weight in infants and adults. High maternal levels of arsenic have been associated with reduced infant birth weight, but the mechanism of action is not yet understood. This study aimed to investigate the association between *in utero* arsenic exposure and infant cord blood leptin concentrations within 156 mother–infant pairs from the New Hampshire Birth Cohort Study (NHBCS) who were exposed to low to moderate levels of arsenic through well water and diet. *In utero* arsenic exposure was obtained from maternal second trimester urinary arsenic concentration, and plasma leptin levels were assessed through immunoassay. Results indicate that urinary arsenic species concentrations were predictive of infant cord blood leptin levels following adjustment for creatinine, infant birth weight for gestational age percentile, infant sex, maternal pregnancy-related weight gain, and maternal education level amongst 149 white mother–infant pairs in multivariate linear regression models. A doubling or 100% increase in total urinary arsenic concentration (iAs+MMA+DMA) was associated with a 10.3% (95% CI: 0.8–20.7%) increase in cord blood leptin levels. A 100% increase in either monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA) was also associated with an 8.3% (95% CI: −1.0–18.6%) and 10.3% (95% CI: 1.2–20.2%) increase in cord blood leptin levels, respectively. The association between inorganic arsenic (iAs) and cord blood leptin was of similar magnitude and direction as other arsenic species (a 100% increase in iAs was associated with a 6.5% (95% CI: −3.4–17.5%) increase in cord blood leptin levels), *albeit* not significant. These results suggest *in utero* exposure to low levels of arsenic influences cord blood leptin concentration and presents a potential mechanism by which arsenic may impact early childhood growth.

1. Introduction

New research suggests an association between arsenic exposure and leptin (Ahmed et al., 2011; Fei et al., 2013). Leptin is an adipocyte hormone encoded by the *LEP* gene in humans (Li, 2011) that is well known for its role in regulating satiety, energy expenditure, and fat mass in both adults and infants (Friedman, 2002). Prenatal exposure to arsenic during pregnancy has also been associated with decreased fetal size at birth–specifically in the lower ranges of arsenic exposure (Rahman et al., 2009). In a Bangladeshi cohort of mother–infant pairs, arsenic exposure during pregnancy was found to be positively associated with leptin expression in the placenta as detected by immunohistochemistry, when using measures of maternal urinary arsenic collected at 30 weeks of gestation as the exposure (Ahmed et al., 2011). To our

Abbreviations: 95% CI, 95% confidence interval; AGA, average for gestational age; As, arsenic; BMI, body mass index; DMA, dimethylarsinic acid; iAs, inorganic arsenic; IQR, interquartile range; LEP, leptin gene; LGA, large for gestational age; MMA, monomethylarsonic acid; NHBCS, New Hampshire Birth Cohort Study; SGA, small for gestational age

☆This study was reviewed and approved by the Committee for the Protection of Human Subjects (CPHS) at Dartmouth College, Hanover, NH, and all participants in the study provided informed consent in accordance with CPHS guidelines.

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knowledge, no studies have investigated the association between maternal urinary arsenic and leptin expression in infant cord blood.

The placenta (Linnemann et al., 2004) and fetal adipose tissues (Lepercq et al., 2001) produce the majority of leptin that circulates in the fetus; in addition, some leptin is actively transported across the placenta from the mother (Linnemann et al., 2004). Cord blood leptin levels have been shown to closely reflect intravenous leptin levels in infants within the first 6 h of life, indicating that cord blood concentrations are similar to circulating leptin levels within the infant at birth (Harigaya et al., 1997). Interestingly, female infants have been shown to have higher cord blood leptin levels at birth when compared to male infants (Alexe et al., 2006). Cord blood leptin levels are positively correlated with birth weight as well as birth weight for gestational age (Fonseca et al., 2004; Forhead and Fowden, 2009; Geary et al., 1999; Karakosta et al., 2013; Mantzoros et al., 2009; Mullis and Tonella, 2008; Schubring et al., 1997) and explain ~21% of the variation in birth weight between infants (Karakosta et al., 2011). Fetal leptin levels seem to reflect fetal adiposity rather than affect fetal weight gain as infants with congenital leptin deficiency (Montague et al., 1997; Strobel et al., 1998) or leptin receptor mutations (Clement et al., 1998) are born with normal birth weights.

Leptin plays an important role in the regulation of weight gain during infancy and early childhood growth (Fonseca et al., 2004; Mantzoros et al., 2009; Montague et al., 1997; Ong et al., 1999; Strobel et al., 1998). Infants with leptin deficiency or non-functional leptin receptors have rapid weight gain in early life (Clement et al., 1998; Montague et al., 1997; Strobel et al., 1998). Amongst healthy and preterm infants (Fonseca et al., 2004) without monogenic forms of obesity, English and American studies have associated higher cord blood leptin with less weight gain or catch-up growth up to 6 months (Mantzoros et al., 2009) and 2 years of age (Ong et al., 1999), and lower adiposity at 3 years of age (Mantzoros et al., 2009). This work suggests that slight imbalances to infant leptin levels due to gestational exposures may influence future weight phenotypes.

Arsenic (As) is a metalloid element that is ubiquitous in the environment (Jomova et al., 2011; Watanabe and Hirano, 2012), and has well-documented toxicity in humans (Chen et al., 2007; Watanabe and Hirano, 2012). The most common routes of non-occupational arsenic exposure is through food and drinking water (Jomova et al., 2011; Watanabe and Hirano, 2012). In the state of New Hampshire, unregulated private wells are commonly used as the home water supply, and an estimated one in ten New Hampshire homes rely on a well with water arsenic concentrations exceeding the Environmental Protection Agency's recommended maximum of 10 μg/L (Environmental Protection Agency, 2001; Jomova et al., 2011; Karagas et al., 2002; Karagas et al., 1998). Upon ingestion, inorganic arsenic (iAs) is sequentially converted to monomethylated (MMA) and dimethylated (DMA) arsenic species, and all three species are primarily excreted through urine (Jomova et al., 2011; Watanabe and Hirano, 2012).

Arsenic is a contaminant of particular concern for pregnant women, as it is known to freely transport from mother to fetus through the placenta, with *in utero* arsenic exposure concentrations approximating those experienced by the mother throughout pregnancy (Concha et al., 1998; Vahter, 2009). Furthermore, even low levels of *in utero* exposure to arsenic may result in decreased fetal birth size (Rahman et al., 2009). The previously observed associations between leptin and infant weight, and prenatal arsenic exposure and infant birth weight, support the investigation of a relationship between arsenic exposure and infant leptin levels, as arsenic may influence infant weight through leptin.

This study investigates whether exposure to arsenic *in utero* (as measured through maternal excretion of arsenic species in urine) can predict infant cord blood leptin levels in a population exposed to relatively low levels of arsenic through diet and well water.

2. Materials and methods

2.1. Study population

The study population consisted of 156 mother–infant pairs who were participants in the ongoing New Hampshire Birth Cohort Study (NHBCS), and were enrolled between January 2009 and June 2009. The mother–infant pairs chosen for this study represent a convenience sample of the first 156 mother–infant pairs from the NHBCS with sufficient maternal and cord blood plasma for leptin analysis, as well as sufficient maternal urine samples for arsenic analysis. The NHBCS has been described in detail elsewhere (Farzan et al., 2013; Fei et al., 2013; Gilbert-Diamond et al., 2011; Koestler et al., 2013). Briefly, mothers were enrolled at ~24–28 gestational weeks at study clinics in New Hampshire, USA, beginning January, 2009. Mothers included in the cohort were literate in English, mentally competent, between 18–45 years old, and reported using a private, unregulated well for their home drinking water since their last menstrual period. Infants included in the cohort were singleton, live pregnancies. Self-reported sociodemographic (age, race/ethnicity, marital status, level of education), lifestyle (including tobacco and alcohol use, previous pregnancies, complications, birth outcomes), and clinical data were derived from pre- and post-delivery questionnaires and a medical records review. Participants provided informed consent in accordance with the policies set up by the Institutional Review Board (IRB) and Dartmouth College.

2.2. Sample collection

Infant cord blood samples were collected at the time of delivery by trained obstetrical staff. Cord blood samples were centrifuged and separated into components which were then aliquoted, and the plasma component was stored at −80 °C in liquid nitrogen until plasma leptin concentrations were assayed.

Urine samples were requested from study participants during a routine second trimester prenatal visit. Maternal spot urine samples were collected from study participants at the time of enrollment (between ~24–28 weeks of gestation). Samples were collected in pre-labeled, acid washed bottles containing 30 μL of 10 nM diammonium diethyldithiocarbamate to stabilize arsenic species, and were subsequently frozen within 24 h of sample collection at −80 °C until analysis. Analysis occurred at the University of Arizona Hazard Identification Core.

2.3. Leptin concentration analysis

Infant cord blood leptin concentration levels (pg/mL) were measured using the standard manufacturer's protocol for the MILLIPLEX MAP® Human Adipokine Magnetic Bead Panel 2 (Millipore, Billerica, Massachusetts) by DartLab, Geisel School of Medicine at Dartmouth College. Standards and spikes were measured in triplicate, undiluted cord blood samples were measured once, and blank values were subtracted from all readings. The mean intra-plate and inter-plate assay quality controls were 8.27% and 13.51%, respectively. In total, $n=156$ infant cord blood samples were assayed for leptin protein levels. All leptin concentrations were within the detection range of the assay (0.192–600 ng/mL) and fell within the quality control/assurance acceptability limits of 70–130%.