EARLY LIFE EXPOSURE TO LEAD, IRON METABOLISM GENE VARIANTS, AND IMPACTS ON REPRODUCTIVE AND INFANT OUTCOMES

by

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CHAPTER I

Introduction

Fetal Origins of Adult Disease

As of 2006, roughly 87,000 chemical substances were registered for commercial use in the United States (USEPA 2006). Humans are exposed daily to mixtures of these environmental contaminants through air, water, food, and consumer products. Thousands of these substances are produced in high volume in the US and worldwide, yet surprisingly few have any basic toxicological data regarding developmental or neonatal toxicity (USEPA 1998). Route of exposure, dose of toxicant, and interpersonal variations in susceptibly all alter how an individual may respond to these potential environmental toxicants. Of particular interest recently in environmental epidemiology has been identifying particular exposure time points during development which are most critical for toxicant impacts on particular health outcomes and how personal susceptibility to environmental toxicants may be modified by common genetic mutations. This dissertation addresses these pertinent issues by expanding the scientific understanding of prenatal susceptibility to lead exposure

Lead Exposure: Public Health Significance

Although tremendous reductions in lead exposure to the general population have been achieved in most of North America and Europe, worldwide adults and children continue to be exposed to elevated levels through a variety of media and informal sector occupations (Meyer et al. 2008). Lead has been documented to impact a variety of health outcomes including, but not limited to; neurodevelopment (Lamphear et al. 2005; Bellinger 2008), cardiovascular disease (Navas-Acien et al. 2007), neurodegenerative diseases and cognitive decline (Schwartz et al. 2000; Weisskopf et al. 2004), immune system impairment (Dietert and Piepenbrink 2006), renal system function (Kim et al. 1996; Weaver et al. 2009), and adverse birth outcomes (Andrews et al. 1994). Many of these health impacts have been shown to occur at increasingly lower exposures suggesting there is no 'safe' threshold to lead exposure.

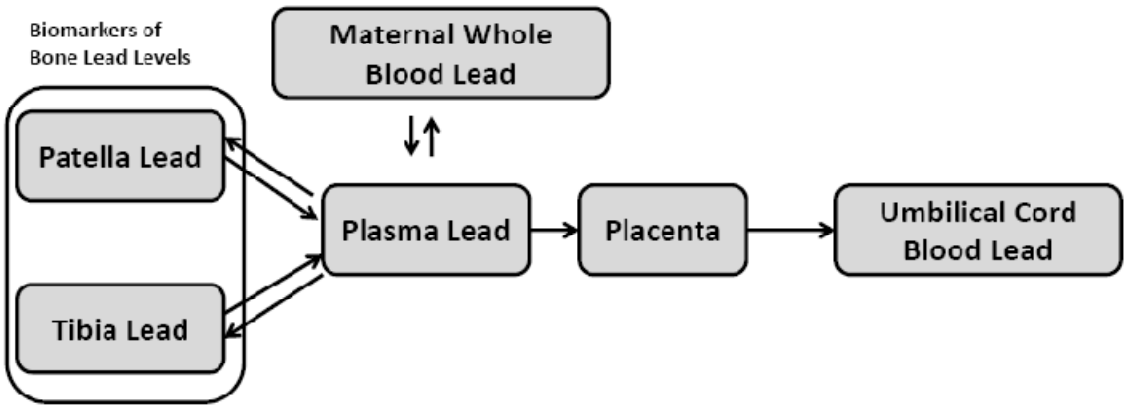
The estimated economic impacts of a population-level reduction in lead exposure are staggering. Grosse et al.'s model of economic gain due to a nationwide reduction in blood lead over the last 23 years and subsequent loss of IQ predicted a 213.8 billion dollar gain over the lifetime of a yearly birth cohort (Grosse et al. 2002). Rothenberg and Rothenberg built upon this conservative estimate to show that a log linear dose–response relationship between blood lead reduction and IQ doubled (2.2 times) the economic gains of Grosse et al.'s estimates (Rothenberg and Rothenberg 2005). Of particular concern recently has been research documenting that long-term (potentially lifetime) storage of lead in cortical and trabecular bone sites continue to be a significant internal sources of

exposure, especially during times of increased bone turnover such as pregnancy and lactation (Hu and Hernandez-Avila 2002; Gulson et al. 2003).

Routes of Exposure and Internal Distribution of Lead

The principle routes of external exposure to and intake of lead usually occur through ingestion and inhalation (White et al. 1998; Hu et al. 2007). Absorption can be influenced by a host of factors such as nutritional, metabolic, and genetic factors (Mahaffey 1995; Philip and Gerson 1994; Gulson et al. 2004; Ettinger et al. 2009; Hopkins et al. 2008). This is of particular concern during times of altered physiology, like pregnancy, because the demands of the fetus can enhance exposure and absorption. Measurement of internal lead dose has been assessed through a variety of biomarkers (See figure I-1), though the bulk of human epidemiological studies have utilized two critical biomarkers: whole blood and tibia/patella bone lead.

Figure I-1: Biomarkers of lead exposure



Whole Blood Lead

Once lead is absorbed externally, or internal stores are mobilized, it enters into a flux between plasma and erythrocytes, with over 95% of lead bound to erythrocytes (Simons 1988; Rabinowitz et al. 1976; deSilva 1981; Barltrop and Smith 1972; Bergdahl and Skerfving 1997; Church et al. 1993; O'Flaherty 1993; Rabinowitz 1991; Simons 1984). Whole blood lead is commonly thought of as a biological marker of external lead dose representing recent exposure, with a half-life of approximately 30-35 days (Rabinowitz 1991; Sakai 2000). Bone lead stores have been shown to contribute as much as 40-70% of the lead found in whole blood, suggesting that internal exposure arising from stored lead in the skeleton will additionally be reflected in this biomarker (Smith et al. 1996). Umbilical cord blood lead measured at delivery has also been widely utilized as a surrogate of fetal exposure (Gardella 2001). Maternal blood lead concentration is widely thought to readily cross the placenta and is highly correlated with cord blood lead (Goyer 1996). However, pregnancy is a dynamic state with increases in bone resorption, intestinal absorption, and hemodilution which requires that studies of prenatal lead exposure and health effects require a more complex study design than one using a single biomarker of exposure.

Plasma Lead

While ~95% of lead is bound to erythrocytes, the rest present in plasma is considered the fraction of biologically active lead, able to readily cross cell membranes and exert toxicological effects upon cellular systems (Cavalleri et al.

1978; Smith et al. 2002; Smith et al. 1998). The pattern of association between plasma and whole blood lead remains largely unclear, but recent studies indicate that the relationship not only follows an exponential pattern, but overall, whole blood lead levels are neither an accurate nor precise reflection of plasma lead levels (Smith et al. 2002; Lamadrid-Figueroa et al. 2006). Hernandez-Avila et al. concluded in their study that bone lead levels were exerting a stronger influence on plasma lead levels than whole blood lead (Hernandez-Avila et al. 1998). In follow-up studies, it was found that the plasma-to-whole blood ratio had wide variation among individuals providing additional evidence that during the dynamic period of pregnancy there is wide-interpersonal variation resulting in exposure discrepancies from internal bone lead sources (Lamadrid-Figueroa et al. 2006).

Bone Lead

Lead residing in bone represents approximately 90–95% of an adult's current body burden of lead (Barry 1975; Wittmers et al. 1988). The half-life of lead in bone varies from years (in patella) to decades (in tibia) and therefore bone lead is considered an estimate of lifetime cumulative lead dose (Brito et al. 2000; Brito et al. 2001). Bone may release it's lead content into the bloodstream in the course of normal bone metabolism or at increased rates during active bone demineralization, such as occurs during pregnancy (Gulson et al. 2003). Measurement of lead in bone has mainly been conducted by the use of a noninvasive, in vivo X-ray fluorescence (KXRF) technique which has been utilized in a variety of epidemiological studies (Schwartz et al. 2000; Aro et al.

2000; Hoppin et al. 2000; Hu et al. 2007). Recent studies have shown that during times of critical fetal development internal sources of lead are significant predictors of negative health effects in later life, such as decreased cognitive function, even after controlling for the traditional measure of external exposure, the blood lead level (Hernandez-Avila et al. 2002; Gomaa et al. 2002).

Lead Health Impacts: Focus on Neurotoxicity and Adverse Birth Outcomes

Although lead exposure has been associated, and causally linked, with a number of health impacts, for the purpose of this thesis we will focus upon two particular early life outcomes: neurocognition and birth outcomes (gestational length and birthweight).

Neurocognition

Lead continues to be one of the most prevalent neurotoxic environmental contaminants worldwide (Fewtrell et al. 2004). Experimental studies have provided convincing evidence that lead can adversely impact N-methyl D-aspartate receptors, dopaminergic receptors, neurotransmitter release, and integrity of the blood-brain barrier in animal models (Cory-Slechta 1995; Cory-Slechta et al. 1997; Cory-Slechta et al. 1996; Lasley and Gilbert 1999; White et al. 2007; Yi and Lim 1998; Bressler and Goldstein 1991; Goldstein 1990). Evidence has also linked these neurochemical alterations induced by lead in animal models to functional deficits in a wide variety of memory and learning tasks (Rice 1993).

Early epidemiological evidence indicates that prenatal lead exposure (assessed through either cord blood or maternal blood at delivery) and postnatal lead exposure both independently impact a variety of early infant cognition outcomes (Shen et al. 1998; Lanphear et al. 2000; Needleman and Gatsonis 1990; Bellinger et al. 1987; Ernhart et al. 1987; Dietrich et al. 1987). In a meta-analysis of epidemiological studies done during the 1990s, a 10 μg/dL increase of blood lead level resulted in a linear decline of 2-3 points of children's IQ (Schwartz 1994).

Few of these previous studies used designs that allow for neurocognition impacts of prenatal lead exposure to be distinguished from those of postnatal lead exposure, with two important exceptions. Gomaa et al. found that maternal bone lead levels and infant cord blood independently predicted lower scores on the Bayley scales of infant development (BSID), highlighting the importance of assessing lead exposure during pregnancy with both biomarkers in order to capture early and late effects of lead exposure (Gomaa et al. 2002). In addition, Hu et al. showed that, after controlling for cord blood lead and infant blood lead at 24 months, early prenatal (1^{st} and 2^{nd} trimester) lead exposure significantly reduces infant BSID at 24 months by approximately 4 points (95%CI –8.10 to –0.17), which suggests that only using cord blood lead as a biomarker may not adequately capture the most sensitive time point for lead's prenatal effects upon neurocognition (Hu et al. 2006).

As epidemiological research has advanced, many researchers have argued that there is no apparent threshold for lead's effects upon infant neurocognition

(Bellinger 2008; Tellez-Rojo et al. 2006; Schwartz et al. 1988; Schwartz et al. 1993). Low dose effects (<10 μg/dL) occurring from postnatal lead exposure have consistently been associated with decrements in childhood IQ ((Tellez-Rojo et al. 2006; Lanphear et al. 2005; Lanphear et al. 2000; Bellinger and Needleman 2003) Researchers utilizing a log-linear model, building upon the previous linear model meta-analysis, predicted that steeper declines in children's IQ, approximately 6 points, occurred at blood lead levels less than 10 μg/dL than at blood lead levels higher than 10 μg/dL (Lanphear et al. 2005).

In conclusion, animal and human epidemiological studies have provided consistent, compelling evidence that lead exposure is adversely associated with infant cognition. Recent epidemiological research has suggested that these adverse health effects may be due to exposure occurring earlier in pregnancy and to lower than previously documented lead levels.

Birth Outcomes

It is estimated that approximately 17% (more than 20 million) of all neonates in developing countries and 8% of neonates in the United States are classified as low birth weight infants, defined as weighing less than 2,500 grams at birth (UNICEF 2004; IOM 2007). Low birth weight has been well established as a predictor of higher infant mortality, morbidity, and adverse developmental outcomes such as lower cognitive performance, and increased risk of chronic disease into adulthood (Tamakoshi et al. 2006; Tong et al. 2006). An infant may be low birth weight because she/he is either born small for gestational age (a

measure of intrauterine growth restriction) or she/he is born prematurely. Of particular concern recently has been the steady rise in premature births accounting for 12.5% of all births in the United States during 2004 (IOM 2007). Many factors such as maternal nutrition, maternal genitourinary infection, maternal and paternal genetics, pregnancy-induced hypertension, and maternal cigarette smoking are associated with elevated risks for low birth weight, small for gestational age, and preterm delivery. Although this list is not all inclusive, there has been a recognized gap in knowledge concerning the role of suspected environmental factors, such as exposure to toxic metals (IOM 2007).

Decreases in birth weight and gestational length have been independently associated in epidemiological studies with cord blood lead (Factor-Litvak et al. 1991; Bellinger et al. 1991) and maternal blood lead at delivery (McMichael et al. 1986). Andrews et. al. reviewed 25 epidemiological studies published prior to 1994 investigating the relationship between lead exposure, mean birthweight, gestational length, and risk of either low birth weight or preterm delivery, but due to the inconsistency between studies and failure of many other studies to account for potential confounders they concluded it was impossible to judge if an association existed between lead exposure and many birth outcomes, with the exception of risk for preterm delivery (Andrews et al. 1994). A common hallmark of these past studies and even the recent ones (Torres-Sanchez et al. 1999; Odland et al. 1999; Falcon et al. 2003; West et al. 1994; Berkowitz et al. 2006; Chen et al. 2006) has been a failure to account for potential internal sources of exposure resulting from higher cumulative bone stores. Only one study has been

published to date indicating a significant negative association between maternal bone lead levels and birthweight (Gonzalez-Cossio et al. 1997).

There are few prior studies which assess the relationship between whole blood lead levels at multiple times during gestation and either birthweight or gestational length (Factor-Litvak et al. 1991; Sowers et al. 2002; Jelliffe-Pawlowski et al. 2006). In Jelliffe-Pawlowski et al.'s study of 262 mother-infant pairs who gave birth between 1996-2002 in California, it found that a significant decrease in length of gestation is associated with 2nd trimester maternal blood lead (1.0 day decrease in length of gestation per 1 μg/dL blood lead level over 10 μg/dL), though they observed no effect in relation to birthweight (Jelliffe-Pawlowski et al. 2006). In contrast, Sowers et. al., 2002 found no significant relationship between risk of preterm delivery or low birthweight and trimester-specific maternal blood lead, though it is important to note that blood lead levels were low (1.2μg/dL+/-SE 0.03) (Sowers et al. 2002). Factor-Litvak et. al. also found no significant relationship between length of gestation or birthweight and maternal blood lead measured at mid-pregnancy (Factor-Litvak et al. 1991).

In conclusion, though there have been a variety of epidemiological studies attempting to define the relationship between lead exposure and adverse birth outcomes, a vast majority of these studies cannot address critical issues such as windows of susceptibility or contribution of internal lead stores due to original design. Additionally, with the inconsistency in effects seen across epidemiological studies and the relative lack of studies addressing potential biological mechanisms, the available data are inadequate to establish the

presence or absence of an association between lead exposure and adverse birth outcomes.

Iron During Pregnancy/Early Infancy: Public Health Significance

Anemia has been recognized by the World Health Organization to be one the major contributing factors to the global burden of disease (WHO 2002). Globally, anemia affects 1.62 billion people (95% CI 1.50–1.74), which corresponds to 24.8% of the total population, though pregnant women (41.8%) and preschool aged children (47.4%) are significantly more affected (WHO 2002). Anemia, which is defined as a shortage in hemoglobin, may result from various factors but a major contributor is iron deficiency (WHO 2004). Worldwide, 50% of women of reproductive age are iron deficient, with estimates as high as 80% in pregnant women from developing countries (WHO 2004).

Assessing iron status in individuals is a complicated process for which any single biomarker may not be an accurate estimate of iron body stores (WHO 2004). To further compound the issue, a reliable assessment of iron status is not possible in the presence of inflammation or infection. Hematological parameters such as mean corpuscular volume, free erythrocyte protoporphyrin, serum ferritin, transferrin saturation, and serum ferritin receptor have all been used independently to assess iron status, (WHO 2004) but some researchers have advocated for using multiple parameters such as mean corpuscular volume, free erythrocyte protoporphyrin, and serum ferritin together to better explain potential iron status (Lozoff et al. 2006). The WHO suggests using serum ferritin to assess

iron status since it is relatively stable in healthy people. Due to plasma volume expansion serum ferritin is only accurate during pregnancy up to the 2nd trimester and not recommended for children under 18 months (WHO 2004).

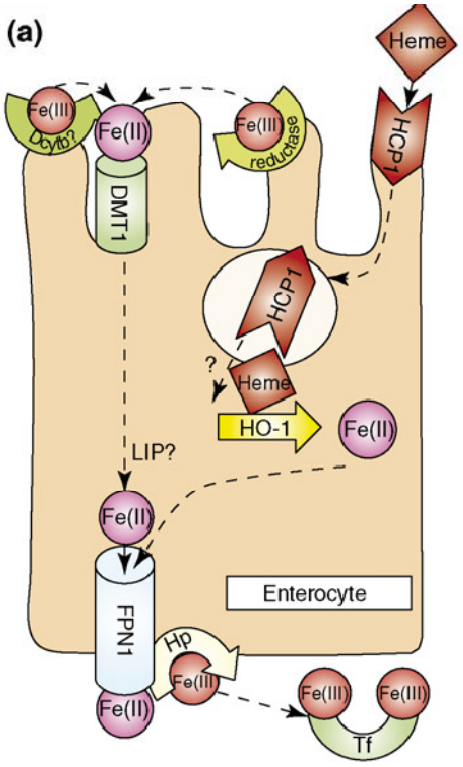
Iron is a critical participant in many important biochemical processes (Dunn et al. 2007). Achieving cellular iron homeostasis within a "normal" functional range is essential since excess iron can react with oxygen via the Fenton reaction producing hydroxyl radicals capable of free radical damage. Abnormal levels of iron, either excess or shortage, have been shown to adversely impact neurodevelopment (Lozoff 2007; Beard 2007), birth outcomes (Lao et al. 2000), and neurocognitive declines (Lehmann et al. 2006; Pulliam et al. 2003). Iron uptake across cell membranes is complex and regulated by multiple proteins including transport proteins such as transferrin, divalent metal transporter-1, and ferroportin, their receptors, as well as regulatory proteins such as *HFE* and hepcidin (Rivers et al. 2007). Briefly, we will discuss the normal cellular mechanisms involved in iron absorption, cellular transport and uptake, and control of iron homeostasis.

Iron Absorption

Though organisms preserve a majority of internal body iron during erythrocyte recycling, daily losses occur through a variety of mechanisms, necessitating absorption from dietary sources. Iron exists in two main forms: the ferric (+3) and ferrous (+2) form. Before intestinal absorption iron (+3) must be reduced, to +2, which occurs on the apical surface of enterocytes by

ferrireductase duodenal cytochrom-b (Dcytb) (Figure I-2). Iron (+2) is then transported across the apical surface of the enterocytes via the divalent metal transporter-1 (DMT-1). Iron, bound within the heme molecule, can also be transported across the apical membrane of enterocytes via the recently identified heme carrier protein-1 (HCP-1).

Figure I-2: Intestinal iron absorption



(From ref: Dunn et al. 2007)

Through a currently unknown mechanism these intercellular sources of iron are transported to the basal lateral membrane. They are then actively transported across the apical membrane via ferroportin-1 (FPN-1), and subsequently oxidized to iron (+3) by ferroxidase hephaestin (Ganz 2007). This extracellular source of iron is then quickly bound by the serum iron transport protein transferrin (TF) and moved into circulation (de Jong et al. 1990). Control of

absorption occurs by regulation in expression of DMT-1, FPN-1, and HCP-1, becuase any intercellular enterocyte iron stores are lost during normal intestinal shedding every 2-3 days (Anderson et al. 2007). Ferroportin has been shown to be negatively regulated by hepcidin, an iron regulatory hormone secreted mainly from hepatocytes (Ganz 2006; Nemeth et al. 2004). Additionally, it has been shown that DMT-1 expression can be regulated by *HFE* (Ganz 2007).

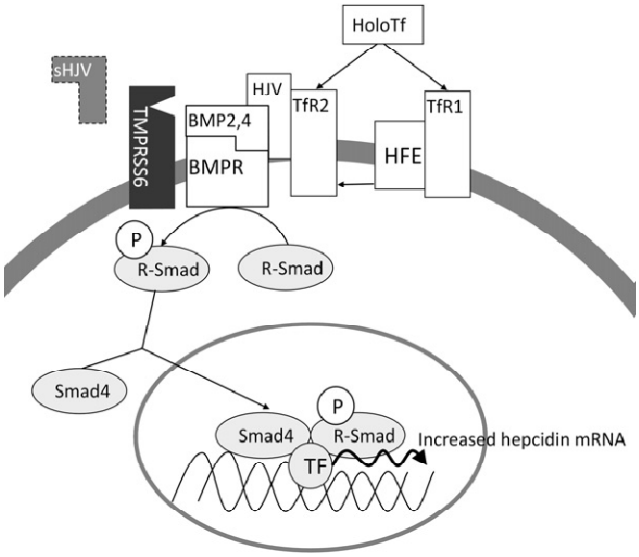
Cellular Iron Transport and Uptake

Transferrin (TF), one of the most important plasma proteins involved in iron transport, is thought to be the major regulator of iron movement between sites of absorption, storage, and utilization (de Jong et al. 1990). Transferrin has the ability to bind reversibly two iron (+3) ions with high affinity at neutral pH, as well as a wide range of other metal ions though at a reduced affinity (Sargent et al. 2005). Transferrin is encoded by a gene on chromosome 3q21 and has over 36 different protein variants (Beckman et al. 1998). The *TF P570S* variant arises when a proline in the C-terminal lobe of the native *TF* (position 570) is replaced by a serine (Beckman et al. 1998). Approximately 14%-20% individuals of European descent carry the *TF P570S* variant (Zatta et al. 2005). It is still being debated whether the *TF P570S* polymorphism decreases the iron binding capacity of transferrin when compared to wildtype individuals (Beckman et al. 1998; Zatta et al. 2005).

Iron Homeostasis

Sensing sufficient iron body stores is critical to proper iron homeostasis. When this system is disrupted, altered expression of transporters, such as DMT-1 and ferroportin, results. Gao et al. showed that the TfR2/HFE/Tf complex is intimately involved in sensing TF saturation which in turn regulates hepcidin expression and ultimately breakdown of ferroportin (Gao et al. 2009). In addition, it has been shown that when *HFE* is dissociated from TfR1, hepcidin production is stimulated (Schmidt et al. 2008; Ganz 2008). It has been postulated when these phenomena are connected together, it is likely that transferrin interacts with TfR1, which in turn frees *HFE* to interact with TfR2 resulting in increased hepcidin production and subsequent breakdown of ferroportin (Figure I-3).

Figure I-3: Molecular mechanism of hepcidin regulation



(From ref Ganz 2008)

Hemochromatosis

Adult onset hemochromatosis is an autosomal recessive disease in which normal iron absorption is altered leading to increased body stores of unbound iron (Drakesmith and Townsend 2000). Without proper treatment, affected individuals develop iron overload, which eventually leads to death via liver cancer, liver cirrhosis, diabetes, or cardiomyopathy due to the generation of iron free radicals by the Fenton reaction (Drakesmith and Townsend 2000; Deugnier and Turlin 2007; Olynyk et al. 1999).

Polymorphisms in the *HFE* gene are found in 85% of adult onset hemochromatosis cases (Jackson et al. 2001). While the penetrance for clinical disease is low (about 0.4% in those with European ancestry) the *HFE* gene is of particular interest in public health research as it contains two functional variants. In the general population, approximately 9.2% and 22.0% carry a variant copy of either the *C282Y* or *H63D* polymorphism respectively (Hanson et al. 2001).

Mutations in the *HFE* gene have been shown to disrupt the binding of HFE to TfR1 (Schmidt et al. 2008) and TfR2/HFE Tf-dependent regulation of hepcidin expression (Gao et al. 2009), thus leading to more iron bound transferrin entering cells and increased ferroportin-mediated iron import (Ganz 2006). In the C282Y polymorphism, a cysteine is replaced with a tyrosine, disrupting a disulfide bridge necessary for *HFE* binding to the β2-microglobulin within the TF receptor (Feder 1999). The *HFE H63D* mutation occurs within a histidine rich encoding patch on the *HFE* DNA while it is thought that the interaction with the transferrin receptor occurs within this patch, researchers doubt that this effects the function of *HFE*

(Lebron et al. 1998; Davies and Enns 2004). A secondary feature of *HFE* mutations is that expression of DMT-1 is subsequently increased (Ganz 2007). It is well agreed upon that homozygotes for the *HFE* C282Y mutation and compound heterozygotes for the *HFE C282Y* and *HFE H63D* mutations, are associated with elevated transferrin saturations and serum ferritin levels (Jackson et al. 2001; Beutler 2006; Beutler et al. 2002). Research has suggested that individuals heterozygous for either the *HFE H63D* or *HFE C282Y* mutation have slightly elevated makers of iron status when compared to wildtype individuals (Jackson et al. 2001; Pedersen and Milman 2009) but these results need to be validated.

Iron Health Impacts: Focus on Neurotoxicity and Adverse Birth Outcomes

Neurocognition

There is compelling animal and epidemiological evidence to indicate that early developmental iron deficiency (6-24 months) leads to irreversible changes in brain structure and function (Lozoff 2007; Beard 2007; Lozoff and Georgieff 2006; McCann and Ames 2007). Three interconnected aspects of brain biology impacted by iron deficiency shown by animal and human studies are: neurogenesis and differentiation of certain brain cells and brain regions (specifically the hippocampus, striatum, and oligodendrocytes cells), neurochemistry (specifically the monoaminergic pathways), and neurometabolism (Beard 2007).

There have been a vast number of cross-sectional, longitudinal, randomized controlled trial (RCT), and double-blind randomized controlled trial (DBRCT) studies of iron status post-delivery and infant cognition conducted around the world which have been summarized in more detail elsewhere (Lozoff 2007; Grantham-McGregor and Ani 2001; Sachdev et al. 2005). Summaries from these studies indicate that if an infant is found to be iron deficient at delivery and subsequently has it's iron status brought back to normal, then maintained through early childhood, it still suffers from adverse neurological effects such as slower neural transmission in the auditory system and visual system (Peirano et al. 2009), social-emotional behavior alternations (i.e. greater incidence of shyness) (Lozoff et al. 2008), and cognitive deficits (Beard 2007). Though there have been several longitudinal (Colomer et al. 1990; Kilbride et al. 1999; Miller et al. 2003) and few randomized iron supplementation controlled trials (Preziosi et al. 1997) during pregnancy and subsequent effects upon infant iron status none of these studies assessed cognitive, social-emotional behavior, or motor development.

Birth Outcomes

Birthweight has been shown to have an inverse "U" shaped pattern with hemoglobin, where concentrations above 14.5 g/dL (Steer 2000; Steer et al. 1995; Sagen et al. 1984) and below 8.5 g/dL (Lao et al. 2000; Murphy et al. 1986) are associated with decreases in birthweight. It has been speculated that the relationship between high hemoglobin concentrations and decreased birthweight may result from an increase in blood viscosity, which in turn may

reduce placental perfusion and growth of the fetus (Sagen et al. 1984). As noted earlier, it is difficult to assess proper iron status during pregnancy due to complex physiological changes, such as plasma volume expansion. Although there have been several large double-blind randomized controlled trials of multimicronutrient supplementation during pregnancy, these studies were not designed to assess iron supplementation per se(Chan et al. 2009; Zeng et al. 2008; Zagre et al. 2007).

In conclusion, approximately 42% of pregnant women worldwide are anemic with half of these cases attributable to iron deficiency (WHO 2002). A number of studies have shown that iron deficient anemic mothers give birth to infants with significantly reduced iron stores (Halvorsen 2000). Recent research has also suggested that prenatal iron status is extremely important for subsequent neurological effects, and potentially adverse birth outcomes, since iron is prioritized to erythrocytes at the expense of other tissues, including brain tissue (WHO 1998). Future research into prenatal iron status and subsequent health outcomes is critically needed.

Lead/Iron Interactions

For the last several decades a variety of cross-sectional epidemiological studies have found associations between iron deficiency and increased blood lead burdens (Yip et al. 1981; Wright et al. 1999; Kim et al. 2003; Bradman et al. 2001). In addition to the cross-sectional studies, Wright et al. demonstrated that

there is a longitudinal association between iron deficiency and a subsequent increase in blood lead levels (Wright et al. 2003).

Previous molecular stuides between increased lead uptake during iron deficient conditions have recognized that both lead and iron compete for the common DMT-1 transporter (Bressler et al. 2004; Bannon et al. 2002). Evidence of this interaction has been shown in yeast and mammalian cell lines, where lead and iron both use DMT-1 (Bannon et al. 2002). Iron binds with more affinity to DMT-1 than lead suggesting that under conditions of higher iron concentration lead absorption through this system might be restricted (Garrick et al. 2006). In support of this, lead transport through DMT-1 was found to be 80% inhibited by a 25 fold increase in iron (Bannon et al. 2002).

HFE/TF Modification of Lead Absorption and Health Effects

Lead absorption has also been shown to be affected by mutations in the *HFE* gene. It was shown by Barton et al. that individuals homozygous for hereditary hemochromatosis had significantly higher blood lead than normal controls (5.6 ± 0.6 µg/dL compared to 3.6 ± 0.5 µg/dL), while Akesson et al. found no difference in blood lead levels between variants (Barton et al. 1994; Akesson et al. 2000). Recently, it was demonstrated that both separate and joint effects of iron metabolism gene variants *HFE* and *TF* were associated with increased blood lead levels in Mexican children after controlling for either hemoglobin or ferritin levels (Hopkins et al. 2008). Conversely, Wright et. al. showed that older adult carriers of either *HFE* variant genotype (*H63D* or

C282Y), had lower blood, patella bone, and tibia bone lead stores when compared to wildtype individuals (Wright et al. 2004).

Although existing evidence for *HFE* modification of lead body burden is inconsistent and conflicting, many of the studies were unable to provide an accurate picture of internal iron status (Hopkins et al. 2008; Barton et al. 1994; Wright et al. 2004) or did not have enough power to perform meaningful statistical testing (Akesson et al. 2000). Wang et al. recently showed in an elderly male population that presence of any *HFE* variant genotype had an independent effect upon cognition resulting in a -1.77 points/year (95% CI -3.88, 0.35) decline for variant carriers when compared to wildtype individuals (Wang et al. 2007). Additionally, they found that *HFE* variant carriers had an enhanced effect (curvilinear appearance) upon tibia lead induced cognitive decline (Wang et al. 2007). Although this study hints at the importance of these polymorphisms as potential effect modifiers of health outcomes, it was also limited by the lack biomarkers for iron status (Wang et al. 2007).

Overview of the Thesis

Chapter 2 of this dissertation explores the concept of prenatal windows of susceptibility to environmental toxicants by assessing lead exposure at three distinct time points during gestation and associating these exposure windows with gestational length. As described earlier, lead has been shown to be adversely associated with gestational length and increased risk for premature delivery (<37 weeks), though many of these studies are inconsistent with regards

to strength and direction of association (Andrews et al. 1994). The potential design weakness in this relationship has been the use of blood lead at delivery, or cord blood lead, as a biomarker of exposure. Toxicokinetic studies have shown that the half-life of lead in RBCs is approximately 30 days (+/-) indicating that these study designs were only able to assess direct lead exposure in the last trimester of pregnancy (Rabinowitz 1991; Sakai 2000). Researchers have also shown that whole blood lead levels are neither an accurate nor precise reflection of plasma lead levels, which are considered the fraction of biologically active lead, able to readily cross cell membranes and exert toxicological effects upon cellular systems. Several recent papers have suggested (in concert with plausible biological mechanisms) that lead can exert adverse effects early in gestation which may alter the normal course of pregnancy (Hu et al. 2006; Cory-Slechta et al. 2008; Smith and Nicholson 2007). To circumvent potential limitations in earlier research, validate results of more recent study designs, and expand upon the literature, this study sought to utilize multiple biomarkers of lead exposure (blood, plasma, and bone lead) at biologically relevant time points (trimesters 1-3) during gestation. The a priori hypothesis was that lead exposure early in gestation will be associated with a stronger adverse effect upon gestational length.

Chapters 3 & 4 address interpersonal variations in susceptibility to environmental toxicants by exploring gene-environment interactions between biomarkers of lead exposure and genetic polymorphisms in the iron regulatory and transport system. Due to the observed relationship between iron deficiency

and increased lead absorption (Wright et al. 2003) and research showing that *HFE* polymorphisms can alter blood and bone lead levels (Hopkins et al. 2008; Wright et al. 2004), alterations to the iron sensing system should impact transport of lead across barriers, such as the blood-brain-barrier or placenta potentially altering site-specific lead induced damage to the developing brain and fetal growth. As described earlier, mechanistic studies indicate that lead and iron can compete for the common transporter DMT-1 which is involved in the transport of divalent metal ions across barriers in the intestine, placenta, and brain. Lead transported via DMT-1 was found to be 80% inhibited by a 25-fold increase in iron (Bannon et al. 2002), and iron supplementation was shown to reduce brain lead levels (Wang et al. 2007). Our a priori hypothesis in both these chapters is that genetic polymorphisms in *HFE* will attenuate lead's adverse effects upon infant neurocognition and birthweight.

Literature Cited

Akesson A, Stal P, Vahter M. 2000. Phlebotomy increases cadmium uptake in hemochromatosis. Environ Health Perspect 108(4): 289-291.

Anderson GJ, Darshan D, Wilkins SJ, Frazer DM. 2007. Regulation of systemic iron homeostasis: how the body responds to changes in iron demand. Biometals.

Andrews KW, Savitz DA, Hertz-Picciotto I. 1994. Prenatal lead exposure in relation to gestational age and birth weight: A review of epidemiologic studies. American Journal of Industrial Medicine 26(1): 13-32.

Aro A, Amarasiriwardena C, Lee ML, Kim R, Hu H. 2000. Validation of K x-ray fluorescence bone lead measurements by inductively coupled plasma mass spectrometry in cadaver legs. Medical physics 27(1): 119-123.

Bannon D, Portnoy ME, Olivi L, Lees PS, Culotta VC, Bressler JP. 2002. Uptake of lead and iron by divalent metal transporter 1 in yeast and mammalian cells. Biochem Biophys Res Commun 295(4): 978-984.

Barltrop D, Smith A. 1972. Lead binding to human haemoglobin. Experientia 28(1): 76-77.

Barry PS. 1975. A comparison of concentrations of lead in human tissues. British journal of industrial medicine 32(2): 119-139.

Barton JC, Patton MA, Edwards CQ, Griffen LM, Kushner JP, Meeks RG, et al. 1994. Blood lead concentrations in hereditary hemochromatosis. J Lab Clin Med 124(2): 193-198.

Beard J. 2007. Recent evidence from human and animal studies regarding iron status and infant development. The Journal of nutrition 137(2): 524S-530S.

Beckman LE, Van Landeghem GF, Sikstrom C, Beckman L. 1998. DNA polymorphisms and haplotypes in the human transferrin gene. Hum Genet 102(2): 141-144.

Bellinger D, Leviton A, Rabinowitz M, Allred E, Needleman H, Schoenbaum S. 1991. Weight gain and maturity in fetuses exposed to low levels of lead. Environmental Research 54(2): 151-158.

Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. 1987. Longitudinal analyses of prenatal and postnatal lead exposure and early cognitive development. N Engl J Med 316(17): 1037-1043.

Bellinger DC, Needleman HL. 2003. Intellectual impairment and blood lead levels. N Engl J Med 349(5): 500-502; author reply 500-502.

Bellinger DC. 2008. Very low lead exposures and children's neurodevelopment. Current opinion in pediatrics 20(2): 172-177.

Bergdahl IA, Skerfving S. 1997. Partition of circulating lead between plasma and red cells does not seem to be different for internal and external sources of lead. Am J Ind Med 32(3): 317-318.

Berkowitz Z, Price-Green P, Bove FJ, Kaye WE. 2006. Lead exposure and birth outcomes in five communities in Shoshone County, Idaho. Int J Hyg Environ Health 209(2): 123-132.

Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. 2002. Penetrance of 845G--> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 359(9302): 211-218.

Beutler E. 2006. Hemochromatosis: genetics and pathophysiology. Annu Rev Med 57: 331-347.

Bradman A, Eskenazi B, Sutton P, Athanasoulis M, Goldman LR. 2001. Iron deficiency associated with higher blood lead in children living in contaminated environments. Environ Health Perspect 109(10): 1079-1084.

Bressler JP, Goldstein GW. 1991. Mechanisms of lead neurotoxicity. Biochem Pharmacol 41(4): 479-484.

Bressler JP, Olivi L, Cheong JH, Kim Y, Bannona D. 2004. Divalent metal transporter 1 in lead and cadmium transport. Annals of the New York Academy of Sciences 1012: 142-152.

Brito JA, McNeill FE, Chettle DR, Webber CE, Vaillancourt C. 2000. Study of the relationships between bone lead levels and its variation with time and the cumulative blood lead index, in a repeated bone lead survey. J Environ Monit 2(3): 271-276.

Brito JA, McNeill FE, Stronach I, Webber CE, Wells S, Richard N, et al. 2001. Longitudinal changes in bone lead concentration: implications for modelling of human bone lead metabolism. J Environ Monit 3(4): 343-351.

Cavalleri A, Minoia C, Pozzoli L, Polatti F, Bolis PF. 1978. Lead in red blood cells and in plasma of pregnant women and their offspring. Environ Res 17(3): 403-408.

Chan K, Chan B, Lam K, Tam S, Lao T. 2009. Iron supplement in pregnancy and development of gestational diabetes-a randomised placebo-controlled trial. BJOG 116(6): 789-798.

Chen PC, Pan IJ, Wang JD. 2006. Parental exposure to lead and small for gestational age births. Am J Ind Med 49(6): 417-422.

Church HJ, Day JP, Braithwaite RA, Brown SS. 1993. Binding of lead to a metallothionein-like protein in human erythrocytes. J Inorg Biochem 49(1): 55-68.

Colomer J, Colomer C, Gutierrez D, Jubert A, Nolasco A, Donat J, et al. 1990. Anaemia during pregnancy as a risk factor for infant iron deficiency: report from the Valencia Infant Anaemia Cohort (VIAC) study. Paediatr Perinat Epidemiol 4(2): 196-204.

Cory-Slechta DA, Garcia-Osuna M, Greenamyre JT. 1997. Lead-induced changes in NMDA receptor complex binding: correlations with learning accuracy and with sensitivity to learning impairments caused by MK-801 and NMDA administration. Behav Brain Res 85(2): 161-174.

Cory-Slechta DA, Pokora MJ, Johnson JL. 1996. Postweaning lead exposure enhances the stimulus properties of N-methyl-D-aspartate: possible dopaminergic involvement? Neurotoxicology 17(2): 509-521.

Cory-Slechta DA, Virgolini MB, Rossi-George A, Thiruchelvam M, Lisek R, Weston D. 2008. Lifetime consequences of combined maternal lead and stress. Basic Clin Pharmacol Toxicol 102(2): 218-227.

Cory-Slechta DA. 1995. Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions. Annu Rev Pharmacol Toxicol 35: 391-415.

Davies PS, Enns CA. 2004. Expression of the hereditary hemochromatosis protein HFE increases ferritin levels by inhibiting iron export in HT29 cells. J Biol Chem 279(24): 25085-25092.

de Jong G, van Dijk JP, van Eijk HG. 1990. The biology of transferrin. Clin Chim Acta 190(1-2): 1-46.

deSilva PE. 1981. Determination of lead in plasma and studies on its relationship to lead in erythrocytes. British journal of industrial medicine 38(3): 209-217.

Deugnier Y, Turlin B. 2007. Pathology of hepatic iron overload. World J Gastroenterol 13(35): 4755-4760.

Dietert RR, Piepenbrink MS. 2006. Lead and immune function. Crit Rev Toxicol 36(4): 359-385.

Dietrich KN, Krafft KM, Shukla R, Bornschein RL, Succop PA. 1987. The neurobehavioral effects of early lead exposure. Monogr Am Assoc Ment Defic(8): 71-95.

Drakesmith H, Townsend A. 2000. The structure and function of HFE. Bioessays 22(7): 595-598.

Dunn LL, Rahmanto YS, Richardson DR. 2007. Iron uptake and metabolism in the new millennium. Trends Cell Biol 17(2): 93-100.

Ernhart CB, Morrow-Tlucak M, Marler MR, Wolf AW. 1987. Low level lead exposure in the prenatal and early preschool periods: early preschool development. Neurotoxicol Teratol 9(3): 259-270.

Ettinger AS, Lamadrid-Figueroa H, Tellez-Rojo MM, Mercado-Garcia A, Peterson KE, Schwartz J, et al. 2009. Effect of calcium supplementation on blood lead levels in pregnancy: a randomized placebo-controlled trial. Environ Health Perspect 117(1): 26-31.

Factor-Litvak P, Graziano JH, Kline JK, Popovac D, Mehmeti A, Ahmedi Shrout GP, et al. 1991. A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. International Journal of Epidemiology 20(3): 722-728.

Falcon M, Vinas P, Luna A. 2003. Placental lead and outcome of pregnancy. Toxicology 185(1-2): 59-66.

Feder JN. 1999. The hereditary hemochromatosis gene (HFE): a MHC class I-like gene that functions in the regulation of iron homeostasis. Immunol Res 20(2): 175-185.

Fewtrell LJ, Pruss-Ustun A, Landrigan P, Ayuso-Mateos JL. 2004. Estimating the global burden of disease of mild mental retardation and cardiovascular diseases from environmental lead exposure. Environ Res 94(2): 120-133.

Fleming RE. 2009. Iron sensing as a partnership: HFE and transferrin receptor 2. Cell Metab 9(3): 211-212.

Ganz T. 2006. Hepcidin and its role in regulating systemic iron metabolism. Hematology Am Soc Hematol Educ Program: 29-35.

Ganz T. 2007. Molecular control of iron transport. J Am Soc Nephrol 18(2): 394-400.

Ganz T. 2008. Iron homeostasis: fitting the puzzle pieces together. Cell Metab 7(4): 288-290.

Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, Enns CA. 2009. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metab 9(3): 217-227.

Gardella C. 2001. Lead exposure in pregnancy: a review of the literature and argument for routine prenatal screening. Obstet Gynecol Surv 56(4): 231-238.

Garrick MD, Singleton ST, Vargas F, Kuo HC, Zhao L, Knopfel M, et al. 2006. DMT1: which metals does it transport? Biol Res 39(1): 79-85.

Goldstein GW. 1990. Lead poisoning and brain cell function. Environ Health Perspect 89: 91-94.

Gomaa A, Hu H, Bellinger D, Schwartz J, Tsaih SW, Gonzalez-Cossio T, et al. 2002. Maternal bone lead as an independent risk factor for fetal neurotoxicity: a prospective study. Pediatrics 110(1 Pt 1): 110-118.

Gonzalez-Cossio T, Peterson KE, Sanin LH, Fishbein E, Palazuelos E, Aro A, et al. 1997. Decrease in birth weight in relation to maternal bone-lead burden. Pediatrics 100(5): 856-862.

Goyer RA. 1996. Results of lead research: prenatal exposure and neurological consequences. Environ Health Perspect 104(10): 1050-1054.

Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368(9553): 2167-2178.

Grantham-McGregor S, Ani C. 2001. A review of studies on the effect of iron deficiency on cognitive development in children. The Journal of nutrition 131(2S-2): 649S-666S; discussion 666S-668S.

Grosse SD, Matte TD, Schwartz J, Jackson RJ. 2002. Economic gains resulting from the reduction in children's exposure to lead in the United States. Environ Health Perspect 110(6): 563-569.

Gulson BL, Mizon KJ, Korsch MJ, Palmer JM, Donnelly JB. 2003. Mobilization of lead from human bone tissue during pregnancy and lactation--a summary of long-term research. Sci Total Environ 303(1-2): 79-104.

Gulson BL, Mizon KJ, Palmer JM, Korsch MJ, Taylor AJ, Mahaffey KR. 2004. Blood lead changes during pregnancy and postpartum with calcium supplementation. Environ Health Perspect 112(15): 1499-1507.

Halvorsen S. 2000. Iron balance between mother and infant during pregnancy and breastfeeding. Acta Paediatr 89:625-7.

Hanson EH, Imperatore G, Burke W. 2001. HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology. Am J Epidemiol 154(3): 193-206.

Hernandez-Avila M, Peterson KE, Gonzalez-Cossio T, Sanin LH, Aro A, Schnaas L, et al. 2002. Effect of maternal bone lead on length and head circumference of newborns and 1-month-old infants. Arch Environ Health 57(5): 482-488.

Hernandez-Avila M, Smith D, Meneses F, Sanin LH, Hu H. 1998. The influence of bone and blood lead on plasma lead levels in environmentally exposed adults. Environ Health Perspect 106(8): 473-477.

Hopkins MR, Ettinger AS, Hernandez-Avila M, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, et al. 2008. Variants in iron metabolism genes predict higher blood lead levels in young children. Environ Health Perspect 116(9): 1261-1266.

Hoppin JA, Aro A, Hu H, Ryan PB. 2000. Measurement variability associated with KXRF bone lead measurement in young adults. Environ Health Perspect 108(3): 239-242.

Hu H, Hernandez-Avila, M. 2002. Invited commentary: lead, bones, women, and pregnancy – the poison within? Am J Epidemiol 156(12): 1088-91.

Hu H, Shih R, Rothenberg S, Schwartz BS. 2007. The epidemiology of lead toxicity in adults: Measuring dose and consideration of other methodologic issues. Environmental Health Perspectives 115(3): 455-462.

Hu H, Tellez-Rojo MM, Bellinger D, Smith D, Ettinger AS, Lamadrid-Figueroa H, et al. 2006. Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. Environ Health Perspect 114(11): 1730-1735.

IOM, ed. 2007. Preterm Birth: Causes, Consequences, and Prevention.

Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD, et al. 2001. HFE mutations, iron deficiency and overload in 10,500 blood donors. Br J Haematol 114(2): 474-484.

Jelliffe-Pawlowski LL, Miles SQ, Courtney JG, Materna B, Charlton V. 2006. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. Journal of Perinatology 26(3): 154-162.

Kilbride J, Baker TG, Parapia LA, Khoury SA, Shuqaidef SW, Jerwood D. 1999. Anaemia during pregnancy as a risk factor for iron-deficiency anaemia in infancy: a case-control study in Jordan. Int J Epidemiol 28(3): 461-468.

Kim HS, Lee SS, Hwangbo Y, Ahn KD, Lee BK. 2003. Cross-sectional study of blood lead effects on iron status in Korean lead workers. Nutrition 19(7-8): 571-576.

Kim R, Rotnitsky A, Sparrow D, Weiss S, Wager C, Hu H. 1996. A longitudinal study of low-level lead exposure and impairment of renal function. The Normative Aging Study. Jama 275(15): 1177-1181.

Lamadrid-Figueroa H, Tellez-Rojo MM, Hernandez-Cadena L, Mercado-Garcia A, Smith D, Solano-Gonzalez M, et al. 2006. Biological markers of fetal lead exposure at each stage of pregnancy. J Toxicol Environ Health A 69(19): 1781-1796.

Lanphear BP, Dietrich K, Auinger P, Cox C. 2000. Cognitive deficits associated with blood lead concentrations <10 microg/dL in US children and adolescents. Public Health Rep 115(6): 521-529.

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. 2005. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 113(7): 894-899.

Lao TT, Tam KF, Chan LY. 2000. Third trimester iron status and pregnancy outcome in non-anaemic women; pregnancy unfavourably affected by maternal iron excess. Hum Reprod 15(8): 1843-1848.

Lasley SM, Gilbert ME. 1999. Lead inhibits the rat N-methyl-d-aspartate receptor channel by binding to a site distinct from the zinc allosteric site. Toxicol Appl Pharmacol 159(3): 224-233.

Lebron JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, Mintier GA, et al. 1998. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell 93(1): 111-123.

Lehmann DJ, Worwood M, Ellis R, Wimhurst VL, Merryweather-Clarke AT, Warden DR, et al. 2006. Iron genes, iron load and risk of Alzheimer's disease. Journal of medical genetics 43(10): e52.

Lozoff B, Clark KM, Jing Y, Armony-Sivan R, Angelilli ML, Jacobson SW. 2008. Dose-response relationships between iron deficiency with or without anemia and infant social-emotional behavior. J Pediatr 152(5): 696-702, 702 631-693.

Lozoff B, Georgieff MK. 2006. Iron deficiency and brain development. Semin Pediatr Neurol 13(3): 158-165.

Lozoff B, Kaciroti N, Walter T. 2006. Iron deficiency in infancy: applying a physiologic framework for prediction. Am J Clin Nutr 84(6): 1412-1421.

Lozoff B. 2007. Iron deficiency and child development. Food and nutrition bulletin 28(4 Suppl): S560-571.

Mahaffey KR. 1995. Nutrition and lead: strategies for public health. Environ Health Perspect 103 Suppl 6: 191-196.

McCann JC, Ames BN. 2007. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 85(4): 931-945.

McMichael AJ, Vimpani GV, Robertson EF. 1986. The Port Pirie cohort study: Maternal blood lead and pregnancy outcome. Journal of Epidemiology and Community Health 40(1): 18-25.

Meyer PA, Brown MJ, Falk H. 2008. Global approach to reducing lead exposure and poisoning. Mutat Res 659(1-2): 166-175.

Miller MF, Stoltzfus RJ, Mbuya NV, Malaba LC, Iliff PJ, Humphrey JH. 2003. Total body iron in HIV-positive and HIV-negative Zimbabwean newborns strongly predicts anemia throughout infancy and is predicted by maternal hemoglobin concentration. The Journal of nutrition 133(11): 3461-3468.

Murphy JF, O'Riordan J, Newcombe RG, Coles EC, Pearson JF. 1986. Relation of haemoglobin levels in first and second trimesters to outcome of pregnancy. Lancet 1(8488): 992-995.

Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. 2007. Lead exposure and cardiovascular disease--a systematic review. Environ Health Perspect 115(3): 472-482.

Needleman HL, Gatsonis CA. 1990. Low-level lead exposure and the IQ of children. A meta-analysis of modern studies. JAMA 263(5): 673-678.

Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, et al. 2004. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306(5704): 2090-2093.

Odland JO, Nieboer E, Romanova N, Thomassen Y, Lund E. 1999. Blood lead and cadmium and birth weight among sub-arctic and arctic populations of Norway and Russia. Acta Obstet Gynecol Scand 78(10): 852-860.

O'Flaherty EJ. 1993. Physiologically based models for bone-seeking elements. IV. Kinetics of lead disposition in humans. Toxicology and Applied Pharmacology 118(1): 16-29.

Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. 1999. A population-based study of the clinical expression of the hemochromatosis gene. N Engl J Med 341(10): 718-724.

Pedersen P, Milman N. 2009. Genetic screening for HFE hemochromatosis in 6,020 Danish men: penetrance of C282Y, H63D, and S65C variants. Ann Hematol. ISSN: 1432-0584 (Electronic)

Peirano PD, Algarin CR, Chamorro R, Reyes S, Garrido MI, Duran S, et al. 2009. Sleep and neurofunctions throughout child development: lasting effects of early iron deficiency. J Pediatr Gastroenterol Nutr 48 Suppl 1: S8-15.

Philip AT, Gerson B. 1994. Lead poisoning--Part I. Incidence, etiology, and toxicokinetics. Clin Lab Med 14(2): 423-444.

Preziosi P, Prual A, Galan P, Daouda H, Boureima H, Hercberg S. 1997. Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 66(5): 1178-1182.

Pulliam JF, Jennings CD, Kryscio RJ, Davis DG, Wilson D, Montine TJ, et al. 2003. Association of HFE mutations with neurodegeneration and oxidative stress in Alzheimer's disease and correlation with APOE. Am J Med Genet B Neuropsychiatr Genet 119(1): 48-53.

Rabinowitz MB, Wetherill GW, Kopple JD. 1976. Kinetic analysis of lead metabolism in healthy humans. J Clin Invest 58(2): 260-270.

Rabinowitz MB. 1991. Toxicokinetics of bone lead. Environ Health Perspect 91: 33-37.

Rice DC. 1993. Lead-induced changes in learning: evidence for behavioral mechanisms from experimental animal studies. Neurotoxicology 14(2-3): 167-178.

Rivers CA, Barton JC, Gordeuk VR, Acton RT, Speechley MR, Snively BM, et al. 2007. Association of ferroportin Q248H polymorphism with elevated levels of serum ferritin in African Americans in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. Blood Cells, Molecules, and Diseases 38(3): 247-252.

Robson KJ, Lehmann DJ, Wimhurst VL, Livesey KJ, Combrinck M, Merryweather-Clarke AT, et al. 2004. Synergy between the C2 allele of transferrin and the C282Y allele of the haemochromatosis gene (HFE) as risk factors for developing Alzheimer's disease. Journal of medical genetics 41(4): 261-265.

Rothenberg SJ, Rothenberg JC. 2005. Testing the dose-response specification in epidemiology: public health and policy consequences for lead. Environ Health Perspect 113(9): 1190-1195.

Sachdev H, Gera T, Nestel P. 2005. Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. Public Health Nutr 8(2): 117-132.

Sagen N, Nilsen ST, Kim HC, Bergsjo P, Koller O. 1984. Maternal hemoglobin concentration is closely related to birth weight in normal pregnancies. Acta Obstet Gynecol Scand 63(3): 245-248.

Sakai T. 2000. Biomarkers of lead exposure. Ind Health 38(2): 127-142.

Sargent PJ, Farnaud S, Evans RW. 2005. Structure/function overview of proteins involved in iron storage and transport. Curr Med Chem 12(23): 2683-2693.

Schmidt PJ, Toran PT, Giannetti AM, Bjorkman PJ, Andrews NC. 2008. The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. Cell Metab 7(3): 205-214.

Schwartz BS, Bolla KI, Stewart W, Ford DP, Agnew J, Frumkin H. 1993. Decrements in neurobehavioral performance associated with mixed exposure to organic and inorganic lead. Am J Epidemiol 137(9): 1006-1021.

Schwartz BS, Stewart WF, Bolla KI, Simon PD, Bandeen-Roche K, Gordon PB, et al. 2000. Past adult lead exposure is associated with longitudinal decline in cognitive function. Neurology 55(8): 1144-1150.

Schwartz J, Landrigan PJ, Feldman RG, Silbergeld EK, Baker EL, Jr., von Lindern IH. 1988. Threshold effect in lead-induced peripheral neuropathy. J Pediatr 112(1): 12-17.

Schwartz J. 1994. Low-level lead exposure and children's IQ: a meta-analysis and search for a threshold. Environ Res 65(1): 42-55.

Shen XM, Yan CH, Guo D, Wu SM, Li RQ, Huang H, et al. 1998. Low-level prenatal lead exposure and neurobehavioral development of children in the first year of life: a prospective study in Shanghai. Environ Res 79(1): 1-8.

Simons TJ. 1984. Active transport of lead by human red blood cells. FEBS Lett 172(2): 250-254.

Simons TJ. 1988. Active transport of lead by the calcium pump in human red cell ghosts. J Physiol 405: 105-113.

Smith D, Hernandez-Avila M, Tellez-Rojo MM, Mercado A, Hu H. 2002. The relationship between lead in plasma and whole blood in women. Environ Health Perspect 110(3): 263-268.

Smith DR, Ilustre RP, Osterloh JD. 1998. Methodological considerations for the accurate determination of lead in human plasma and serum. Am J Ind Med 33(5): 430-438.

Smith DR, Osterloh JD, Flegal AR. 1996. Use of endogenous, stable lead isotopes to determine release of lead from the skeleton. Environ Health Perspect 104(1): 60-66.

Smith R, Nicholson RC. 2007. Corticotrophin releasing hormone and the timing of birth. Front Biosci 12: 912-918.

Sowers M, Jannausch M, Scholl T, Li W, Kemp FW, Bogden JD. 2002. Blood lead concentrations and pregnancy outcomes. Archives of Environmental Health 57(5): 489-495.

Steer P, Alam MA, Wadsworth J, Welch A. 1995. Relation between maternal haemoglobin concentration and birth weight in different ethnic groups. BMJ 310(6978): 489-491.

Steer PJ. 2000. Maternal hemoglobin concentration and birth weight. Am J Clin Nutr 71(5 Suppl): 1285S-1287S.

Tamakoshi K, Yatsuya H, Wada K, Matsushita K, Otsuka R, Yang PO, et al. 2006. Birth weight and adult hypertension: cross-sectional study in a Japanese workplace population. Circ J 70(3): 262-267.

Tellez-Rojo MM, Bellinger DC, Arroyo-Quiroz C, Lamadrid-Figueroa H, Mercado-Garcia A, Schnaas-Arrieta L, et al. 2006. Longitudinal associations between blood lead concentrations lower than 10 microg/dL and neurobehavioral development in environmentally exposed children in Mexico City. Pediatrics 118(2): e323-330.

Tong S, Baghurst P, McMichael A. 2006. Birthweight and cognitive development during childhood. J Paediatr Child Health 42(3): 98-103.

Torres-Sanchez LE, Berkowitz G, Lo?pez-Carrillo L, Torres-Arreola L, Ri?os C, Lo?pez-Cervantes M. 1999. Intrauterine lead exposure and preterm birth. Environmental Research 81(4): 297-301.

UNICEF. 2004. United Nations Children's Fund and World Health Organization, Low Birthweight: Country regional and global estimates.

USEPA. 1998. Chemical Hazard Data Availablity Study: What do we Really Know About the Safety of High Production Volume Chemicals? Office of Pollution Prevention and Toxics EPA.

USEPA. 2006. What is the TSCA Chemical Substance Inventory. US Environmental Protection Agency 2007.

Wang FT HH, Schwartz J, Weuve J, Spiro AS III, Sparrow D, Nie H, Silverman EK, Weiss ST, Wright RO. . 2007. Modifying Effects of the HFE Polymorphisms

on the Association between Lead Burden and Cognitive Decline. Environ Health Perspect. 115(8): 1210-1215.

Weaver VM, Griswold M, Todd AC, Jaar BG, Ahn KD, Thompson CB, et al. 2009. Longitudinal associations between lead dose and renal function in lead workers. Environ Res 109(1): 101-107.

Weisskopf MG, Wright RO, Schwartz J, Spiro A, 3rd, Sparrow D, Aro A, et al. 2004. Cumulative lead exposure and prospective change in cognition among elderly men: the VA Normative Aging Study. Am J Epidemiol 160(12): 1184-1193.

West WL, Knight EM, Edwards CH, Manning M, Spurlock B, James H, et al. 1994. Maternal low level lead and pregnancy outcomes. The Journal of nutrition 124(6 Suppl): 981S-986S.

White LD, Cory-Slechta DA, Gilbert ME, Tiffany-Castiglioni E, Zawia NH, Virgolini M, et al. 2007. New and evolving concepts in the neurotoxicology of lead. Toxicol Appl Pharmacol 225(1): 1-27.

White PD, Van Leeuwen P, Davis BD, Maddaloni M, Hogan KA, Marcus AH, et al. 1998. The conceptual structure of the integrated exposure uptake biokinetic model for lead in children. Environ Health Perspect 106 Suppl 6: 1513-1530.

WHO. 2004. Assessing the Iron Status of Populations. Geneva: WHO/CDC Joint Report.

WHO. 2002. The World Health Report 2002: Reducing risks, promoting healthy life. Geneva World Health Organization.

WHO. 1998. Global database on child growth and malnutrition. Geneva World Health Organization.

Wigg NR. 2001. Low-level lead exposure and children. J Paediatr Child Health 37(5): 423-425.

Winder C. 1993. Lead, reproduction and development. Neurotoxicology 14(2-3): 303-317.

Wittmers LE, Jr., Aufderheide AC, Wallgren J, Rapp G, Jr., Alich A. 1988. Lead in bone. IV. Distribution of lead in the human skeleton. Arch Environ Health 43(6): 381-391.

Wright RO, Shannon MW, Wright RJ, Hu H. 1999. Association between iron deficiency and low-level lead poisoning in an urban primary care clinic. Am J Public Health 89(7): 1049-1053.

Wright RO, Silverman EK, Schwartz J, Tsaih SW, Senter J, Sparrow D, et al. 2004. Association between hemochromatosis genotype and lead exposure among elderly men: the normative aging study. Environ Health Perspect 112(6): 746-750.

Wright RO, Tsaih SW, Schwartz J, Wright RJ, Hu H. 2003. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. J Pediatr 142(1): 9-14.

Yi EY, Lim DK. 1998. Effects of chronic lead exposure on glutamate release and uptake in cerebellar cells of rat pups. Arch Pharm Res 21(2): 113-119.

Yip R, Norris TN, Anderson AS. 1981. Iron status of children with elevated blood lead concentrations. J Pediatr 98(6): 922-925.

Zagré NM, Desplats G, Adou P, Mamadoultaibou A, Aguayo VM. 2007. Prenatal multiple micronutrient supplementation has greater impact on birthweight than supplementation with iron and folic acid: a cluster-randomized, double-blind, controlled programmatic study in rural Niger. Food Nutr Bull. 2007 Sep;28(3):317-27.

Zatta P, Messori L, Mauri P, van Rensburg SJ, van Zyl J, Gabrielli S, et al. 2005. The C2 variant of human serum transferrin retains the iron binding properties of the native protein. Biochim Biophys Acta 1741(3): 264-270.

Zeng L, Dibley MJ, Cheng Y, Dang S, Chang S, Kong L, et al. 2008. Impact of micronutrient supplementation during pregnancy on birth weight, duration of gestation, and perinatal mortality in rural western China: double blind cluster randomised controlled trial. BMJ 337: a2001.

CHAPTER II

Fetal Lead Exposure, Length of Gestation and Risk of Premature Delivery

Abstract

Introduction: Premature birth is a significant public health problem worldwide. Research on the role of population-level exposure to toxicants, including lead exposure, in the complex etiology of this adverse birth outcome has yielded inconsistent results. We assessed the effect of prenatal lead exposure on gestational age and risk of premature delivery using trimester-specific maternal blood and plasma lead levels as biomarkers of fetal exposure.

Methods: Whole blood and plasma were collected from 243 pregnant women recruited in Mexico City from 1997-1999 using strict procedures to prevent contamination and hemolysis of the samples and were analyzed for lead content by inductively coupled plasma high-resolution mass spectrometer (ICP-MS.) Data were analyzed using multiple linear and logistic regression to examine the associations of trimester-specific lead exposure, gestational age, and risk of premature delivery adjusting for infant sex, maternal age, maternal education, history of prior adverse birth outcome, cigarette smoking during pregnancy, and parity.

Results: Of the 243 participants, there were 20 (8.2%) preterm deliveries (<37 weeks of gestation). In single-trimester models, gestational age was most strongly associated with 1st trimester blood and plasma lead leading to a decrease of 2.87 days (95%CI -5.14, -0.60) and 2.91 days (95%CI -5.32, -0.50) per 1-SD increase in lead biomarker, respectively. In adjusted logistic regression models, a 1-SD increase in 1st trimester plasma was associated with an odds ratio of prematurity of 1.75 (95%CI 0.92, 3.32).

Conclusion: General population levels of fetal lead exposure in early pregnancy have an inverse association with length of gestation and possibly increased risk of prematurity. This effect was most prominent with respect to maternal plasma or whole blood lead levels measured during the 1st trimester.

Introduction

Premature births worldwide constitute a significant public health problem. Not only is premature delivery a major contributor to an estimated 4 million neonatal deaths per year (Lawn et al. 2005; Lawn et al. 2006), but if preterm infants survive they are at greater risk for a host of health problems in later life when compared to term infants. These include, but are not limited to: neurodevelopmental disabilities (Sesma and Georgieff 2003; Johnson 2007); growth & metabolic disorders (Saigal S 2001; Darendeliler F 2008); and respiratory disorders such as asthma (Jaakkola et al. 2006; Kumar et al. 2008; von Mutius et al. 1993; Schwartz et al. 1990). The role of environmental

toxicants, including lead, in the complex etiology of this adverse birth outcome is unclear as limited research results have been inconsistent (IOM 2007).

Although tremendous reductions in lead exposure to the general population have been achieved in most of North America and Europe, globally adults and children continue to be exposed to elevated levels through a variety of media and informal sector occupations. Lead can persist in bone for decades after exposure, which in turn serves as in internal source of exposure. Endogenous lead exposure is an important independent predictor of adverse health outcomes, such as: cognitive decline (Tellez-Rojo et al. 2006; Lanphear et al. 2005), cardiovascular disease (Hu et al. 2007; Navas-Acien et al. 2007), and decreased fetal growth (Bellinger et al. 1991; Gonzalez-Cossio et al. 1997; Sanin et al. 2001). During pregnancy, these internal stores of lead mobilize to a marked degree, partitioning into red blood cells (~99%) and plasma (~1%). The pattern of association between plasma and whole blood lead remains largely unclear but recent studies indicate that the relationship follows an exponential pattern and whole blood lead levels are not an accurate reflection of plasma lead levels (Lamadrid-Figueroa et al. 2006; Smith et al. 2002). This is of particular note since the lead present in plasma represents the circulating fraction of lead capable of crossing membranes, such as the placenta, and may serve as an important biomarker of fetal lead exposure.

A 2007 Institute of Medicine report on preterm births concluded the available epidemiologic data to date supports an adverse relationship between lead exposure and preterm delivery (IOM 2007). However, given that a common

hallmark of most of the reviewed studies was that lead exposure was assessed at delivery, either by measuring lead levels in cord blood or maternal whole blood, many were unable to address the issue of timing, i.e. what stage of pregnancy is the most vulnerable to lead's impact on risk of prematurity. Of the studies which assessed lead at several time points, one found a significant inverse relationship with second trimester blood lead levels over 10 μg/dl (Jelliffe-Pawlowski et al. 2006) but several others were inconclusive (Factor-Litvak et al. 1991; Sowers et al. 2002).

In the present study we assessed the effect of prenatal lead exposure on length of gestational age and risk of premature delivery using trimester-specific maternal blood and plasma lead levels and K-XRF measured maternal bone lead as biomarkers of fetal exposure to lead.

Methods

Study Population

This analysis was based on data from a parent birth cohort study of fetal lead exposure and offspring cognitive development (Hu et al. 2006). Study subjects were recruited between May 1997 and July 1999 during prenatal visits at one of three clinics of the Mexican Institute of Social Security (IMSS) in Mexico City. Women were eligible if they had a confirmed positive β-human chorionic gonadotropin test, were trying to become pregnant, lived in Mexico City, and were willing to participate in the 3-year follow-up study protocol. Of the 2,273 women approached, 1,502 (66%) declined to be enrolled. We applied the

following exclusion criteria to the 771 (34%) women who were willing to participate: if the mother was planning to leave the area within 5 years, daily consumption of alcoholic beverages, addiction to illegal drugs, continuous use of prescription drugs, diagnosis of multi-fetal, preeclampsia, renal or heart disease, gestational diabetes, seizures that require medical treatment. A total of 280 pregnant women were recruited and 182 women with a negative pregnancy test who declared an intention to become pregnant in the near future were also recruited. Of the 182 women who declared an intention to become pregnant, 47 (26%) became pregnant and agreed to participate giving a total study population of 327.

Of the 327 pregnant women, 277 (85%) had complete information on gestational age, and 243 met the following inclusion criteria: at least one measurement of plasma or blood lead during any of the three prenatal visits, complete information on maternal age, education, adverse birth outcome history, prior pregnancy, and infant sex.

All mothers were given detailed information about the study procedures, provided information on ways to minimize lead exposure and signed a written letter of informed consent. The research protocol was approved by the Ethics and Research Committees of the National Institute of Public Health of Mexico, the Harvard School of Public Health, the Brigham and Women's Hospital, the University of California, the University of Michigan School of Public Health, and the participating hospitals.

Maternal Blood and Plasma Lead Measurements

Maternal whole blood and plasma samples were collected at each prenatal visit at the Center for Environmental Health Research of the American British Cowdray (ABC) Hospital. Prior to venipuncture, each subject's arm was washed with ultrapure water and disinfected with reagent-grade alcohol. An initial whole-blood sample was collected into a low-lead container (BD Vacutainer® #367734, Becton-Dickinson, Franklin Lakes, NJ) for total lead analysis. Subsequently, the catheter tubing was severed at a point distal to the venipuncture, and a second whole-blood sample for plasma separation was collected via gravity-assisted phlebotomy and centrifuged at 800 × *g* for 10 min at room temperature (Smith et al. 1998). The plasma fraction was then transferred using a polyethylene pipette into a polyethylene bottle and immediately frozen. All blood collections, plasma and whole blood processing, and sample analyses were conducted under high-efficiency particulate air (HEPA)- filtered conditions using trace metal clean techniques (Smith et al. 1998).

Plasma lead levels were analyzed using a Finnigan element inductively coupled plasma high-resolution mass spectrometer (ICP-MS; Thermo Finnigan, Bermen, Germany). Plasma hemoglobin and ferritin levels were also measured in order to evaluate the potential contribution of hemolysis to plasma lead levels (Smith et al. 2002).

Bone Lead Measurements

Maternal bone lead measurements were obtained within one month of delivery (+/- 5 days), using a spot-source ^{109}Cd K-XRF instrument, at two bone sites, the mid-tibial shaft (representing cortical bone) and the patella (representing trabecular bone). The physical principles, technical specifications, and validation of this and other similar K-XRF instruments have been described in detail elsewhere (Aro et al. 1994). For purposes of quality control, bone lead measurements with uncertainty estimates greater than 10 and 15 μg/g bone lead were excluded for tibia and patella, respectively.

Measurement of Gestational Length and Potential Confounders

Gestational length was estimated by date of last maternally-recalled menstrual period. Premature delivery was defined as the occurrence of birth prior to 37 completed weeks (259 days) gestation. Information on demographic, socioeconomic, and other factors that could confound the relationship between lead and gestational length was collected through questionnaire.

Statistical Analysis

All descriptive statistics and transformations were performed prior to bivariate analysis. Potential outliers were detected using the ESD (Extreme Studentized Deviate) Many-Outlier procedure (Rosner 1983). Characteristics of the final study population were compared with excluded participants using two sample tests (t-test or chi-squared) as appropriate. All maternal blood and

plasma lead measures were $\log_e$-transformed prior to statistical analysis. Plasma lead samples that had potential contamination due to hemolysis were excluded (N=5). The ratio of plasma lead to blood lead was fitted for each trimester in order to explore the effects of relationship between these two highly correlated exposure measures. Spearman correlation coefficients were calculated on all trimester specific plasma and blood lead measures.

In the initial study design, individuals were seen three times during pregnancy which corresponded to the first visit being < 20 weeks, the second visit between 20-28 weeks, and the last visit after 28 weeks. Due to this specified schedule and to postponed visits for some individuals, the three participant visits did not always correspond to clinical trimesters. Thus, we recoded the participant visits to correspond to the first (<13th week), second (between the 13th-27th week) and third (>27th week) trimester classification. To accommodate the multiple blood and plasma measures occurring for some individuals in the second and third trimesters, after recoding the participant's visits, we took the mean of these two measures.

Multiple linear regression (MLR) models were used to describe the relationships between gestational age and trimester-specific measures of blood, plasma lead, plasma to blood ratio, and cord blood lead adjusted for covariates of interest. Models were also estimated using the mean of available blood or plasma measures as well as the average blood and plasma of those with all three measures. In order to assess the effects of cumulative lead exposure, as reflected by measures of maternal tibia and patella bone lead, on gestational

length, we fit models assessing bone lead as a continuous linear term and divided into quartiles. Potential confounding variables were chosen based on biologic plausibility (regardless of statistical significance) and those significantly associated ($p < 0.1$) with gestational age in bivariate analysis. Covariates of interest included in multiple linear regression models were: maternal age, years of maternal education, history of adverse birth outcome, cigarette smoking during pregnancy, history of previous pregnancy, and infant sex. A similar model building strategy was used for logistic regression models. In order to compare the regression coefficients between the lead biomarkers in our models, we standardized the effect estimates for a 1-SD change in each exposure metric.

Regression diagnostics were performed on all models to evaluate multicollinearity and violations of the linear regression model assumptions. When influential data points were detected new models were fit excluding the observations. Data were analyzed using SAS 9.1, Cary, NC, SAS Institute Inc. 2002-2003 and R 2.6.1, The R Foundation for Statistical Computing, 2007.

Results

Our final study population included 243 pregnant women who had complete birth and covariate information with a total of 20 (8.2%) premature deliveries. There were no significant differences in population characteristics when compared with the 34 mother-infant pairs who were excluded from the analysis (Table II-1). In the 1st, 2nd, and 3rd trimesters, mean levels of lead in plasma and whole blood, respectively, were: 0.17, 0.13, 0.15 μg/dL and 7.3, 6.4,

6.7 µg/dL. As expected, maternal blood and plasma lead levels were moderately correlated (Spearman's Rho: 0.35 – 0.68, all $p<0.05$) between trimesters with the exception of third trimester plasma lead which was not significantly correlated to first trimester blood or plasma lead.

The relationships between gestational age and trimester-specific exposure are given in Table II-2. There was a general negative relationship between biomarkers of lead during each trimester of pregnancy and gestational age after adjusting for maternal age, maternal education, history of adverse birth outcome, cigarette smoking during pregnancy, history of previous pregnancy, and infant sex with the strongest negative effect estimates found in the first trimester. In these trimester-specific models, decreased gestational age was significantly associated with plasma lead during the 1st trimester (standardized coefficient, -2.91 days; 95%CI: -5.32, -0.50) and marginally associated during the 2nd trimester (standardized coefficient, -1.31 days; 95%CI: -2.64, 0.02). Mean blood lead showed a significant association with decreased gestational age in the 1st trimester (standardized coefficient, -2.87 days; 95%CI: -5.14, -0.60).The mean of maternal plasma lead levels over the course of all three trimesters also indicated a significant negative association with gestational age (standardized coefficient -1.37 days, 95%CI: -2.67, -0.07).

A one standard deviation (SD) increase in the first trimester ratio of plasma to blood lead resulted in a 3.04 day decrease (95% CI -5.48, -0.60) in gestational age after controlling for covariates of interest (Table II-3).

For comparison to previous studies, cord blood lead predicted a non-significant decrease of 0.72 days (95%CI: -2.34, 0.90) of gestation per 1-SD increase in exposure (Table II-4). The effect size was similar to effects found for a 1-SD increase in third trimester blood or plasma lead. The association between maternal tibia and patella bone lead levels indicated a general negative relationship with gestational age (Table II-4), although non-significant. No consistent trend was observed between quartiles of maternal bone lead burdens and gestational length.

Logistic regression models were fitted to explore the relationship between the trimester-specific biomarkers of lead exposure and odds of being born prematurely (Figure II-1). The odds ratios of delivering prematurely in relation to lead exposure were consistently positive with the highest odds ratio associated with plasma lead in the 1st trimester: 1.75 (95%CI 0.92, 3.32). None of the odds ratios attained statistical significance; however, the number of premature births was between 7 and 20 for the different trimester specific biomarker logistic regression models.

Discussion

In the present study we examined the relationship between biomarkers of maternal lead exposure at each trimester and subsequent length of gestation. Our study is the first to assess both cumulative lead effects, as reflected by tibia and patella bone lead burdens, and plasma lead effects upon gestational length and risk of prematurity. The results of this study indicate that fetal exposure to

lead negatively affects length of gestation, but is inconclusive with respect to the risk of delivering prematurely. The effects were found to be strongest in the first trimester for plasma and whole blood lead levels. In addition, we found that plasma lead independently predicted the same adverse association with length of gestation as whole blood lead in our population. Cumulative lead burden, as reflected by maternal tibia and patella bone lead levels, had a modest negative relationship with gestational age but warrants further study.

There are few prior studies that assess lead exposure at least once during gestation (Jelliffe-Pawlowski et al. 2006; Factor-Litvak et al. 1991; Sowers et al. 2002). Our study is most similar to the results of Jelliffe-Pawlowski et al. (2006.) In their study of 262 mother-infant pairs who gave birth between 1996-2002 in California, they found that a significant decrease in length of gestation was associated with second trimester measures of maternal blood lead resulting in a 1.0 day decrease in length of gestation per 1 μg/dl blood lead level over 10 μg/dL. In contrast, Sowers et al. (2002) found no significant relationship between risk of preterm delivery and trimester-specific maternal blood lead, though blood lead levels were approximately five times lower in their study compared to ours. Factor-Litvak et al. (1991) also found no significant relationship between length of gestation and maternal blood lead measured at mid-pregnancy. In a majority of the other large epidemiologic studies associating length of gestation and lead exposure measured only at delivery, the direction and strength of effects were inconsistent (Andrews et al. 1994). Several aspects that may have contributed to

this inconsistency include timing of exposure and assessment of various biomarkers of lead exposure.

It has been shown that lead can persist in bone for many years after exposure, serving as an endogenous source of exposure (Hu et al. 2007; Hu et al. 1998). During periods of increased bone turnover, such as pregnancy, these internal stores of lead can mobilize to a marked degree (Gulson et al. 2003; Manton et al. 2003). While in this study, our results were inconclusive regarding the relationship between bone lead (a cumulative lead exposure measure) and length of gestation, we were able to assess plasma lead levels which are a more reflective biomarker of the circulating fraction of lead (Smith et al. 2002) and an intermediate biological marker in the pathway between maternal bone lead and fetal lead exposure. Past studies by Hu and colleagues have documented that substantial changes in plasma lead go unnoticed when lead is measured only in the whole blood fraction, especially at higher blood lead levels (Lamadrid-Figueroa et al. 2006; Smith et al. 2002). We found that as the ratio of plasma to blood lead increases, the length of gestation decreases and this relationship was most prominent with exposures measured in the first trimester.

Preterm delivery is a complex condition with a multifactorial etiology. Potential mechanisms of how lead exposure may impact preterm delivery are unclear but several recent studies provide evidence to support a role of lead in altering the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the fetal HPA axis has been shown to be one of the major events in eliciting the activation of parturition through fetal hypothalamus and/or the placenta increase in secretion

of corticotropin-releasing hormone (CRH) ultimately leading to the uterine contraction, cervical ripening and decidual/fetal membrane activation (IOM 2007). Altering the trajectory of CRH release during pregnancy has been suggested as one plausible mechanism that can lead to delivering an infant prematurely (Hobel et al. 1999; Leung et al. 2000; McLean and Smith 1999, 2001). Heightened maternal stress is a known risk factor in preterm delivery which is thought to act through the maternal/fetal HPA pathway by altering CRH release (Wadhwa et al. 2002). Lead exposure may play an important role by increasing the overall baseline level of corticosterone (the biologic equivalent to cortisol in humans) in rats and heightening the response to acute stressors (Cory-Slechta et al. 2008; Cory-Slechta et al. 2004). It is then plausible to speculate that increased lead exposure early in gestation may alter CRH release alone or in concert with heightened maternal stress responses.

Our study has several limitations. The primary aim of the original study was to investigate the impacts of prenatal and postnatal lead exposure on infant neurodevelopment (Hu et al. 2006), and a focus on prematurity was only an exploratory aim. Gestational length was estimated by date of last maternally recalled menstrual period which may be an unreliable measure, varying as much as ±7-21 days, and depending on a host of factors including nutrition, physical activity, smoking, alcohol consumption, stress, and inter-pregnancy interval (Kato et al. 1999; Munster et al. 1992; Rowland et al. 2002; Liu et al. 2004). Unfortunately, it is not standard practice in Mexico to assess gestational length through ultrasound for non-high risk pregnancies, but there are no indications

that our population differs significantly from other lower/middle income pregnant women residing in Mexico City. Since the aims of the parent study related to infant cognition, not prematurity, another limitation is the small number (N=20, 8.2%) of preterm births we had, which constrained our power for examining this as an outcome. Despite these limitations, the strength and direction of our results are consistent with previous research findings in populations with similar maternal blood lead levels.

Conclusions

In conclusion, the present study provides evidence that length of gestation is adversely impacted by whole blood and plasma lead levels and this effect is strongest early in pregnancy. If future studies confirm this finding it will be critical to consider implementing screening for pre-pregnancy interventions to prevent exposure since this and other studies (Jelliffe-Pawlowski et al. 2006; Hu et al. 2006) have indicated that adverse infant health effects associated with *in-utero* lead exposure may arise early in pregnancy.

Table II-1: Characteristics of the study population of mother-infant pairs

	Included		Not Included		
	No	Mean ± SD	No	Mean ± SD	P-value
Maternal Characteristics					
Age (years)	243	27.0 ± 5.3	216	27.3 ± 5.2	0.52
Education (years)	243	10.7 ± 3.1	214	10.6 ± 3.2	0.79
Cigarette smoking during pregnancy (%)	243	4.0%	75	4.1 %	0.96
Number of prior pregnancies	243	1.8 ± 1.0	219	1.6 ± 1.2	0.02
Primiparity (%)	243	55.0%	211	58.9 %	0.41
History of adverse birth outcome (%)	243	12.4%	162	8.2 %	0.17
Married (%)	243	70.8%	216	73.7 %	0.50
Weight change during pregnancy (kg/day)	176	0.40 ± 0.16	10	0.36 ± 0.18	0.77
Blood lead (ug/dL)					
Trimester 1	98	7.3 ± 5.2	6	5.4 ± 3.5	0.24
Trimester 2	219	6.4 ± 4.2	12	6.0 ± 3.2	0.62
Trimester 3	207	6.7 ± 4.3	6	7.7 ± 4.3	0.41
Plasma Lead (ug/dL)					
Trimester 1	87	0.17 ± 0.16	6	0.10 ± 0.06	0.13
Trimester 2	231	0.13 ± 0.10	14	0.18 ± 0.24	0.75
Trimester 3	208	0.15 ± 0.25	12	0.22 ± 0.38	0.79
Tibia Bone Lead (ug/g)	180	11.5 ± 9.9	156	13.3 ± 12.9	0.28
Patella Bone Lead (ug/g)	181	14.0 ± 10.4	153	15.8 ± 11.2	0.29
Infant Characteristics					
Gestational Age (days)	243	271.4 ± 10.3	34	270.5 ± 11.4	0.66
Birthweight (grams)	243	3349.5 ± 680.0	42	2966.7±740.8	0.004
Sex (% Male)	243	52.4%	42	51.9 %	0.95
Umbilical Cord Blood Lead (ug/dL)	126	6.0 ± 3.8	12	5.5 ± 4.8	0.22

Table II-2: Multivariate linear regression models for gestational age comparing markers of lead exposure at different times for blood lead and plasma lead

Variable	No	β	p-Value	95% CI
Blood Lead (ug/dL)				
Trimester 1	97	-2.87	0.01	-5.14, -0.60
Trimester 2	218	-1.10	0.12	-2.49, 0.29
Trimester 3	207	-0.35	0.60	-1.64, 0.94
Average	89	-1.23	0.24	-3.33, 0.86
Mean of†	224	-1.05	0.13	-2.43, 0.32
Plasma Lead (ug/dL)				
Trimester 1	87	-2.91	0.02	-5.32, -0.50
Trimester 2	231	-1.31	0.05	-2.64, 0.02
Trimester 3	204	-0.85	0.20	-2.16, 0.47
Average	73	-0.39	0.75	-2.85, 2.08
Mean of†	239	-1.37	0.04	-2.67, -0.07

CI, confidence interval. Each model is adjusted for sex, maternal age, education, history of adverse birth outcome, cigarette smoking, and parity. Logarithmically transformed lead concentrations were used.

Each line in the table represents a different model

† Models were estimated using the mean of available blood or plasma measures.

Table II-3: Multivariate linear regression models for gestational age comparing the ratio of plasma lead to blood lead at different trimesters

Variable	No	B	p-Value	95% CI
Plasma Lead/Blood Lead Ratio				
Trimester 1	83	-3.04	0.02	-5.48, -0.60
Trimester 2	211	-0.78	0.27	-2.18, 0.61
Trimester 3	201	-0.25	0.70	-1.57, 1.06
Average	73	-1.30	0.28	-3.69, 1.09
Mean of†	220	-1.30	0.06	-2.65, 0.06

CI, confidence interval. Each model is adjusted for sex, maternal age, education, history of adverse birth outcome, cigarette smoking, and parity. Logarithmically transformed lead concentrations were used.

Each line in the table represents a different model

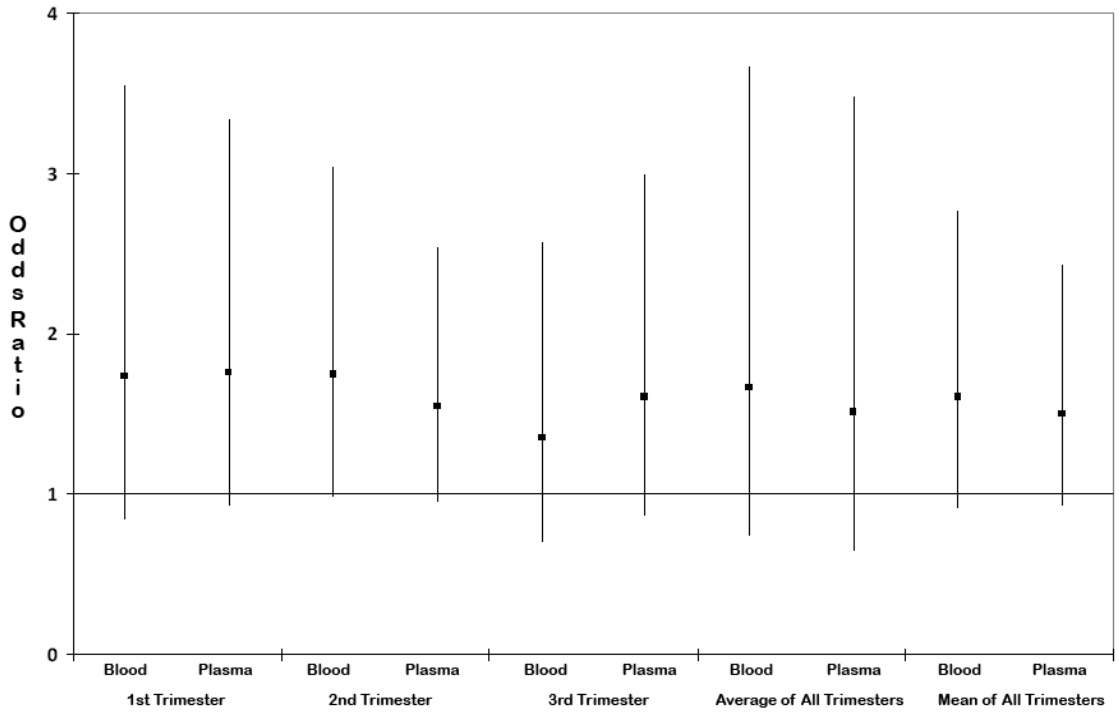
† Models were estimated using the mean of available blood or plasma measures.

Table II-4: Multivariate linear regression models for gestational age comparing biological markers of cumulative lead exposure

Variable	No	β	p-Value	95% CI
Cord Blood Lead (ug/dL)	125	-0.72	0.38	-2.34, 0.90
Tibia Lead (ug/g)	180	-0.54	0.49	-2.06, 0.99
Tibia Lead (quartiles)				
Q1 (<1 – 5.0)	45	Ref		
Q2 (5.0 – 12.0)	45	-3.70	0.09	-7.96, 0.56
Q3 (12.0 – 18.0)	45	-0.14	0.95	-4.45, 4.17
Q4 (18.0 – 44.0)	45	-1.66	0.45	-5.97, 2.65
			p-trend 0.85	
Patella Lead (ug/g)	181	-0.74	0.35	-2.31, 0.82
Patella Lead (quartiles)				
Q1 (<1 – 6.4)	45	Ref		
Q2 (6.4 – 13.5)	45	-0.09	0.97	-4.36, 4.18
Q3 (13.5 – 21.2)	46	-3.64	0.10	-8.00, 0.72
Q4 (21.2 – 43.0)	45	-1.97	0.38	-6.40, 2.46
			p-trend 0.16	

Each model is adjusted for sex, maternal age, education, history of adverse birth outcome, cigarette smoking, and parity.
Ref = Reference value

Figure II-1: Logistic Regression Analysis of Trimester-Specific and Average Biomarkers of Lead and Odds of Delivering Prematurely



Literature Cited

Andrews KW, Savitz DA, Hertz-Picciotto I. 1994. Prenatal lead exposure in relation to gestational age and birth weight: A review of epidemiologic studies. American Journal of Industrial Medicine 26(1): 13-32.

Aro AC, Todd AC, Amarasiriwardena C, Hu H. 1994. Improvements in the calibration of 109Cd K x-ray fluorescence systems for measuring bone lead in vivo. Physics in medicine and biology 39(12): 2263-2271.

Bellinger D, Leviton A, Rabinowitz M, Allred E, Needleman H, Schoenbaum S. 1991. Weight gain and maturity in fetuses exposed to low levels of lead. Environmental Research 54(2): 151-158.

Cory-Slechta DA, Virgolini MB, Rossi-George A, Thiruchelvam M, Lisek R, Weston D. 2008. Lifetime consequences of combined maternal lead and stress. Basic Clin Pharmacol Toxicol 102(2): 218-227.

Cory-Slechta DA, Virgolini MB, Thiruchelvam M, Weston DD, Bauter MR. 2004. Maternal stress modulates the effects of developmental lead exposure. Environ Health Perspect 112(6): 717-730.

Darendeliler F CA, Baş F, Bundak R, Dişçi R, Sükür M, Ince Z, Can G. 2008. Catch-up growth in appropriate- or small-for-gestational age preterm infants. Turk J Pediatr 50(3): 207-213.

Factor-Litvak P, Graziano JH, Kline JK, Popovac D, Mehmeti A, Ahmedi Shrout GP, et al. 1991. A prospective study of birthweight and length of gestation in a population surrounding a lead smelter in Kosovo, Yugoslavia. International Journal of Epidemiology 20(3): 722-728.

Gonzalez-Cossio T, Peterson KE, Sanin LH, Fishbein E, Palazuelos E, Aro A, et al. 1997. Decrease in birth weight in relation to maternal bone-lead burden. Pediatrics 100(5): 856-862.

Gulson BL, Mizon KJ, Korsch MJ, Palmer JM, Donnelly JB. 2003. Mobilization of lead from human bone tissue during pregnancy and lactation--a summary of long-term research. Sci Total Environ 303(1-2): 79-104.

Hobel CJ, Arora CP, Korst LM. 1999. Corticotrophin-releasing hormone and CRH-binding protein. Differences between patients at risk for preterm birth and hypertension. Annals of the New York Academy of Sciences 897: 54-65.

Hu H, Rabinowitz M, Smith D. 1998. Bone lead as a biological marker in epidemiologic studies of chronic toxicity: conceptual paradigms. Environ Health Perspect 106(1): 1-8.

Hu H, Shih R, Rothenberg S, Schwartz BS. 2007. The epidemiology of lead toxicity in adults: Measuring dose and consideration of other methodologic issues. Environmental Health Perspectives 115(3): 455-462.

Hu H, Tellez-Rojo MM, Bellinger D, Smith D, Ettinger AS, Lamadrid-Figueroa H, et al. 2006. Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. Environ Health Perspect 114(11): 1730-1735.

IOM, ed. 2007. Preterm Birth: Causes, Consequences, and Prevention.

Jaakkola JJ, Ahmed P, Ieromnimon A, Goepfert P, Laiou E, Quansah R, et al. 2006. Preterm delivery and asthma: a systematic review and meta-analysis. The Journal of allergy and clinical immunology 118(4): 823-830.

Jelliffe-Pawlowski LL, Miles SQ, Courtney JG, Materna B, Charlton V. 2006. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. Journal of Perinatology 26(3): 154-162.

Johnson S. 2007. Cognitive and behavioural outcomes following very preterm birth. Semin Fetal Neonatal Med 12(5): 363-373.

Kato I, Toniolo P, Koenig KL, Shore RE, Zeleniuch-Jacquotte A, Akhmedkhanov A, et al. 1999. Epidemiologic correlates with menstrual cycle length in middle aged women. Eur J Epidemiol 15(9): 809-814.

Kumar R, Yu Y, Story RE, Pongracic JA, Gupta R, Pearson C, et al. 2008. Prematurity, chorioamnionitis, and the development of recurrent wheezing: a prospective birth cohort study. The Journal of allergy and clinical immunology 121(4): 878-884.

Lamadrid-Figueroa H, Tellez-Rojo MM, Hernandez-Cadena L, Mercado-Garcia A, Smith D, Solano-Gonzalez M, et al. 2006. Biological markers of fetal lead exposure at each stage of pregnancy. J Toxicol Environ Health A 69(19): 1781-1796.

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. 2005. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 113(7): 894-899.

Lawn JE, Cousens S, Zupan J. 2005. 4 million neonatal deaths: when? Where? Why? Lancet 365(9462): 891-900.

Lawn JE, Wilczynska-Ketende K, Cousens SN. 2006. Estimating the causes of 4 million neonatal deaths in the year 2000. Int J Epidemiol 35(3): 706-718.

Leung TN, Chung TK, Madsen G, Lam CW, Lam PK, Walters WA, et al. 2000. Analysis of mid-trimester corticotrophin-releasing hormone and alpha-fetoprotein concentrations for predicting pre-eclampsia. Hum Reprod 15(8): 1813-1818.

Liu Y, Gold EB, Lasley BL, Johnson WO. 2004. Factors affecting menstrual cycle characteristics. Am J Epidemiol 160(2): 131-140.

Manton WI, Angle CR, Stanek KL, Kuntzelman D, Reese YR, Kuehnemann TJ. 2003. Release of lead from bone in pregnancy and lactation. Environ Res 92(2): 139-151.

McLean M, Smith R. 1999. Corticotropin-releasing Hormone in Human Pregnancy and Parturition. Trends Endocrinol Metab 10(5): 174-178.

McLean M, Smith R. 2001. Corticotrophin-releasing hormone and human parturition. Reproduction 121(4): 493-501.

Munster K, Schmidt L, Helm P. 1992. Length and variation in the menstrual cycle--a cross-sectional study from a Danish county. Br J Obstet Gynaecol 99(5): 422-429.

Navas-Acien A, Guallar E, Silbergeld EK, Rothenberg SJ. 2007. Lead exposure and cardiovascular disease--a systematic review. Environ Health Perspect 115(3): 472-482.

Rosner B. 1983. Percentage Points for a Generalized ESD Many-Outlier Procedure. Technometrics 25(2): 165-172.

Rowland AS, Baird DD, Long S, Wegienka G, Harlow SD, Alavanja M, et al. 2002. Influence of medical conditions and lifestyle factors on the menstrual cycle. Epidemiology 13(6): 668-674.

Saigal S SB, Streiner DL, Burrows E. 2001. Physical growth and current health status of infants who were of extremely low birth weight and controls at adolescence. Pediatrics 108: 407–415.

Sanin LH, Gonzalez-Cossio T, Romieu I, Peterson KE, Ruiz S, Palazuelos E, et al. 2001. Effect of maternal lead burden on infant weight and weight gain at one month of age among breastfed infants. Pediatrics 107(5): 1016-1023.

Schwartz J, Gold D, Dockery DW, Weiss ST, Speizer FE. 1990. Predictors of asthma and persistent wheeze in a national sample of children in the United States. Association with social class, perinatal events, and race. The American review of respiratory disease 142(3): 555-562.

Sesma HW, Georgieff MK. 2003. The effect of adverse intrauterine and newborn environments on cognitive development: the experiences of premature delivery and diabetes during pregnancy. Dev Psychopathol 15(4): 991-1015.

Smith D, Hernandez-Avila M, Tellez-Rojo MM, Mercado A, Hu H. 2002. The relationship between lead in plasma and whole blood in women. Environ Health Perspect 110(3): 263-268.

Smith DR, Ilustre RP, Osterloh JD. 1998. Methodological considerations for the accurate determination of lead in human plasma and serum. Am J Ind Med 33(5): 430-438.

Sowers M, Jannausch M, Scholl T, Li W, Kemp FW, Bogden JD. 2002. Blood lead concentrations and pregnancy outcomes. Archives of Environmental Health 57(5): 489-495.

Tellez-Rojo MM, Bellinger DC, Arroyo-Quiroz C, Lamadrid-Figueroa H, Mercado-Garcia A, Schnaas-Arrieta L, et al. 2006. Longitudinal associations between blood lead concentrations lower than 10 microg/dL and neurobehavioral development in environmentally exposed children in Mexico City. Pediatrics 118(2): e323-330.

Von Mutius E, Nicolai T, Martinez FD. 1993. Prematurity as a risk factor for asthma in preadolescent children. J Pediatr 123(2): 223-229.

Wadhwa PD, Glynn L, Hobel CJ, Garite TJ, Porto M, Chicz-DeMet A, et al. 2002. Behavioral perinatology: biobehavioral processes in human fetal development. Regul Pept 108(2-3): 149-157.

CHAPTER III

HFE Gene Variants Modify the Association between Maternal Lead Burden and Infant Birth Weight

Abstract

Background: Neonatal growth is a complex process involving genetic and environmental factors. Polymorphisms in the hemochromatosis (*HFE*) iron regulatory gene have been demonstrated to modify transport of metals and, in recent articles, to modify lead's toxicity. We investigated the role of genetic polymorphisms *C282Y* and *H63D* of the *HFE* gene and *P570S* of the transferrin (*TF*) gene in modifying lead biomarker effects upon infant birthweight in Mexican mother-infant pairs.

Methods: Subjects were recruited from three maternity hospitals in Mexico City, from 1994 to 1995. Of the initial 1382 eligible mother/infant pairs, 617 agreed to participate, and 411 infants /565 mothers had archived blood available for genotyping. Multiple linear regression models, stratified by either maternal/infant *HFE* or *TF* genotype and then combined with interaction terms, were constructed examining the relationship of birthweight to biomarkers of lead exposure after controlling for maternal age, maternal education, arm circumference, smoking

during pregnancy, gestational age, maternal 1 month PP hemoglobin levels, parity, marital status, and sex.

Results: The presence of infant and maternal variant *HFE H63D* genes independently predicted 110.3 g (95% CI -216.1, -4.6) and 52.0 g (95% CI -147.3 to 43.2) decreases in birthweight respectively. Stratified and interaction models suggest that both maternal and infant *HFE H63D* genotype may modify tibia lead's effect upon infant birthweight in opposing ways. In our interaction models, maternal *HFE H63D* variant carriers had an enhanced negative association ($p_{interaction}$=0.07) between tibia lead and birthweight.

Conclusions: These preliminary results suggest that the *HFE H63D* genotype modifies lead's effects on infant birthweight in a complex fashion that may reflect maternal-fetal interactions with respect to the metabolism and transport of metals.

Introduction

Decreased birth weight has been established as a predictor of infant mortality, morbidity, developmental outcomes such as cognitive performance, and chronic disease into adulthood (Tamakoshi et al. 2006; Tong et al. 2006). Both environmental and genetic factors contribute to the weight of an infant at birth. Environmental factors that have been association with birth weight include maternal nutritional status (Ramakrishnan, 2004), maternal infections (Rees and Harding, 2004), parity, and exposure to toxicants such as lead (Gonzalez-Cossio et al. 1997). Two recent population studies have estimated that approximately

50% of the variation in birth weight is due to heritable maternal/infant genetic factors (Lunde et al. 2007; Dunger et al. 2006), though it is interesting to note that some of this genetic heritability may be due to epigenetic influences, (Cutfield et al. 2007; Nafee et al. 2008) such as effects on the maternal insulin-like growth factor system (Olausson et al. 2008) or alterations to placental growth (Mitchell 2006). Genetic components which metabolize, respond to, or regulate these environmental factors would be strong candidates for gene-environment interactions.

Environmental exposure to lead (Tong et. al. 2000) and iron deficiency (Walker et al. 2007; WHO, 2004) continue to contribute significantly to worldwide infant mortality and developmental retardation. Decreases in birth weight have been independently associated with increased lead exposure (Gonzalez-Cossio et al. 1997; Jelliffe-Pawlowski et al. 2006) and extremes in iron status (Lozoff et al. 2006; Milman 2006). These effects may be compounded when women are both iron deficient and exposed to lead during pregnancy, because lead absorption is upregulated during iron deficiency (Wright et al. 1999; Wright et al. 2003; Wolf et al. 2003).

Iron uptake across cell membranes is complex and regulated by multiple proteins including transport proteins such as transferrin (*TF*), divalent metal transporter-1 (DMT-1), and ferroportin, their receptors, as well as regulatory proteins such as *HFE* and hepcidin (Rivers et al. 2007). The *HFE* gene is of particular interest in public health research because it contains two functional variants, *C282Y* and *H63D*, which are common (approximately 9.2% and 22.0% respectively) among individuals of European descent (Hanson et al. 2001). It has

been previously reviewed (Onalaja and Claudio 2000), and recent studies have indicated (Hopkins et al. 2008; Wang FT 2007), that *HFE* variants alone can be important modifiers of lead susceptibility. Lead absorption has also been shown to be affected by disruptions in *HFE*. Wright et. al. showed that older adult carriers of either *HFE* variant genotype (*H63D* or *C282Y*), had lower blood, patella bone, and tibia bone lead stores when compared to wildtype individuals (Wright et al. 2004). Recently, it was demonstrated that both separate and joint effects of iron metabolism gene variants *HFE* and *TF* were associated with increased blood lead levels in this cohort of Mexican children (Hopkins et al. 2008).

The aim of the present study was to explore the interaction between variants in the iron regulatory protein *HFE* (*C282Y* and *H63D*), the iron transport protein *TF* (*P570S*), and biomarkers of neonatal lead exposure upon infant birth weight. We hypothesized that 1) the maternal/infant *HFE* and *TF* variant genotypes would both independently and jointly modify infant birthweight and 2) *HFE* variant and *TF* variant genotypes could be protective against the negative effects of lead exposure upon birth weight given adequate iron stores do to competition for common transport receptors.

Methods

Sample Population

Maternal/infant pairs were recruited between 1994 and 1995 from three hospitals in Mexico City which serve low to moderate income populations as part of a clinical trial to assess calcium supplementation effects on bone lead

mobilization during lactation. Of the initial 1382 mothers who didn't fit any of the exclusion criteria which included factors that could interfere with maternal calcium metabolism, medical conditions that could cause low birth weight (<2000g), logistic reasons that would interfere with data collection (households living outside the metropolitan area), delivering a premature neonate (<37 weeks) or an infant with an Apgar score at 5 minutes of 6 or under, conditions requiring placement in a neonatal intensive care unit, a physician's diagnosis of multiple fetuses, intention not to breastfeed, preeclampsia, psychiatric, kidney, or cardiac diseases, gestational diabetes, history of repeated urinary infections, family or personal history of kidney stone formation, seizure disorder requiring daily medication, ingestion of corticosteroids or blood pressure >140mmHg systolic or >90mmHg diastolic, 617 agreed to participate. Of those who participated, 411 infants and 565 mothers had blood that was genotyped.

The study protocol was approved by the Ethics Committee of the National Institute of Public Health of Mexico, the participating hospitals, the Brigham and Women's Hospital, and the Harvard School of Public Health. All participating mothers received a detailed explanation of the study intent, research procedures, as well as counseling on how to reduce environmental lead exposure.

Anthropometric Measurements

Neonates were weighed within 12 hours of delivery by experienced obstetric nurses using calibrated beam scales (Oken, Model TD16, Naucalpan, Mexico) read to the nearest 10 grams. Maternal anthropometric measures were collected by our trained project personnel and standardized according to the

technique described by Habicht (Habicht 1974).These standardization exercises were performed until the project staff reached imprecision errors equal to or below those reported by Lohman and coworkers (Lohman et al. 1988). Accepted technical errors were 0.3 cm for arm circumference and 0.22 cm for height.

Blood Lead Measurements

Umbilical cord blood samples were collected in trace metal-free tubes at delivery. Blood samples were analyzed using an atomic absorption spectrometry instrument (Perkin-Elmer 3000, Chelmsford, MA, USA) at the metals laboratory of the American British Cowdray Hospital in Mexico City. External blinded quality-control samples were provided throughout the study period by the Maternal and Child Health Bureau (MCHB) and the Wisconsin State Laboratory of Hygiene Cooperative Blood Lead Proficiency Testing Program (WSLH PBPTP.)

Bone Lead Measurements

In vivo maternal bone lead measurements were taken within 1 month of delivery at 2 bone sites, the mid-tibial shaft (cortical bone) and the patella (trabecular bone). Bone lead was measured non-invasively using a spot-source ^{109}Cd K-XRF instrument constructed at Harvard University and installed in a research facility in the American British Cowdray Medical Center. The physical principles, technical specifications, and validation of this and other similar K-XRF instruments have been described in detail elsewhere (Aro et al. 1994). For this study, 30-minute measurements were taken at the midshaft of the left tibia and

the left patella. Analysis of means and standard deviations of phantom-calibrated measurements did not disclose any significant shift in accuracy or precision.

Genotyping Methods

DNA extraction and genotyping were performed in The Harvard-Partners Center for Genetics and Genomics. High-molecular-weight DNA was extracted with commercially available PureGene Kits (Gentra Systems, Minneapolis, MN) from the white blood cells of archived maternal and umbilical cord blood samples. Genotyping for the hemochromotosis *HFE C282Y* (RS1800562), *HFE H63D* (RS1799945) and transferrin *TF P570S* (RS1049296) variants was performed using Sequenom MALDI-TOF (Matrix-assisted laser desorption ionization – time of flight) mass spectrometry according to the following methods.

The PCR was carried out in 384-well reaction plates in a volume of 5 µl using 2.5 ηg genomic DNA. Multiplex PCR was carried out to generate short PCR products (> 100 bp) containing one SNP or insertion-deletion. Briefly, 2.5 ηg genomic DNA was amplified in a 5 µl reaction containing 1 x HotStar Taq PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 200 µM each dNTP, 50 ηM each PCR primer, 0.1 U HotStar Taq (Qiagen). The reaction was incubated at 95^{o}C for 15 minutes followed by 45 cycles of 95^{o}C for 20 seconds, 56^{o}C for 30 seconds, 72^{o}C for 1 minute, followed by 3 minutes at 72^{o}C. Excess dNTPs were then removed from the reaction by incubation with 0.3 U shrimp alkaline phosphatase (USB) at 37^{o}C for 20 minutes followed by 5 minutes at 85^{o}C to deactivate the enzyme. Single primer extension over the SNP or insertion-deletion was carried out in a final concentration of 600 ηM each extension primer, 50 µM d/ddNTP

and 0.126 U Thermosequenase (Solis Biodyne) and incubated at 94^{o}C for 2 minutes followed by 45 cycles of 94 ^{o}C for 5 seconds, 52 ^{o}C for 5 seconds, and 72 ^{o}C for 5 seconds. The reaction was then desalted by addition of a cation exchange resin followed by mixing and centrifugation to settle the contents of the tube. The extension product was then spotted onto a 384 well spectroCHIP before being flown in the MALDI-TOF mass spectrometer.

Statistical Analyses

Descriptive statistics and identification of outliers, using the generalized extreme studentized deviation (ESD) method (Rosner 1983) were performed. Distribution of *HFE* and *TF* alleles and genotypes were examined and frequencies were tested using a chi-square statistic to compare observed and expected counts according to principles of Hardy-Weinberg equilibrium. Individuals with heterozygous variant genotypes (CY, HD, and PS) were compared separately to participants with wild type genotypes. Three Infant and four maternal homozygous *HFE H63D* individuals (DD) as well as three infant and one maternal compound heterozygous individuals (CY HD) were removed from analysis due to population studies that demonstrate those individuals may have significantly increased iron levels when compared to carrier individuals (Jackson et al. 2001).

Demographic characteristics and bone/blood lead levels by genotype (wild-type vs. variant carriers) were examined, and mean differences were tested by chi-square or Student's *t*-test (2-tailed) as appropriate. Potential non-linearity between continuous predictor variables and birth weight was explored by plotting

generalized additive models (GAM) using R 2.9.1 (The R Foundation for Statistical Computing) software that included smoothing parameters for continuous variables. Resulting non-linear associations were controlled for in multiple linear regression (MLR) models that included the maternal *HFE H63D* or *TF P570S* genotype by adding a squared term for maternal age and dummy variables for maternal education. MLR was then used to model the relationship between birthweight, maternal and infant *HFE H63D* and *TF P570S* genotypes, and biomarkers of lead exposure, after controlling for potential confounding variables. The potential confounding variables considered in our model were based on biologic plausibility or those significantly associated with birthweight ($p<0.1$) in bivariate analysis; variables included were: maternal age at delivery (years), maternal education (years), maternal postpartum arm circumference (cm)((which served as proxy for gestational weight gain), cigarette smoking during pregnancy (yes/no), gestational age (weeks), infant gender (female gender as reference group), maternal hemoglobin at 1 month post-partum, parity (total number of live births), and marital status (single/partnered.) To examine the potential modifying effect of the variant genotypes we initially ran separate MLR models stratified by maternal or infant variant genotype. On the basis of the differences in effect estimates of bone lead on birthweight in stratified models, we fitted MLR models that included an interaction term between the variant genotype and lead biomarker.

Regression diagnostics were performed on all models to evaluate multicollinearity and violations of the linear regression model assumptions. Data

were analyzed using SAS 9.1, Cary, NC, SAS Institute Inc. 2002-2003 and R 2.9.1 (The R Foundation for Statistical Computing) 2007.

Results

Our final study population included 390 genotyped children, of which, 3.1%, 16.8%, and 17.5% carried the *HFE C282Y, HFE H63D*, and *TF P570S* variants, respectively. Additionally, 533 genotyped mothers were included in the final study population and 1.9%, 14.5%, and 18.9% carried the *HFE C282Y*, *HFE H63D*, and *TF P570S* variant genotype (Table III-1). All genotype distributions were found to conform to Hardy-Weinberg equilibrium expectations. After taking into consideration both maternal and infant *HFE H63D* genotype status 20 (5.4%) mothers carried the *HFE H63D* variant while their infants were wildtype, 32 (8.6%) mothers were wildtype while their infants carried the *HFE H63D* variant, and 40 (10.8%) were both heterozygous for *HFE H63D* (Table III-1).

Table III-2 shows the distribution of lead biomarkers and covariates stratified by infant and mother *HFE H63D* variant/wildtype individuals. Mean infant birth weight was significantly lower in infant *HFE H63D* carriers when compared to infant *HFE H63D* wild-type individuals. Maternal hemoglobin at one month post partum was increased significantly in infant genotype *HFE H63D* variants 14.1 g/dL (95% CI 13.7 to 14.3) when compared to infant *HFE H63D* wild-type individuals 13.5 g/dL (95% CI 13.3 to 13.7) There were no significant differences between maternal *HFE H63D* variants and maternal wild-type individuals. Patella bone lead levels were significantly increased in infant and

maternal genotype *TF P570S* variants when compared to infant and maternal *TF P570S* wild-type individuals respectively (Data not shown).

There were 59 (12.6%) infants and 132 (27.9%) mothers with cord blood lead levels or blood lead levels at delivery exceeding 10µg/dL. Additionally, 147 (26.0%) mothers were anemic based on recommendations for lactating women living at an altitude of 7,000 – 7,999 feet above sea level (CDC 1998). Cord blood lead, maternal blood lead at 1 month post-partum, and maternal anemia status at 1 month post-partum all failed to predict a significant association with birthweight (Table III-3). A one unit change in tibia bone lead predicted a decrease of 4.4 g (95%CI -7.9 to -0.9) in birthweight after controlling for covariates of interest. Using the lowest quartile of tibia lead as the reference group, the highest quartile of tibia lead predicted a 95.4 gram (95%CI -189.9, -0.8) gram decrease in birthweight and the trend in the quartiles had a p-value of 0.06.

Results from generalized additive models indicated that gestational age had a slight non-linear association with birthweight. Additionally, in models with the maternal *HFE H63D* or *TF P570S* variants, maternal age and maternal education had significant non-linear relationships. To control for this effect, any models including the maternal *HFE H63D* or *TF P570S* included additional covariates: maternal age squared and tertiles of maternal education.

After controlling for tibia bone lead and potential confounding variables of birth weight in multivariate analysis (maternal age at delivery in years, maternal years of education, maternal arm circumference, cigarette smoking during pregnancy (yes/no), gestational age in weeks, infant gender, maternal

hemoglobin at 1 month post-partum, and parity (# previous children)), the presence of the infant and maternal variant *HFE H63D* gene independently predicted a 129.5 g (95% CI -236.4, -22.6) and 53.7 g (95% CI -148.9, 41.5) decrease in birthweight respectively (Table III-4). The presence of both a maternal and infant *HFE H63D* variant genotype predicted a decrease of 176.9 g (95% CI -318.6 to -35.3) in birthweight. Main effects of maternal and infant transferrin variant genotypes upon birthweight were non-significant. Due to small number of heterozygous *HFE C282Y* variant mothers and infants results were not reported.

We next examined effect modification by *HFE* and *TF* genotype on the relationship between tibia/patella lead levels with birthweight. We found that the maternal *HFE H63D* genotype may modify the relationship between tibia lead and birthweight by enhancing the negative effect in maternal *HFE H63D* variants. The interaction term coefficient for tibia lead and maternal *HFE H63D* genotype was -10.3 (p=0.05) (Table III-5). This relationship persisted when we modeled infant/maternal genotype interactions; maternal *HFE H63D* variants who gave birth to infant *HFE H63D* wildtypes had an enhanced negative effect of tibia lead upon birthweight with an interaction term of -28.3 (p=0.003). Effect modification by infant *HFE H63D* and *TF P570S* status upon the tibia lead/birthweight relationship were not significant, but suggested a positive direction for effect modification.

Discussion

Our research demonstrates that the presence of the infant *HFE H63D* variant genotype predicts a decrease in birth weight after adjusting for tibia lead and potential confounding variables. In our MLR models, having the *HFE H63D* mutation in infants led to a decrease of 129.5 grams (95%CI -236.4, -22.6) in infant birth weight after controlling for covariates of interest. The combined effects of a maternal and infant *HFE H63D* variant genotype predicted a greater decrease in birthweight of 176.9 grams (95%CI -318.6, -35.3). Furthermore, this preliminary research suggests that the presence of the infant *HFE H63D* variant genotype may provide a protective effect against the negative effects of lead upon birth weight while the maternal *HFE H63D* variant genotype enhances the negative effects of lead upon birthweight. To our knowledge this study is the first to observe effect modification of lead exposure and birth weight by *HFE* genotype status.

The independent effects of *HFE* upon birth weight have only been studied in one other study (Maier et al. 1999). In Maier et. al.'s study, very low birth weight infants (<1500g) were assessed for *HFE C282Y* genotype and transferrin saturation. Although the study observed no association between *HFE C282Y* genotype, transferrin saturation and very low birth weight (VLBW), it should be noted that only six infants were heterozygous for the *HFE C282Y* mutation. Our study differs from Maier et. al. in that we had no very low birth weight infants and instead chose to look at the continuous measure of birth weight.

A mechanistic role for the interaction between *HFE*, lead, and birth weight has yet to be established. Previous research has shown that both maternal iron

deficiency and iron excess can increase risk of preterm delivery and decrease infant birthweight (Casanueva and Viteri 2003; Swain et al. 1994; Ronnenberg et al. 2004; Lee et al. 2006; Lao et al. 2000). The production of reactive oxygen species (ROS) resulting from reactive iron species, like unbound iron, is thought to be the major mechanistic contributor to the damage done by iron excess and possibly iron deficiency (Casanueva and Viteri 2003; Lund et al. 2001; Srigiridhar et al. 2001). Exposure to lead has also been shown to disrupt the balance between ROS and antioxidant cellular defenses (Casado et al. 2007; El-Sayed et al. 2006). It is hypothesized that any disruption in this delicate balance of pro-oxidant/antioxidant molecules either through excess/deficiency of iron and exposure to lead could impair infant development. If we stratify by maternal anemia status we find that mothers that are anemic no longer have a significant interaction term between maternal *HFE H63D* genotype and tibia lead and have a reduced beta estimate, but those mothers that are non-anemic have a significant negative interaction term (results not shown). The enhancement of lead's negative effects by maternal *HFE H63D* variant status indicated by our results may be mediated through such a mechanism.

Due to the dietary importance of iron for establishing normal neonatal growth and decreased lead absorption under iron sufficient conditions, it is plausible to speculate that the protective effects of the infant *HFE H63D* variant genotypes may be the result of competition between iron and lead for common transportation receptors across the placenta. On the apical plasma membrane of syncytiotrophoblastic (STB) cells in human placenta, *HFE* associates with the transferrin receptor (TFr) and on the basal side with ferroprotin and DMT-1,

suggesting a major role in iron transport across the placenta (Georgieff et al. 2000; Gruper et al. 2005; Bastin et al. 2006; Parkkila et al. 1997; Ganz 2007).

Previous associations between increased lead uptake during iron deficient conditions have recognized that both lead and iron compete for the common DMT-1 transporter (Bannon et al. 2002; Bressler et al. 2004). Evidence of this interaction has been shown in cell lines with lead and iron both using DMT-1 (Bannon et al. 2002). Iron binds with more affinity to DMT-1 than lead suggesting that under conditions of higher iron concentration less lead would cross cells in the duodenum and placenta (Garrick et al. 2006). Lead transport through DMT-1 was found to be 80% inhibited by a 25 fold increase in the presence of iron (Bannon et al. 2002).

In human epidemiological studies, Wright et al. found that older male adults carriers of the *HFE* variant genotype had lower bone and blood lead levels (Wright et al. 2004). Contrary to what was found in the latter aging population, it was recently shown that infant carriers of either *HFE H63D* or *HFE C282Y* had increased blood lead levels (Hopkins et al. 2008). In our study we did not have a direct measure of maternal iron status during pregnancy and instead used maternal hemoglobin at 1 month post-partum as an indirect measure. While research has indicated that maternal hemoglobin during pregnancy can predict decreases in birthweight, it has been extensively discussed that a single biomarker of iron status is insufficient in determining true iron status (WHO 2004). Further research will be needed to in order to gain a greater understanding of how biologically useful metals (iron, magnesium, selenium)

interact with toxic metals (lead, cadmium) and how these interactions may modify health effects.

As with any epidemiologic study there are limitations. Given the small number of subjects who carry an either the infant or maternal *HFE H63D* variant genotype (n=68 and n=85 respectively, *HFE C282Y* variant (n=11 and n=9 respectively), or *TF P570S* variant (n=77 and n=117) these results should be considered preliminary, since the protective effects of the infant *HFE H63D* genotype upon lead's decrease of birth weight, and enhancement by maternal *HFE H63D* genotype, maybe due to chance. This is critically important for the interpretation of any results of our interacting maternal/infant genotype populations. We were also unable to take measures of ferritin, hemoglobin, mean corpuscular volume, free erythrocyte protoporphyrin, and other markers of iron status during pregnancy due to logistical and financial reasons. Without proper measures of iron status we were unable to assess iron deficient or iron excess which have both been linked with decreased birthweight in numerous studies (Wright et al. 2003; Wolf et al. 2003; Casanueva and Viteri 2003; Lao et al. 2000; England et al. 2001). As a surrogate measure for iron status we used hemoglobin measures at one month post partum which was significantly associated with a decrease in birthweight in our regression models suggesting a role of iron excess. Previous studies have indicated that iron levels are slightly elevated in heterozygous carriers of either *HFE C282Y* or *HFE H63D* genotypes (Jackson et al. 2001). In our study maternal carriers of *HFE H63D* variant genotypes did not have significantly higher 1 month post-partum hemoglobin

measures (13.7±1.6 µg/dL) when compared to wildtype individuals (13.5±1.5 µg/dL, p-value=0.13).

In summary, we found in this study that infants or mother/infant pairs who both carry the *HFE H63D* variant genotype have lower birth weight babies. Additionally, infant *HFE H63D* variants may modify the negative effects of lead biomarkers on birth weight by decreasing lead biomarker effects in infants who carry an *HFE H63D* variant genotype. Conversely, mothers who carry an *HFE H63D* variant genotype may enhance the negative effects upon birthweight from bone lead exposure which may arise from increased oxidative damage during fetal development.

Table III-1: Maternal and Infant *HFE* and *TF* Genotype Frequencies

Infant Genotype Frequencies			Maternal Genotype Frequencies		
Genotype	**Number**	**%**	**Genotype**	**Number**	**%**
HFE (C282Y)			**HFE (C282Y)**		
C282Y Wildtype (CC)	378	96.9	C282Y Wildtype (CC)	523	98.1
C282Y Heterozygous (CY)	12	3.1	C282Y Heterozygous (CY)	10	1.9
C282Y Homozygous (YY)	0	0	C282Y Homozygous (YY)	0	0
Hardy–Weinberg equilibrium: $\chi 2 = 0.10$, $p = 0.76$			Hardy–Weinberg equilibrium: $\chi 2 = 0.05$, $p = 0.83$		
HFE (H63D)			**HFE (H63D)**		
H63D Wildtype (HH)	319	82.2	H63D Wildtype (HH)	452	85.0
H63D Heterozygous (HD)	65	16.8	H63D Heterozygous (HD)	77	14.5
H63D Homozygous (DD)	4	1.0	H63D Homozygous (DD)	3	0.5
Hardy–Weinberg equilibrium: $\chi 2 = 0.11$, $p = 0.74$			Hardy–Weinberg equilibrium: $\chi 2 = 0.02$, $p = 0.87$		
TF (P570S)			**TF (P570S)**		
P570S Wildtype (PP)	319	82.0	P570S Wildtype (PP)	429	80.9
P570S Heterozygous (PS)	68	17.5	P570S Heterozygous (PS)	100	18.9
P570S Homozygous (SS)	2	0.5	P570S Homozygous (SS)	1	0.2
Hardy–Weinberg equilibrium: $\chi 2 = 0.65$, $p = 0.42$			Hardy–Weinberg equilibrium: $\chi 2 = 3.81$, $p = 0.05$		

	Inf. Wildtype / Mat. Wildtype	Inf. Variant / Mat. Wildtype	Inf. Wildtype / Mat. Variant	Inf. Variant / Mat. Variant
HFE H63D N(%):	279 (75.2%)	32 (8.6%)	20 (5.4%)	40 (10.8%)
TF P570S N(%):	276 (73.6%)	36 (9.6%)	30 (8.0%)	33 (8.8%)

Table III-2: Study Population Characteristics by *HFE H63D* Genotype

	Infant *H63D* Wildtype		Infant *H63D* Variant		Maternal *H63D* Wildtype		Maternal *H63D* Variant	
	No.	Mean ± SD (Range)	No.	Mean ± SD (Range)	No.	Mean ± SD (Range)	No.	Mean ± SD (Range)
MATERNAL CHARACTERISTICS								
Maternal age at delivery (yrs)	317	24.6 ± 5.0 (44 – 15)	63	24.9±5.0 (38 – 16)	489	24.5±5.1 (44 – 14	85	24.4±5.5 (39 – 15)
Maternal years of education	317	9.6±3.0 (18 – 1)	63	9.9±3.1 (17 – 3)	479	9.3±3.1 (18 – 1)	82	9.4±2.9 (17 – 1)
Arm circumference (cm)	316	26.3±2.7 (35.4 – 20.4)	63	26.4±2.2 (31.5 – 21.5)	472	26.3±2.7 (35.4 – 20.4)	79	26.2±2.5 (33.2 – 22.0)
Cigarette smoking (%)	343	3.8	68	0	485	4.9	85	2.4
Primiparity (%)	343	43.5	68	36.8	485	43.6	85	45.9
Marital Status (% Not Married)	319	9.4	63	4.8	452	8.4	76	10.5
Maternal hemoglobin 1mo PP (g/dL)	317	13.5±1.5 (16.7 – 8.3)	63	14.0±1.3** (16.0 – 10.2)	479	13.6±1.5 (16.7 – 8.3)	84	13.7±1.6 (16.9 – 9.0)
Maternal blood lead delivery (µg/dl)	315	8.6±4.1 (23.7 – 2.1)	63	9.1±5.1 (35.4 – 3.3)	484	8.6±3.9 (23.7 –1.8)	85	8.1±4.7 (35.4 – 1.8)
Maternal patella lead (µg/g)	287	14.3±13.8 (50.1 -- < 1)	58	17.3±15.7 (65.5 -- < 1)	448	14.0±14.1 (53.8 -- <1)	81	15.9±13.6 (54.7 -- <1)
Maternal tibia lead (µg/g)	309	10.4±9.5 (43.2 -- < 1)	61	9.2±10.1 (38.6 -- < 1)	472	9.8±9.3 (39.9 -- <1)	83	8.4±9.4 (31.2 -- <1)
INFANT CHARACTERISTICS								
Umbilical cord blood lead (µg/dl)	279	6.6±3.6 (26.3 – 1.2)	54	7.1±4.6 (29.9 – 2.2)	405	6.6±3.5 (26.3 – 1.2)	72	6.4±4.0 (29.9 – 1.9)
Infant birth weight (g)	317	3166.6±407.0 (4450 – 1850)	63	3056.4±429.3* (4100 – 1950)	489	3148.1±414.7 (4450 – 1650)	85	3093.8±431.3 (4125 – 1950)
Gestational age (wks)	314	39.3±1.4 (44 – 35)	63	39.1±1.5 (41 – 35)	481	39.2±1.5 (44 – 35)	85	39.1±1.5 (42 – 35)
Infant Gender (% male)	342	54.1	68	64.7	405	54.7	84	52.4

** P-value < 0.05, * P-value < 0.1

Table III-3: Adjusted parameter estimates for birthweight by biological markers of lead exposure in separate linear regression models†

Variable	No	β	p-Value	95% CI
Cord Blood Lead (µg/dL)	464	-31.1	0.41	-105.4, 43.3
Maternal Blood Lead at Delivery (µg/dL)	550	-8.2	0.82	-80.2, 63.8
Maternal Anemia at 1 mo PP (<13 µg/L)^	554	59.2	0.12	-15.9, 134.3
Tibia Lead (µg/g)	538	-4.4	0.01	-7.9, -0.9
Tibia Lead (quartiles)				
Q1 (<1 – 4.1)	137	Ref		
Q2 (4.1 – 9.2)	137	17.2	0.72	-75.6, 110.1
Q3 (9.2 – 15.4)	138	-19.1	0.69	-112.1, 73.9
Q4 (15.4 – 43.2)	137	-95.4	0.05	-189.9, -0.8
		p-trend	0.06	
Patella Lead (µg/g)	507	0.16	0.89	-2.2, 2.5
Patella Lead (quartiles)				
Q1 (<1 – 4.5)	129	Ref		
Q2 (4.5 – 14.0)	130	-16.2	0.74	-113.7, 81.3
Q3 (14.0 – 23.9)	130	-23.6	0.63	-119.6, 72.5
Q4 (23.9 – 65.5)	130	13.9	0.78	-82.3, 110.0
		p-trend	0.35	

†All models are adjusted for maternal age, years of maternal education, infant gender, maternal arm circumference, gestational age, smoking status during pregnancy, marital status, maternal hemoglobin at 1 month PP, and parity.
^N=147 (26.0%)
** P-value < 0.05, * P-value < 0.1

Table III-4: Adjusted parameter estimates for birthweight by *HFE H63D and TF P570S* genotype status, in separate linear regression models†

Variable	N	Beta Coef.	95% CI
Infant H63D HFE Genotype	367	-129.5**	-236.4, -22.6
Infant P570S Tf Genotype	371	34.9	-68.3,138.2
Maternal H63D HFE Genotype^	502	-53.7	-148.9, 41.5
Maternal P570S Tf Genotype^	503	62.6	-148.5, 23.4
$H63D_{Inf.}$ Wildtype / $H63D_{Mat.}$Variant	332	94.7	-99.0, 288.4
$H63D_{Inf.}$ Variant / $H63D_{Mat.}$Wildtype	332	-77.4	-240.9, 86.1
$H63D_{Inf.}$ Variant / $H63D_{Mat.}$Variant	332	-176.9**	-318.6, -35.3
$Tf_{Inf.}$ Wildtype / $Tf_{Mat.}$Variant	337	-63.2	-207.2, 80.8
$Tf_{Inf.}$ Variant / $Tf_{Mat.}$Wildtype	337	66.9	-88.6, 222.3
$Tf_{Inf.}$ Variant / $Tf_{Mat.}$Variant	337	-36.4	-190.1,117.2

†All models are adjusted for maternal age, years of maternal education, infant gender, maternal arm circumference, gestational age, smoking status during pregnancy, marital status, maternal tibia lead, maternal hemoglobin at 1 month post-partum, and parity.
^All models additionally adjusted for maternal age^2, and dummy variables for maternal education (years of education: <8, 8 -11, 11>) to account for the non-linear nature of these covariates.
** P-value < 0.05, * P-value < 0.1

Table III-5: Adjusted regression coefficients of *HFE H63D* and *TF P570S* genotype, lead biomarker, and the interaction term in association with birthweight†

		Tibia Bone Lead			Patella Bone Lead	
Genotype	Genotype Variant	Tibia Lead	Interaction Term	Genotype Variant	Patella lead	Interaction Term
Infant H63D HFE	-127.5**	-6.5**	4.5	-114.2**	-1.5	1.2
Infant P570S Tf	34.9	-5.8**	0.9	5.6	-2.7	7.5**
Maternal H63D HFE^	-62.7	-3.0	-10.3**	-56.9	-0.2	5.9
Maternal P570S Tf^	-60.8	-5.7**	6.8	76.6*	-0.4	4.3
Maternal/Infant H63D Interactions						
$H63D_{Inf.}$ Wildtype / $H63D_{Mat.}$Wildtype	Referent					
$H63D_{Inf.}$ Wildtype / $H63D_{Mat.}$Variant	143.0	-4.5*	-28.7**	99.0	-2.0	-1.4
$H63D_{Inf.}$ Variant / $H63D_{Mat.}$Wildtype	-64.2		6.2	-38.3		-10.7
$H63D_{Inf.}$ Variant / $H63D_{Mat.}$Variant	-177.2**		-6.0	-192.3**		6.8
Maternal/Infant Tf Interactions						
$Tf_{Inf.}$ Wildtype / $Tf_{Mat.}$Wildtype	Referent					
$Tf_{Inf.}$ Wildtype / $Tf_{Mat.}$Variant	-51.7	-7.2**	10.3	-42.9	-3.6*	2.9
$Tf_{Inf.}$ Variant / $Tf_{Mat.}$Wildtype	66.7		-4.5	66.4		3.7
$Tf_{Inf.}$ Variant / $Tf_{Mat.}$Variant	-40.5		9.3	-76.1		11.4**

†All models are adjusted for maternal age, years of maternal education, infant gender, maternal arm circumference, gestational age, smoking status during pregnancy, maternal hemoglobin one month post-partum, and parity.
* P-value <0.1, ** P-value<0.05

Literature Cited

Aro AC, Todd AC, Amarasiriwardena C, Hu H. 1994. Improvements in the calibration of 109Cd K x-ray fluorescence systems for measuring bone lead in vivo. Physics in medicine and biology 39(12): 2263-2271.

Bannon D, Portnoy ME, Olivi L, Lees PS, Culotta VC, Bressler JP. 2002. Uptake of lead and iron by divalent metal transporter 1 in yeast and mammalian cells. Biochem Biophys Res Commun 295(4): 978-984.

Bastin J, Drakesmith H, Rees M, Sargent I, Townsend A. 2006. Localisation of proteins of iron metabolism in the human placenta and liver. Br J Haematol 134(5): 532-543.

Bressler JP, Olivi L, Cheong JH, Kim Y, Bannona D. 2004. Divalent metal transporter 1 in lead and cadmium transport. Annals of the New York Academy of Sciences 1012: 142-152.

Casado MF, Cecchini AL, Simao AN, Oliveira RD, Cecchini R. 2007. Free radical-mediated pre-hemolytic injury in human red blood cells subjected to lead acetate as evaluated by chemiluminescence. Food Chem Toxicol 45(6): 945-952.

Casanueva E, Viteri FE. 2003. Iron and oxidative stress in pregnancy. Journal of Nutrition 133(5 SUPPL. 1): 1700S-1708S.

CDC. 1998. Recommendations to prevent and control iron deficiency in the United States. MMWR Recomm Rep 47(RR-3): 1-29.

Cutfield WS, Hofman PL, Mitchell M, Morison IM. 2007. Could epigenetics play a role in the developmental origins of health and disease? Pediatric Research 61(5 PART 2 SUPPL.).

Dunger DB, Petry CJ, Ong KK. 2006. Genetic variations and normal fetal growth. Horm Res 65 Suppl 3: 34-40.

El-Sayed IH, Lotfy M, El-Khawaga OA, Nasif WA, El-Shahat M. 2006. Prominent free radicals scavenging activity of tannic acid in lead-induced oxidative stress in experimental mice. Toxicology and industrial health 22(4): 157-163.

England LJ, Kendrick JS, Wilson HG, Merritt RK, Gargiullo PM, Zahniser SC. 2001. Effects of smoking reduction during pregnancy on the birth weight of term infants. Am J Epidemiol 154(8): 694-701.

Ganz T. 2007. Molecular control of iron transport. J Am Soc Nephrol 18(2): 394-400.

Garrick MD, Singleton ST, Vargas F, Kuo HC, Zhao L, Knopfel M, et al. 2006. DMT1: which metals does it transport? Biol Res 39(1): 79-85.

Georgieff MK, Wobken JK, Welle J, Burdo JR, Connor JR. 2000. Identification and localization of divalent metal transporter-1 (DMT-1) in term human placenta. Placenta 21(8): 799-804.

Gonzalez-Cossio T, Peterson KE, Sanin LH, Fishbein E, Palazuelos E, Aro A, et al. 1997. Decrease in birth weight in relation to maternal bone-lead burden. Pediatrics 100(5): 856-862.

Gruper Y, Bar J, Bacharach E, Ehrlich R. 2005. Transferrin receptor co-localizes and interacts with the hemochromatosis factor (HFE) and the divalent metal transporter-1 (DMT1) in trophoblast cells. J Cell Physiol 204(3): 901-912.

Habicht JP. 1974. [Standardization of quantitative epidemiological methods in the field]. Boletin de la Oficina Sanitaria Panamericana 76(5): 375-384.

Hanson EH, Imperatore G, Burke W. 2001. HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology. Am J Epidemiol 154(3): 193-206.

Hopkins MR, Ettinger AS, Hernandez-Avila M, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, et al. 2008. Variants in iron metabolism genes predict higher blood lead levels in young children. Environ Health Perspect 116(9): 1261-1266.

Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD, et al. 2001. HFE mutations, iron deficiency and overload in 10,500 blood donors. Br J Haematol 114(2): 474-484.

Jelliffe-Pawlowski LL, Miles SQ, Courtney JG, Materna B, Charlton V. 2006. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. J Perinatol 26(3): 154-162.

Lao TT, Tam KF, Chan LY. 2000. Third trimester iron status and pregnancy outcome in non-anaemic women; pregnancy unfavourably affected by maternal iron excess. Hum Reprod 15(8): 1843-1848.

Lee HS, Kim MS, Kim MH, Kim YJ, Kim WY. 2006. Iron status and its association with pregnancy outcome in Korean pregnant women. European journal of clinical nutrition 60(9): 1130-1135.

Lohman, Roche, Martorell. 1988. Anthropometric standarization reference manual. In: Human Kinetics Book. Champaign: Human Kinetics Publishers.

Lozoff B, Kaciroti N, Walter T. 2006. Iron deficiency in infancy: applying a physiologic framework for prediction. Am J Clin Nutr 84(6): 1412-1421.

Lund EK, Fairweather-Tait SJ, Wharf SG, Johnson IT. 2001. Chronic exposure to high levels of dietary iron fortification increases lipid peroxidation in the mucosa of the rat large intestine. The Journal of nutrition 131(11): 2928-2931.

Lunde A, Melve KK, Gjessing HK, Skjaerven R, Irgens LM. 2007. Genetic and Environmental Influences on Birth Weight, Birth Length, Head Circumference, and Gestational Age by Use of Population-based Parent-Offspring Data. Am J Epidemiol. 165(7): 734-741.

Maier RF, Witt H, Buhrer C, Monch E, Kottgen E. 1999. HFE gene mutation and transferrin saturation in very low birthweight infants. Archives of disease in childhood 81(2): F144-145.

Milman N. 2006. Iron and pregnancy--a delicate balance. Ann Hematol 85(9): 559-565.

Mitchell MD. 2006. Unique suppression of prostaglandin H synthase-2 expression by inhibition of histone deacetylation, specifically in human amnion but not adjacent choriodecidua. Mol Biol Cell 17(1): 549-553.

Nafee TM, Farrell WE, Carroll WD, Fryer AA, Ismail KM. 2008. Epigenetic control of fetal gene expression. BJOG 115(2): 158-168.

Olausson H, Lof M, Brismar K, Lewitt M, Forsum E, Sohlstrom A. 2008. Longitudinal study of the maternal insulin-like growth factor system before, during and after pregnancy in relation to fetal and infant weight. Horm Res 69(2): 99-106.

Parkkila S, Waheed A, Britton RS, Bacon BR, Zhou XY, Tomatsu S, et al. 1997. Association of the transferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. Proc Natl Acad Sci U S A 94(24): 13198-13202.

Rivers CA, Barton JC, Gordeuk VR, Acton RT, Speechley MR, Snively BM, et al. 2007. Association of ferroportin Q248H polymorphism with elevated levels of serum ferritin in African Americans in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. Blood Cells, Molecules, and Diseases 38(3): 247-252.

Ronnenberg AG, Wood RJ, Wang X, Xing H, Chen C, Chen D, et al. 2004. Preconception hemoglobin and ferritin concentrations are associated with pregnancy outcome in a prospective cohort of Chinese women. The Journal of nutrition 134(10): 2586-2591.

Rosner B. 1983. Percentage Points for a Generalized ESD Many-Outlier Procedure. Technometrics 25(2): 165-172.

Srigiridhar K, Nair KM, Subramanian R, Singotamu L. 2001. Oral repletion of iron induces free radical mediated alterations in the gastrointestinal tract of rat. Molecular and cellular biochemistry 219(1-2): 91-98.

Swain S, Singh S, Bhatia BD, Pandey S, Krishna M. 1994. Maternal hemoglobin and serum albumin and fetal growth. Indian pediatrics 31(7): 777-782.

Tamakoshi K, Yatsuya H, Wada K, Matsushita K, Otsuka R, Yang PO, et al. 2006. Birth weight and adult hypertension: cross-sectional study in a Japanese workplace population. Circ J 70(3): 262-267.

Tong S, Baghurst P, McMichael A. 2006. Birthweight and cognitive development during childhood. J Paediatr Child Health 42(3): 98-103.

Wang FT HH, Schwartz J, Weuve J, Spiro AS III, Sparrow D, Nie H, Silverman EK, Weiss ST, Wright RO. . 2007. Modifying Effects of the HFE Polymorphisms on the Association between Lead Burden and Cognitive Decline. Environ Health Perspect.

WHO. 2004. Assessing the Iron Status of Populations. Geneva: WHO/CDC Joint Report.

Wolf AW, Jimenez E, Lozoff B. 2003. Effects of iron therapy on infant blood lead levels. J Pediatr 143(6): 789-795.

Wright RO, Shannon MW, Wright RJ, Hu H. 1999. Association between iron deficiency and low-level lead poisoning in an urban primary care clinic. Am J Public Health 89(7): 1049-1053.

Wright RO, Silverman EK, Schwartz J, Tsaih SW, Senter J, Sparrow D, et al. 2004. Association between hemochromatosis genotype and lead exposure among elderly men: the normative aging study. Environ Health Perspect 112(6): 746-750.

Wright RO, Tsaih SW, Schwartz J, Wright RJ, Hu H. 2003. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. J Pediatr 142(1): 9-14.

CHAPTER IV

Hemochromatosis and Transferrin Genotype Modification of Lead Biomarker Effects upon Infant Neurodevelopment

Abstract

Background: Critical periods of infant neurodevelopment influenced by environmental events irrefutably alter the neuronal architecture leading to permanent developmental disability. Gene-environment interactions which accelerate neuronal destruction are of particular interest for identifying new susceptible aging populations, but little work has been done on potential interactions in young populations. We examined the modifying effect of the hemochromatosis gene (*HFE*) *C282Y* and *H63D* variants and transferrin gene (*TF*) *P570S* variants upon the impact of lead exposure on infant neurodevelopment at 24 months.

Methods: This was a pilot study of 203 mother/infant pairs nested within a larger birth cohort living in Mexico City. Bayley Scales of Infant Development-II were administered at 24 months as the primary outcome of interest. Multiple linear regression models, stratified by either infant/maternal *HFE* or *TF* genotype and interaction terms models, were constructed examining the relationship of infant

mental development at 24 months and biomarkers of lead exposure after controlling for maternal age, maternal IQ, calcium supplementation, gestational age, marital status, parity, child hemoglobin levels at 24 months, and infant sex.

Results: Our final study population included 199 genotyped children, of which, 4.0%, 13.6%, and 19.3% carried the *HFE C282Y, HFE H63D*, and *TF P570S* variants, respectively. The presence of the infant or maternal *HFE H63D* variant genotype in separate MLR models predicted a 5.88 point (95%CI: -0.13 to 11.89) and 3.68 point (95%CI: -1.42, 8.78) increase in 24-month MDI score, respectively. Interaction terms between umbilical cord blood or tibia bone lead and presence of the either the maternal or infant *HFE H63D* variant genotype were consistently positive (i.e., lower adverse lead effect in carriers), but failed to reach statistical significance at the 0.05 level.

Conclusions: Although not statistically significant, these pilot findings suggest that infant *HFE H63D* genotype may attenuate lead's adverse effects upon MDI scores. This preliminary study is being followed up by a larger study that will address our sample size limitations.

Introduction

Lead continues to be one of the most prevalent neurotoxic environmental contaminants worldwide (Fewtrell et al. 2004). Increasing informal sector exposure (i.e., home-based lead smelting) in developing countries, the continued use in many consumer products, and the legacy left behind from past sources

(such as lead-based paint) will continue to impact global populations well into the future (Meyer et al. 2008).

It has been extensively documented that lead exposure has significant adverse effects upon both neurodevelopment and neurodegeneration (Schwartz et al. 2000; Weisskopf and Myers 2006; Lanphear et al. 2005; Grandjean and Landrigan 2006; Bellinger 2008; Wigg 2001; Shih et al. 2007). Research has demonstrated that adverse early neurodevelopmental effects are associated with infant blood lead levels (BLLs) < 10 μg/dL (Lanphear et al. 2005; Tellez-Rojo et al. 2006) and independently with cumulative maternal bone lead levels (Gomaa et al. 2002). In aging populations, cognitive decline has been extensively studied and associated with both increasing blood (Wright et al. 2003a) and bone lead levels (Schwartz et al. 2000; Weisskopf et al. 2007). In a recent review, Shih et al. concluded that there is “sufficient evidence” for a causal relationship between lead exposure and decrements in adult cognitive function (Shih et al. 2007).

Critical periods of infant neurodevelopment influenced by environmental events can irrefutably alter the neuronal architecture leading to permanent developmental disability. In proposed mechanistic pathways, early environmental damage can drastically reduce the number of neurons in critical brain areas, thus leading to an earlier onset of neurodegenerative diseases (Landrigan et al. 2005). Gene-environment interactions that accelerate this neuronal destruction are of particular interest for identifying new susceptible aging populations, yet little work has been done to recognize how these gene-environment “risk” situations interplay with a developing neurologic system.

Recent research has linked common genetic polymorphisms in iron metabolism (*HFE* gene variants *C282Y* and *H63D*) as well as iron transport (*TF P570S*) to increased risk for neurodegenerative diseases, which has been hypothesized to result from free radical damage arising from increased unbound iron (Robson et al. 2004; Lee and Andersen 2006; Lehmann et al. 2006). Additionally, it has been shown that *HFE* polymorphisms may interact with environmental toxicants, such as cumulative bone lead, to enhance adverse effects upon mental function in older adults (Wang FT 2007).

Lead absorption has also been shown to be affected by mutations in *HFE*. Wright et. al. showed that older adult carriers of *HFE* variant genotype *H63D* or *C282Y* had lower blood, patella bone, and tibia bone lead stores when compared to wildtype individuals (Wright et al. 2004). Alternatively, it was demonstrated that both separate and joint effects of iron metabolism gene variants *HFE* and *TF* were associated with increased blood lead levels in Mexican children (Hopkins et al. 2008). Though these polymorphisms may alter lead absorption and interact with lead in older adults to enhance decline in mental functioning, evidence is lacking for how these polymorphisms affect fetal neurodevelopment.

Early developmental iron deficiency (6-24 months) can lead to irreversible changes in brain structure and function (Hokama et al. 2005; Lozoff and Georgieff 2006; Beard 2007; Akman et al. 2004). Early cognitive effects may be compounded when infants are both iron deficient and exposed to lead, as lead absorption is upregulated during iron deficiency (Wright et al. 1999; Wright et al. 2003b; Wolf et al. 2003). Consequently, genetic polymorphisms which lead to

increased iron absorption and availability of unbound iron may provide early protective effects on cognition.

In this exploratory study, we examined the potential modifying effect of the hemochromatosis gene (*HFE*) variants *C282Y* and *H63D,* and the transferrin gene (*TF*) variant *P570S* on the impact of lead exposure to infant neurodevelopment. We hypothesized that infant variant genotypes would improve 24-month MDI scores due to increased iron absorption. Furthermore, we hypothesized that lead's negative effects upon children's cognitive ability would be decreased, under adequate iron conditions, by the presence of at least one variant genotype (*HFE H63D, HFE C282Y* and/or *TF P570S*), since lead and iron compete for common absorption/transportation receptors.

Methods

Sample Population

Maternal/infant pairs were recruited between 1994 and 1995 from three hospitals in Mexico City which serve low to moderate income populations as part of a clinical trial to assess calcium supplementation on bone lead mobilization during lactation. Of the initial 1382 mothers who didn't fit any of the exclusion criteria which included factors that could interfere with maternal calcium metabolism, medical conditions that could cause low birth weight (<2000g), logistic reasons that would interfere with data collection (households living outside the metropolitan area), delivering a premature neonate (<37 weeks) or an infant with an Apgar score at 5 minutes of 6 or under, conditions requiring

placement in a neonatal intensive care unit, a physician's diagnosis of multiple fetuses, intention not to breastfeed, preeclampsia, psychiatric, kidney, or cardiac diseases, gestational diabetes, history of repeated urinary infections, family or personal history of kidney stone formation, seizure disorder requiring daily medication, ingestion of corticosteroids or blood pressure >140mmHg systolic or >90mmHg diastolic, 617 agreed to participate. Of those who participated, 411 infants and 565 mothers had blood that was genotyped.

The study protocol was approved by the Ethics Committee of the National Institute of Public Health of Mexico, the participating hospitals, the Brigham and Women's Hospital, and the Harvard School of Public Health. All participating mothers received a detailed explanation of the study intent, research procedures, as well as counseling on how to reduce environmental lead exposure.

Blood Lead Measurements

All participant blood samples were collected in trace metal-free tubes (BD Vacutainer® #368381, Becton-Dickinson, Franklin Lakes, NJ) at delivery, 12, and 24 months. Blood samples were subsequently analyzed using an atomic absorption spectrometry instrument (Perkin-Elmer 3000, Chelmsford, MA, USA) at the metals laboratory of the American British Cowdray (ABC) Hospital in Mexico City. External blinded quality-control samples were provided throughout the study period by the Maternal and Child Health Bureau (MCHB) and the Wisconsin State Laboratory of Hygiene Cooperative Blood Lead Proficiency Testing Program (WSLH PBPTP).

Bone Lead Measurements

In vivo maternal bone lead measurements were obtained within one month of delivery (+/- 5 days), using a spot-source ^{109}Cd K-XRF instrument, at two bone sites, the mid-tibial shaft (representing cortical bone) and the patella (representing trabecular bone). The physical principles, technical specifications, and validation of this and other similar K-XRF instruments have been described in detail elsewhere (Aro et al. 1994). In this study, 30-minute measurements were taken at the midshaft of the left tibia and the left patella. For purposes of quality control, bone lead measurements with uncertainty estimates greater than 10 and 15 μg/g bone lead were excluded for tibia and patella, respectively.

Assessment of Child Development

The Bayley Scales of Infant Development-Second Edition (BSID-II) is a revision and restandardization of the BSID, the most widely used test of infant development. The revised scale can be used to assess the development of children between the ages of 1 and 42 months. Scores have been shown to be sensitive to a variety of prenatal, perinatal, and postnatal insults, including lead exposure (Tellez-Rojo et al. 2006; Gomaa et al. 2002). The BSID-II has also been used in numerous cross-cultural studies of lead and child development and a Spanish version of the BSID-II was developed by our research group before this study. The team that administered the BSID-II Spanish Version was led and trained by our group (Drs. Schnaas and Bellinger, respectively), with

standardization and quality control checks conducted through reviews of videotaped interviews. Mental Development Index (MDI) scores at 24 months of age were used as the primary child development endpoints in this study. Maternal IQ was assessed using the Information, Comprehension, Similarities, and Block Design components of the Wechsler Adult Intelligence Score, which has been translated into Spanish and used in Mexico.

Genotyping Methods

DNA extraction and genotyping were performed in The Harvard-Partners Center for Genetics and Genomics. High-molecular-weight DNA was extracted with commercially available PureGene Kits (Gentra Systems, Minneapolis, MN) from the white blood cells of archived maternal and umbilical cord blood samples. Genotyping for the *HFE C282Y* (RS1800562), *HFE H63D* (RS1799945) and *TF P570S* (RS1049296) variants was performed using Sequenom MALDI-TOF (Matrix-assisted laser desorption ionization – time of flight) mass spectrometry according to methods outlined in previous publications by our study group (Hopkins et al. 2008).

Statistical Analyses

Descriptive statistics and identification of outliers, using the generalized extreme studentized deviation (ESD) method (Rosner, 1983) were performed. Distribution of *HFE* and *TF* alleles and genotypes were examined and frequencies were tested using a chi-square statistic to compare observed and

expected counts according to principles of Hardy-Weinberg equilibrium. Individuals with heterozygous variant genotypes (CY, HD, and PS) were compared separately to participants with wild-type genotypes. Three infant and one maternal homozygous *HFE H63D* individuals (DD), as well as two infant and one maternal compound heterozygous individuals (CY HD), were removed from analysis due to population studies that demonstrate those individuals may have significantly increased iron levels when compared to carrier individuals (Jackson et al. 2001). Demographic characteristics, mental development index (MDI), bone and blood lead levels by inclusion and exclusion criteria were examined, then mean differences were tested by chi-square or Student's *t*-test (2-tailed) as appropriate. Potential non-linearity between continuous predictor variables and MDI at 24 months were explored by plotting generalized additive models (GAM) using R 2.9.1 (The R Foundation for Statistical Computing) software that included smoothing parameters for continuous variables.

Multiple linear regression (MLR) was then used to model the relationship between MDI at 24 months, maternal and infant *HFE H63D* and *TF P570S* genotypes, and biomarkers of lead exposure, after controlling for potential confounding variables. The potential confounding variables considered in our model were based on biologic plausibility or those significantly associated with MDI at 24 months ($p<0.1$) in bivariate analysis; variables included were: maternal age at delivery (years), maternal IQ, gestational age (weeks), maternal calcium supplementation during breast feeding (yes/no), infant gender (male gender as

reference group), parity (first birth/not first birth), marital status (single/partnered), and child hemoglobin at 24 months.

To examine the potential modifying effect of the variant genotypes we initially ran separate MLR models stratified by maternal or infant variant genotype. On the basis of the differences in effect estimates of the different lead biomarkers upon MDI at 24 months in stratified models, we fitted MLR models that included an interaction term between the variant genotype and lead biomarker.

Regression diagnostics were performed on all models to evaluate multicollinearity and violations of the linear regression model assumptions. Data were analyzed using SAS 9.2 (SAS Institute Inc., Cary, NC) 2002-2003 and R 2.9.1 (The R Foundation for Statistical Computing) 2007.

Results

Our final study population included 199 genotyped children, of which, 4.0%, 13.6%, and 19.3% carried the *HFE C282Y, HFE H63D*, and *TF P570S* variants, respectively. Additionally, 188 genotyped mothers were included in the final study population, and 3.2%, 18.6%, and 19.3% of these mothers carried the *HFE C282Y*, *HFE H63D*, and *TF P570S* variant genotype (Table IV-1). All genotype distributions were found to conform to Hardy-Weinberg equilibrium expectations. Due to small number of heterozygous *HFE C282Y* variant mothers (N=6) and infants (N=8) these results were not reported.

Table IV-2 shows mental development index (MDI), lead biomarker, and potential confounding variable mean and standard deviations for the final study population and those individuals excluded. Maternal arm circumference was slightly higher (p-value=0.07) in the final study population and there were slightly fewer males (p-value=0.10) when compared to the excluded participants. The average infant blood lead level at 24 months in our final study population was 7.9 µg/dL (SD 3.8) and there were 40 (20.0%) infants with a blood lead level exceeding 10 µg/dL. We had 25 (10.3%) anemic infants based on the CDC recommendations of <12.0 g/dL hemoglobin for 1-2 year olds residing at 7,000-7,999 feet (CDC 1998). Results from generalized additive models indicated that the dose-response relationship for lead and iron biomarkers, as well as for continuous covariates of interest, were all linear.

After controlling for potential confounding variables, a unit increase in either log transformed cord blood or tibia bone lead (µg/g) was associated with a 4.48 point (95%CI -8.345, -0.51) and 0.18 point (95%CI -0.38, 0.02) decrease in MDI at 24 months, respectively (Table IV-3). There were no significant relationships between MDI and either of our biomarkers of iron status (hemoglobin or serum ferritin) at 24 months, after controlling for potential confounding variables and cord blood lead.

In our main gene effect models, presence of the infant *HFE H63D* variant genotype predicted a 5.88 point (95%CI: -0.13 to 11.89) increase in 24-month MDI score after controlling for confounding variables (Table IV-4). Neither the maternal *HFE H63D* nor *TF P580S* variant genotype predicted a significant

relationship with infant MDI score at 24 months. These results did not significantly change if we controlled for serum ferritin instead of hemoglobin, or for tibia lead versus log transformed cord blood lead.

We then fitted MLR models that included an interaction term between the variant genotype and lead biomarkers. Interaction terms between umbilical cord blood or tibia bone lead and presence of the either the maternal or infant *HFE H63D* variant genotype were consistently positive (i.e., lower adverse lead effect in carriers), though failed to reach statistical significance at the 0.05 level (Table IV-5). The interaction term for infant *HFE H63D* x tibia bone lead was 0.44 (95%CI -0.09, 0.96: p-value=0.10).

Discussion

Our research indicates that the presence of the infant *HFE H63D* variant genotype predicts an increase in mental development at 24 months of 5.88 points (95%CI -0.13, 11.89) after adjusting for potential confounding variables including biomarkers of lead and iron. Furthermore, this preliminary research suggests that the presence of the infant *HFE H63D* variant genotype may provide a protective effect against the negative effects of cumulative bone lead upon MDI at 24 months. To our knowledge this study is the first to observe both main effects of *HFE H63D* genotype status upon MDI as well as a suggested effect modification of lead exposure and MDI by *HFE H63D* genotype status.

This pilot study has several important limitations to acknowledge. The primary aim of the original study was to investigate the impacts of prenatal and

postnatal lead exposure on infant neurodevelopment (Gomaa et al. 2002), and a focus on gene-environment interactions with polymorphisms in the iron regulatory/transport pathway was only an exploratory aim. Given the small number of subjects who carry an either the infant or maternal *HFE H63D* variant genotype (n=27 and n=35 respectively), *HFE C282Y* variant (n=8 and n=6 respectively), or *TF P570S* variant (n=38 and n=36) these results should be considered preliminary, since the protective effects of the infant *HFE H63D* genotype upon lead's decrease of infant mental development at 24 months maybe due to chance. This is critically important for the interpretation of any results of our interaction models. There are several important confounders in our study for which we did not have adequate or any information on such as, direct measures of family socioeconomic status, or home environment. We chose to use hemoglobin at 24 months as a potential confounder of iron status upon MDI instead of serum ferritin due to a consistently better model fit. We were unable to take additional measures of iron status, such as mean corpuscular volume or free erythrocyte protoporphyrin, in these infants and any maternal markers of iron status during pregnancy due to logistical and financial reasons. Without multiple proper measures of iron status we were unable to assess potential iron deficiency anemia and iron deficiency without anemia in our population.

There is strong animal and epidemiological evidence to indicate that early developmental iron deficiency (6-24 months) can lead to irreversible changes in brain structure and function (Lozoff and Georgieff 2006; Beard 2007; McCann and Ames 2007; Lozoff 2007). In our study population only 25 (10.3%) infants

were anemic (<11.0 g/dL Hgb) at 24 months and we lacked multiple biomarkers of iron status which precludes any meaningful interpretation between cognition, anemia, and iron status. It has also been documented that adverse early neurodevelopmental effects are associated with infant blood lead levels (BLLs) < 10 μg/dL (Lanphear et al. 2005; Tellez-Rojo et al. 2006) and independently with cumulative maternal bone lead levels (Gomaa et al. 2002). These early detrimental effects upon infant cognition may be compounded when infants are both iron deficient and exposed to lead, as lead absorption is upregulated during iron deficiency (Wright et al. 1999; Wright et al. 2003b; Wolf et al. 2003; Bradman et al. 2001). Alternatively, supplementation with iron has been shown to reduce lead body burdens (Choi and Kim 2003; Hammad et al. 1996; Kim et al. 2003) and to reduce brain lead levels and damage to the blood brain barrier (Wang et al. 2007; Bressler et al. 2007).

Previous associations between increased lead uptake during iron deficient conditions have recognized that both lead and iron compete for the common DMT-1 transporter (Bressler et al. 2004; Bannon et al. 2002). Evidence of this interaction has been shown in yeast and mammalian cell lines with lead and iron both using DMT-1 during intestinal absorption (Bannon et al. 2002). Iron binds with more affinity to DMT-1 than lead suggesting that under conditions of higher iron concentration lead absorption through this system might be restricted (Garrick et al. 2006). In support of this, lead transport through DMT-1 was found to be 80% inhibited by a 25 fold increase in iron (Bannon et al. 2002).

Sensing sufficient iron body stores is critical to proper iron homeostasis. When this system is disrupted, altered expression of transporters, such as DMT-1 and ferroportin, results. Gao et al. showed that the TfR2/HFE/Tf complex is intimately involved in sensing Tf saturation which in turn regulates hepcidin expression and ultimately breakdown of ferroportin (Gao et al. 2009). In addition, it has been shown that when HFE is dissociated from TfR1, hepcidin production is stimulated (Schmidt et al. 2008; Ganz 2008). It has been postulated that when these studies are put together transferrin interacts with TfR1 which in turn frees HFE to interact with TfR2 resulting in increased hepcidin production and subsequent breakdown of ferroportin (Fleming 2009). Mutations in the *HFE* gene have been shown to disrupt the binding of HFE to TfR1 (Schmidt et al. 2008) and TfR2/HFE Tf-dependent regulation of hepcidin expression (Gao et al. 2009), thus leading to more iron bound transferrin entering cells and increased ferroportin-mediated iron import (Ganz 2006). A secondary feature *HFE* mutations is that expression of DMT-1 is subsequently increased (Ganz 2007).

While existing evidence for *HFE* modification of lead body burden is inconsistent and conflicting, many of the studies were unable to provide an accurate picture of internal iron status (Wright et al. 2004; Hopkins et al. 2008; Barton et al. 1994) or did not have enough power to perform meaningful statistical testing (Akesson et al. 2000). Wang et al. recently showed in an elderly male population that *HFE* variant genotypes had an enhanced effect upon lead induced cognitive decline suggesting the importance of these polymorphisms as potential effect modifiers (Wang FT 2007). As far as we know

our study is the only to date investigating *HFE* effect modification upon lead exposure and infant neurocognitive development.

In summary, we have found the infant *HFE H63D* polymorphism may modify the association between lead burden and infant mental development by attenuating lead's negative effects. Additionally, after controlling for biomarkers of iron and lead, infants who carry a *HFE H63D* variant have higher BSID-II scores. It is plausible to speculate that though mutations in *HFE* may lead to increased body burdens of lead, especially in iron deficient children, under adequate iron conditions internal transport of lead through DMT-1 may be hindered thus leading to more positive health outcomes such as infant cognition. Results from this study will need to be validated, utilizing a larger population with more comprehensive markers of iron status.

Table IV-1: Maternal and Infant *HFE* and *TF* Genotype Frequencies

Infant Genotype Frequencies			Maternal Genotype Frequencies		
Genotype	**Number**	**%**	**Genotype**	**Number**	**%**
HFE (C282Y)			**HFE (C282Y)**		
C282Y Wildtype (CC)	191	96.0	C282Y Wildtype (CC)	182	96.8
C282Y Heterozygous (CY)	8	4.0	C282Y Heterozygous (CY)	6	3.2
C282Y Homozygous (YY)	0	0	C282Y Homozygous (YY)	0	0
Hardy–Weinberg equilibrium: $\chi 2 = 0.08$, $p = 0.77$			Hardy–Weinberg equilibrium: $\chi 2 = 0.05$, $p = 0.82$		
HFE (H63D)			**HFE (H63D)**		
H63D Wildtype (HH)	169	84.9	H63D Wildtype (HH)	152	80.9
H63D Heterozygous (HD)	27	13.6	H63D Heterozygous (HD)	35	18.6
H63D Homozygous (DD)	3	1.5	H63D Homozygous (DD)	1	0.5
Hardy–Weinberg equilibrium: $\chi 2 = 2.31$, $p = 0.13$			Hardy–Weinberg equilibrium: $\chi 2 = 0.45$, $p = 0.50$		
TF (P570S)			**TF (P570S)**		
P570S Wildtype (PP)	158	80.2	P570S Wildtype (PP)	150	80.7
P570S Heterozygous (PS)	38	19.3	P570S Heterozygous (PS)	36	19.3
P570S Homozygous (SS)	1	0.5	P570S Homozygous (SS)	0	0
Hardy–Weinberg equilibrium: $\chi 2 = 0.65$, $p = 0.42$			Hardy–Weinberg equilibrium: $\chi 2 = 2.14$, $p = 0.14$		

Table IV-2: Characteristics of the Study Population of Mother-Infant Pairs

	Included		Not Included		
	No	Mean ± SD	No	Mean ± SD	P-value
Maternal Characteristics					
Age (years)	203	24.7 ± 5.3	420	24.4 ± 5.0	0.50
Education (years)	203	9.5 ± 3.1	405	9.3 ± 3.0	0.22
Maternal IQ	203	85.1 ± 23.1	279	84.4 ± 24.5	0.48
Cigarette smoking during pregnancy (%)	202	3.0%	365	5.1 %	0.23
Maternal Arm Circumference at Delivery	195	26.7 ± 2.9	406	26.3 ± 2.8	0.07
Primiparity (%)	203	54.7%	414	57.5 %	0.51
Calcium Supplementation	203	49.8%	402	48.3%	0.73
Marital Status (% Not Married)	203	7.9%	414	9.4%	0.53
Tibia Bone Lead (ug/g)	197	10.5 ± 10.3	405	9.7 ± 9.8	0.40
Patella Bone Lead (ug/g)	184	15.8 ± 15.3	391	14.3 ± 15.4	0.27
Infant Characteristics\					
Mental Development Index at 12 Months	172	99.9 ± 9.4	209	99.7 ± 9.3	0.80
Mental Development Index at 24 Months	203	91.3 ± 14.3	137	92.3 ± 13.6	0.43
Gestational Age (days)	203	39.2 ± 1.3	410	39.2 ± 1.6	0.74
Birthweight (grams)	203	3150.6 ± 427.9	417	3124.3±411.9	0.49
Sex (% Male)	203	49.8%	414	56.9 %	0.10
Infant Hemoglobin at 24 Months (g/dL)	203	12.4 ± 1.1	68	12.2 ± 1.4	0.55
Infant Ferritin at 24 Months (µg/L)	187	17.0 ± 13.0	71	16.5 ± 14.0	0.28
Infant Blood Lead 24 Months (µg/dL)	200	7.9 ± 3.8	106	8.8 ± 5.2	0.14
Umbilical Cord Blood Lead (µg/dL)	203	6.6 ± 3.4	315	6.6 ± 3.6	0.71

Table IV-3: Adjusted parameter estimates for 24 month MDI and biomarkers lead and iron exposure in separate linear regression models†

Lead Biomarkers[a]	N	Beta Coef.	95% CI
Cord Blood Lead (µg/dL)^	200	-4.48**	-8.45, -0.51
Child Blood Lead 24 months (µg/dL)^	197	-2.31	-6.71, 2.09
Maternal Tibia Bone Lead (µg/g)	194	-0.18*	-0.38, 0.02
Tibia Bone Lead (quartiles)			
Q1 (<1 – 4.4)		Ref	
Q2 (4.4 – 9.7)		3.13	-2.30, 8.55
Q3 (9.7 – 15.3)		-2.45	-8.05, 3.15
Q4 (15.3 – 43.2)		-3.29	-8.99, 2.42
		p-trend: 0.09	
Maternal Patella Bone Lead (µg/g)	181	-0.11	-0.26, 0.04
Patella Bone Lead (quartiles)			
Q1 (<1 – 4.9)		Ref	
Q2 (4.9 – 15.0)		-3.78	-9.57, 2.02
Q3 (15.0 – 24.4)		-3.74	-9.50, 2.02
Q4 (24.4 – 50.1)		-2.75	-8.60, 3.10
		p-trend: 0.37	
Iron Biomarkers[b]			
Child Hemoglobin at 24 months (g/dL)	200	-0.36	-2.20, 1.49
Child Ferritin at 24 months (µg/L)^	186	0.12	1.63, 1.88
Ferritin at 24 months (quartiles)			
Q1 (0.5 – 8.1)		Ref	
Q2 (8.1 – 13.6)		1.07	-4.61, 6.75
Q3 (13.6 – 22.3)		-0.28	-5.53, 4.97
Q4 (22.3 – 53.1)		0.88	-4.74, 6.50
		p-trend: 0.90	
Infant Anemic at 24 months (<12.0 g/dL Hgb: N=25, 10.3%)	202	1.65	-2.58, 5.87
Infant Ferritin at 24 months (<12 µg/L: N=94, 40.3%)	186	0.98	-3.12, 5.08

†All models are adjusted for maternal age, maternal intelligence, infant gender, gestational age, calcium supplementation, parity, and marital status

[a]All models are additionally adjusted for child hemoglobin at 24 months

[b]All models are additionally adjusted for tibia bone lead

^Log Transformed ** P-value < 0.05, * P-value < 0.1

Table IV-4: Adjusted parameter estimates for 24 month MDI score by *HFE H63D* and *TF P570S* genotype status, in separate linear regression models†

Variable	N	Beta Coef.	95% CI
Infant H63D HFE	191	5.88*	-0.13, 11.89
Infant P570S Tf	193	1.60	-3.36, 6.56
Maternal H63D HFE	183	3.68	-1.42, 8.78
Maternal P570S Tf	183	-0.01	-4.97, 4.95

†All models are adjusted for maternal age, maternal intelligence, infant gender, gestational age, child hemoglobin at 24 months, cord blood lead, calcium supplementation, parity, and marital status.
** P-value < 0.05, * P-value < 0.1

Table IV-5: Adjusted regression coefficients of *HFE H63D* and *TF P570S* genotype, lead biomarker, and the interaction term in association with mental development at 24 months†

	Cord Blood Lead			Tibia Bone Lead			Patella Bone Lead		
Genotype	Genotype Variant	Cord Lead	Interaction Term	Genotype Variant	Tibia Lead	Interaction Term	Genotype Variant	Patella Lead	Interaction Term
Infant H63D HFE	-9.0	-5.5**	8.7	6.5**	-0.22*	0.44*	5.3	-0.13	-0.07
Infant P570S Tf	-14.2	-5.4**	9.3	2.6	-0.11	-0.20	1.6	-0.24**	0.34*
Maternal H63D HFE	-6.9	-5.4**	6.3	4.4*	-0.21*	0.12	3.3	-0.09	0.04
Maternal P570S Tf	-16.3	-5.7**	8.9	0.38	-0.18*	-0.05	-0.83	-0.10	0.04

†All models are adjusted for maternal age, maternal intelligence, infant gender, gestational age, child hemoglobin at 24 months, calcium supplementation, parity, and marital status.
* P-value <0.1, ** P-value<0.05

Literature Cited

Akesson A, Stal P, Vahter M. 2000. Phlebotomy increases cadmium uptake in hemochromatosis. Environ Health Perspect 108(4): 289-291.

Akman M, Cebeci D, Okur V, Angin H, Abali O, Akman AC. 2004. The effects of iron deficiency on infants' developmental test performance. Acta Paediatr 93(10): 1391-1396.

Aro AC, Todd AC, Amarasiriwardena C, Hu H. 1994. Improvements in the calibration of 109Cd K x-ray fluorescence systems for measuring bone lead in vivo. Physics in medicine and biology 39(12): 2263-2271.

Bannon D, Portnoy ME, Olivi L, Lees PS, Culotta VC, Bressler JP. 2002. Uptake of lead and iron by divalent metal transporter 1 in yeast and mammalian cells. Biochem Biophys Res Commun 295(4): 978-984.

Barton JC, Patton MA, Edwards CQ, Griffen LM, Kushner JP, Meeks RG, et al. 1994. Blood lead concentrations in hereditary hemochromatosis. J Lab Clin Med 124(2): 193-198.

Beard J. 2007. Recent evidence from human and animal studies regarding iron status and infant development. The Journal of nutrition 137(2): 524S-530S.

Bellinger DC. 2008. Very low lead exposures and children's neurodevelopment. Current opinion in pediatrics 20(2): 172-177.

Bradman A, Eskenazi B, Sutton P, Athanasoulis M, Goldman LR. 2001. Iron deficiency associated with higher blood lead in children living in contaminated environments. Environ Health Perspect 109(10): 1079-1084.

Bressler JP, Olivi L, Cheong JH, Kim Y, Bannona D. 2004. Divalent metal transporter 1 in lead and cadmium transport. Annals of the New York Academy of Sciences 1012: 142-152.

Bressler JP, Olivi L, Cheong JH, Kim Y, Maerten A, Bannon D. 2007. Metal transporters in intestine and brain: their involvement in metal-associated neurotoxicities. Hum Exp Toxicol 26(3): 221-229.

CDC. 1998. Recommendations to prevent and control iron deficiency in the United States. . MMWR Recomm Rep 47(RR-3): 1-29.

Choi JW, Kim SK. 2003. Association between blood lead concentrations and body iron status in children. Arch Dis Child 88(9): 791-792.

Fewtrell LJ, Pruss-Ustun A, Landrigan P, Ayuso-Mateos JL. 2004. Estimating the global burden of disease of mild mental retardation and cardiovascular diseases from environmental lead exposure. Environ Res 94(2): 120-133.

Fleming RE. 2009. Iron sensing as a partnership: HFE and transferrin receptor 2. Cell Metab 9(3): 211-212.

Ganz T. 2006. Hepcidin and its role in regulating systemic iron metabolism. Hematology Am Soc Hematol Educ Program: 29-35.

Ganz T. 2007. Molecular control of iron transport. J Am Soc Nephrol 18(2): 394-400.

Ganz T. 2008. Iron homeostasis: fitting the puzzle pieces together. Cell Metab 7(4): 288-290.

Gao J, Chen J, Kramer M, Tsukamoto H, Zhang AS, Enns CA. 2009. Interaction of the hereditary hemochromatosis protein HFE with transferrin receptor 2 is required for transferrin-induced hepcidin expression. Cell Metab 9(3): 217-227.

Garrick MD, Singleton ST, Vargas F, Kuo HC, Zhao L, Knopfel M, et al. 2006. DMT1: which metals does it transport? Biol Res 39(1): 79-85.

Gomaa A, Hu H, Bellinger D, Schwartz J, Tsaih SW, Gonzalez-Cossio T, et al. 2002. Maternal bone lead as an independent risk factor for fetal neurotoxicity: a prospective study. Pediatrics 110(1 Pt 1): 110-118.

Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. Lancet 368(9553): 2167-2178.

Hammad TA, Sexton M, Langenberg P. 1996. Relationship between blood lead and dietary iron intake in preschool children. A cross-sectional study. Ann Epidemiol 6(1): 30-33.

Hokama T, Gushi Ken M, Nosoko N. 2005. Iron deficiency anaemia and child development. Asia Pac J Public Health 17(1): 19-21.

Hopkins MR, Ettinger AS, Hernandez-Avila M, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, et al. 2008. Variants in iron metabolism genes predict higher blood lead levels in young children. Environ Health Perspect 116(9): 1261-1266.

Kim HS, Lee SS, Hwangbo Y, Ahn KD, Lee BK. 2003. Cross-sectional study of blood lead effects on iron status in Korean lead workers. Nutrition 19(7-8): 571-576.

Landrigan PJ, Sonawane B, Butler RN, Trasande L, Callan R, Droller D. 2005. Early environmental origins of neurodegenerative disease in later life. Environ Health Perspect 113(9): 1230-1233.

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, et al. 2005. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 113(7): 894-899.

Lee DW, Andersen JK. 2006. Role of HIF-1 in iron regulation: potential therapeutic strategy for neurodegenerative disorders. Curr Mol Med 6(8): 883-893.

Lehmann DJ, Worwood M, Ellis R, Wimhurst VL, Merryweather-Clarke AT, Warden DR, et al. 2006. Iron genes, iron load and risk of Alzheimer's disease. Journal of medical genetics 43(10): e52.

Lozoff B, Georgieff MK. 2006. Iron deficiency and brain development. Semin Pediatr Neurol 13(3): 158-165.

Lozoff B. 2007. Iron deficiency and child development. Food and nutrition bulletin 28(4 Suppl): S560-571.

McCann JC, Ames BN. 2007. An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 85(4): 931-945.

Meyer PA, Brown MJ, Falk H. 2008. Global approach to reducing lead exposure and poisoning. Mutat Res 659(1-2): 166-175.

Robson KJ, Lehmann DJ, Wimhurst VL, Livesey KJ, Combrinck M, Merryweather-Clarke AT, et al. 2004. Synergy between the C2 allele of transferrin and the C282Y allele of the haemochromatosis gene (HFE) as risk factors for developing Alzheimer's disease. Journal of medical genetics 41(4): 261-265.

Schmidt PJ, Toran PT, Giannetti AM, Bjorkman PJ, Andrews NC. 2008. The transferrin receptor modulates Hfe-dependent regulation of hepcidin expression. Cell Metab 7(3): 205-214.

Schwartz BS, Stewart WF, Bolla KI, Simon PD, Bandeen-Roche K, Gordon PB, et al. 2000. Past adult lead exposure is associated with longitudinal decline in cognitive function. Neurology 55(8): 1144-1150.

Shih RA, Hu H, Weisskopf MG, Schwartz BS. 2007. Cumulative lead dose and cognitive function in adults: a review of studies that measured both blood lead and bone lead. Environ Health Perspect 115(3): 483-492.

Tellez-Rojo MM, Bellinger DC, Arroyo-Quiroz C, Lamadrid-Figueroa H, Mercado-Garcia A, Schnaas-Arrieta L, et al. 2006. Longitudinal associations between blood lead concentrations lower than 10 microg/dL and neurobehavioral development in environmentally exposed children in Mexico City. Pediatrics 118(2): e323-330.

Wang FT HH, Schwartz J, Weuve J, Spiro AS III, Sparrow D, Nie H, Silverman EK, Weiss ST, Wright RO. . 2007. Modifying Effects of the HFE Polymorphisms on the Association between Lead Burden and Cognitive Decline. Environ Health Perspect.

Wang Q, Luo W, Zheng W, Liu Y, Xu H, Zheng G, et al. 2007. Iron supplement prevents lead-induced disruption of the blood-brain barrier during rat development. Toxicol Appl Pharmacol 219(1): 33-41.

Weisskopf MG, Myers G. 2006. Cumulative effect of lead on cognition: is bone more revealing than blood? Neurology 67(9): 1536-1537.

Weisskopf MG, Proctor SP, Wright RO, Schwartz J, Spiro A, 3rd, Sparrow D, et al. 2007. Cumulative lead exposure and cognitive performance among elderly men. Epidemiology 18(1): 59-66.

Wigg NR. 2001. Low-level lead exposure and children. J Paediatr Child Health 37(5): 423-425.

Wolf AW, Jimenez E, Lozoff B. 2003. Effects of iron therapy on infant blood lead levels. J Pediatr 143(6): 789-795.

Wright RO, Shannon MW, Wright RJ, Hu H. 1999. Association between iron deficiency and low-level lead poisoning in an urban primary care clinic. Am J Public Health 89(7): 1049-1053.

Wright RO, Silverman EK, Schwartz J, Tsaih SW, Senter J, Sparrow D, et al. 2004. Association between hemochromatosis genotype and lead exposure among elderly men: the normative aging study. Environ Health Perspect 112(6): 746-750.

Wright RO, Tsaih SW, Schwartz J, Spiro A, 3rd, McDonald K, Weiss ST, et al. 2003a. Lead exposure biomarkers and mini-mental status exam scores in older men. Epidemiology 14(6): 713-718.

Wright RO, Tsaih SW, Schwartz J, Wright RJ, Hu H. 2003b. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. J Pediatr 142(1): 9-14.

CHAPTER V

Conclusions

Lead exposure still poses a significant public health issue worldwide. Though there has been a vast amount of research into associated health effects arising from lead toxicity, especially neurodevelopmental outcomes, we are just now beginning to understand the impact of prenatal lead exposure upon the developing fetus, which has been a main focus of this dissertation. One critical limitation to many of the lead/health outcome studies reported to date has been the reliance on umbilical cord blood or maternal blood lead as an evaluation of lead exposure. In this thesis we provide evidence that lead exposure early in gestation, as reflected by either 1st trimester blood or plasma lead, may better predict length of gestation, a previously studied but inconclusive health outcome. Results from this thesis and other recent studies signal the importance of assessing different fetal “windows of susceptibility” to lead exposure.

Many recent environmental epidemiologic studies have illustrated that common genetic polymorphisms in our population can alter susceptibility to toxicants. Interpersonal genetic differences which can impact absorption, distribution, metabolism, and excretion of environmental toxicants are potentially

important modifiers to help explain the large variation and inconsistency in health outcome data. Interestingly, there have been few gene-environment studies which have assessed altered susceptibility to lead toxicity within the context of maternal-fetal unit. In this thesis we explored the potential modifying effect of both infant and maternal polymorphisms in the iron regulatory (*HFE H63D* and *C282Y*) and iron transport system (*TF P570S*) upon prenatal lead's adverse association with birthweight and infant mental development. Though the gene-environment studies in this thesis were limited by the sample size of the population, the patterns we found suggested that maternal polymorphisms may modify lead toxicity to the fetus differently from infant polymorphisms.

Chapter 2 Conclusions

In chapter two we found that lead's adverse effects as reflected by plasma or whole blood lead levels upon gestational length were strongest and most significant during the first trimester when compared to effects found in the second and third trimesters. In addition, we found that plasma lead, a better biomarker of the fraction of unbound circulating lead, independently predicted the same adverse association with length of gestation as whole blood lead in our population. Our results were inconclusive with regards to cumulative lead exposure biomarkers (bone lead levels) and length of gestation. There are few prior published studies which have assessed lead exposure at multiple time points during gestation and associated these biomarkers with adverse birth

outcomes, which highlight an important strength and contribution to the scientific community of chapter two in this thesis.

Preterm delivery is a complex condition with a multifactorial etiology. Potential mechanisms of how lead exposure may impact preterm delivery are very unclear but several recent studies provide evidence to support a role of lead in altering the hypothalamic-pituitary-adrenal (HPA) axis as well as immune system function (Virgolini et al. 2005; Cory-Slechta et al. 2004; Dietert and Piepenbrink 2006).

Altering the trajectory of corticotropin-releasing hormone (CRH) release during partition has been suggested as one plausible mechanism which can lead to delivering an infant prematurely (Hobel et al. 1999; Leung et al. 2000; McLean and Smith 1999, 2001). Heightened maternal stress, a known risk factor in preterm delivery, is thought to act through the maternal/fetal HPA pathway by altering CRH release (Wadhwa et al. 2002). Studies have indicated that stress effects upon CRH levels may be more prominent early in gestation (Sandman et al. 2006). Lead exposure may play an important role in prematurity by increasing the overall baseline level of corticosterone (the biologic equivalent to cortisol in humans) in rats and heightening the response to acute stressors (Cory-Slechta et al. 2004; Cory-Slechta et al. 2008). Therefore, it is plausible to speculate that increased lead exposure early in gestation may alter CRH release alone or in concert with heightened maternal stress responses.

Maternal infection status is considered an aetiological factor in approximately 50% of all preterm deliveries (Laudanski et al. 2007; Goldenberg

et al. 2000). Lead exposure has been shown to alter humoral immune system function by suppressing and skewing antibody isotype production and subsequently increasing susceptibility to common bacterial infections, the most commonly studied pathogen being *Listeria monocytogenes* (Dietert and Piepenbrink 2006). It is hypothetical that chronic sustained lead exposure may increase susceptibly to bacterial vaginosis leading to increased risk for premature delivery.

Additional future directions for this research may involve assessing lead's effects upon stress mechanisms potentially involved in PTD, such as the 11β-hydroxysteroid dehydrogenase (*11βHSD2*) gene which breaks down maternal cortisol at the placenta, thus limiting fetal exposure to cortisol, an event thought to be involved in eliciting fetal HPA axis activity (Weinstock 2005). Alternatively, methodological issues such as maternal plasma volume expansion or infection status during pregnancy need to be addressed in future prenatal environmental epidemiological studies due to potential for differential exposure misclassification or confounding.

Chapter 3 and 4 Conclusions

In chapters 3 and 4 we assessed interpersonal variations in susceptibility to lead exposure by observing how the association between prenatal lead exposure and health outcomes (birthweight and infant mental development) may be altered by either maternal or infant polymorphisms in the iron regulatory (*HFE H63D* and *C282Y*) and transport system (*TF P570S*). In both chapters we found

that infant *HFE H63D* variants maybe protective against lead's adverse effects upon birthweight and mental development. Additionally, we found that the maternal *HFE H63D* polymorphism may enhance lead's negative effects upon birthweight. If confirmed in larger sample size populations, results from these two chapters highlight the importance of the iron regulatory and transport system in altering prenatal and neonatal susceptibility to lead exposure.

Previous associations between increased lead uptake during iron deficient conditions have recognized that both lead and iron compete for the common DMT-1 transporter (Bressler et al. 2004; Bannon et al. 2002). Iron binds with more affinity to DMT-1 than lead (Garrick et al. 2006) and subsequent epidemiological (Wright et al. 2003) and mechanistic studies (Bannon et al. 2002; Wang et al. 2006) indicate that under conditions of higher iron concentration lead absorption is restricted.

There are many mechanistic gaps in our understanding of the relationship between lead and iron and how polymorphisms in the iron regulatory and transport system may modify this relationship. To further compound this relationship, during pregnancy the interaction between fetus and mother genetics present complex problems for epidemiological investigations. It is plausible to speculate that though mutations in *HFE* may lead to increased body burdens of lead, especially in iron deficient women and children, under adequate iron conditions internal transport of lead through DMT-1 may be hindered thus leading to the more positive health outcomes such as infant cognition or birthweight we see in our studies. Alternatively, it has also been shown that excess iron can be

detrimental, leading to an increase in hydroxyl radicals, and may enhance the association between lead exposure and adverse health outcomes. Although we were unable to assess iron status during pregnancy in chapter 3, potentially mothers with *HFE* polymorphisms may have had higher iron levels which could have led to site-specific damage that was further compounded by lead exposure.

Studies in this thesis highlight the importance of the prenatal period when associating environmental toxicants with health outcomes. Controlling for maternal as well as infant interpersonal genetic variations and timing of exposure pose significant challenges for future research in this area.

LITERATURE CITED

Bannon D, Portnoy ME, Olivi L, Lees PS, Culotta VC, Bressler JP. 2002. Uptake of lead and iron by divalent metal transporter 1 in yeast and mammalian cells. Biochem Biophys Res Commun 295(4): 978-984.

Bressler JP, Olivi L, Cheong JH, Kim Y, Bannon D. 2004. Divalent metal transporter 1 in lead and cadmium transport. Annals of the New York Academy of Sciences 1012: 142-152.

Cory-Slechta DA, Virgolini MB, Rossi-George A, Thiruchelvam M, Lisek R, Weston D. 2008. Lifetime consequences of combined maternal lead and stress. Basic Clin Pharmacol Toxicol 102(2): 218-227.

Cory-Slechta DA, Virgolini MB, Thiruchelvam M, Weston DD, Bauter MR. 2004. Maternal stress modulates the effects of developmental lead exposure. Environ Health Perspect 112(6): 717-730.

Dietert RR, Piepenbrink MS. 2006. Lead and immune function. Crit Rev Toxicol 36(4): 359-385.

Faupel-Badger JM, Hsieh CC, Troisi R, Lagiou P, Potischman N. 2007. Plasma volume expansion in pregnancy: implications for biomarkers in population studies. Cancer Epidemiol Biomarkers Prev 16(9): 1720-1723.

Garrick MD, Singleton ST, Vargas F, Kuo HC, Zhao L, Knopfel M, et al. 2006. DMT1: which metals does it transport? Biol Res 39(1): 79-85.

Goldenberg RL, Hauth JC, Andrews WW. 2000. Intrauterine infection and preterm delivery. N Engl J Med 342(20): 1500-1507.

Hobel CJ, Arora CP, Korst LM. 1999. Corticotrophin-releasing hormone and CRH-binding protein. Differences between patients at risk for preterm birth and hypertension. Annals of the New York Academy of Sciences 897: 54-65.

Hu H, Tellez-Rojo MM, Bellinger D, Smith D, Ettinger AS, Lamadrid-Figueroa H, et al. 2006. Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. Environ Health Perspect 114(11): 1730-1735.

Laudanski P, Pierzynski P, Laudanski T. 2007. Reductionist and system approaches to study the role of infection in preterm labor and delivery. BMC Pregnancy Childbirth 7 Suppl 1: S9.

Leung TN, Chung TK, Madsen G, Lam CW, Lam PK, Walters WA, et al. 2000. Analysis of mid-trimester corticotrophin-releasing hormone and alpha-fetoprotein concentrations for predicting pre-eclampsia. Hum Reprod 15(8): 1813-1818.

McLean M, Smith R. 1999. Corticotropin-releasing Hormone in Human Pregnancy and Parturition. Trends Endocrinol Metab 10(5): 174-178.

McLean M, Smith R. 2001. Corticotrophin-releasing hormone and human parturition. Reproduction 121(4): 493-501.

Sandman CA, Glynn L, Schetter CD, Wadhwa P, Garite T, Chicz-DeMet A, et al. 2006. Elevated maternal cortisol early in pregnancy predicts third trimester levels of placental corticotropin releasing hormone (CRH): priming the placental clock. Peptides 27(6): 1457-1463.

Virgolini MB, Chen K, Weston DD, Bauter MR, Cory-Slechta DA. 2005. Interactions of chronic lead exposure and intermittent stress: consequences for brain catecholamine systems and associated behaviors and HPA axis function. Toxicol Sci 87(2): 469-482.

Wadhwa PD, Glynn L, Hobel CJ, Garite TJ, Porto M, Chicz-DeMet A, et al. 2002. Behavioral perinatology: biobehavioral processes in human fetal development. Regul Pept 108(2-3): 149-157.

Wang Q, Luo W, Zheng W, Liu Y, Xu H, Zheng G, et al. 2007. Iron supplement prevents lead-induced disruption of the blood-brain barrier during rat development. Toxicol Appl Pharmacol 219(1): 33-41.

Wang FT HH, Schwartz J, Weuve J, Spiro AS III, Sparrow D, Nie H, Silverman EK, Weiss ST, Wright RO. . 2007. Modifying Effects of the HFE Polymorphisms on the Association between Lead Burden and Cognitive Decline. Environ Health Perspect. 115(8): 1210-1215.

Weinstock M. 2005. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav Immun 19(4): 296-308.

Wright RO, Tsaih SW, Schwartz J, Wright RJ, Hu H. 2003. Association between iron deficiency and blood lead level in a longitudinal analysis of children followed in an urban primary care clinic. J Pediatr 142(1): 9-14.