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Does Low-Level Arsenic Exposure Predict Blood Pressure in Children?

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by

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Abstract

Background & Significance: Inorganic arsenic is a heavy metal naturally found in soil and human exposure to this heavy metal causes adverse effects. Exposure risks are particularly high in developing children. Arsenic is mainly found in bedrock. It is also a by-product of smelting, it is used in cement production, and historically is has been found in crop pesticides. Among other ill-effects, arsenic exposure in humans can cause respiratory diseases, peripheral neuropathy and liver fibrosis. In particular arsenic has been shown in many studies to be a contributing factor in the risk for hypertension in adults however no studies have yet examined possible associations between blood arsenic and blood pressure in children.

Aims & Objectives: The aim of this study was to investigate possible associations between arsenic and systolic and diastolic blood pressure in children. To examine whether possible effects were specific to arsenic, secondary analyses also tested associations between blood lead levels and blood pressure and blood cadmium levels and blood pressure.

Hypothesis: It was hypothesized that blood arsenic predicts systolic and diastolic blood pressure. Because previous studies have not been conducted, no direction of association was predicted.

Methods: This was an observational study of 204 children from two far west Texas rural communities. Children were recruited from two elementary schools and were between the ages of 4 and 11. Parents completed informed consent prior to testing and provided child and family demographic and medical information. Anthropometric measurements were taken for each child and blood pressure was automatically recorded using an electronic sphygmomanometer. Finger stick blood samples were collected, analyzed using Inductive-Coupled Plasma Spectrometry (ICP-MS) and tested for arsenic, lead, cadmium, and iron in whole blood. For the primary

analysis, a generalized linear model was calculated predicting systolic and diastolic blood pressure (in separate models) controlling for sex and age, with school included as a random effect.

Results: Blood arsenic level predicted decreased systolic blood pressure (F=8.19, p=.0047). For every 1 µg/dL of arsenic, child systolic blood pressure decreased by 3.99 mmHg. A positive association was found between blood cadmium level and blood pressure. Cadmium blood level in children predicted an association between cadmium and systolic blood pressure (F=5.45, p=.0205).

Conclusion: Low-level arsenic exposure as determined by blood arsenic level linearly predicts a decrease in blood pressure in young children. This might be explained by arsenic-induced over production of nitric oxide in the endothelium and interference of nitric oxide synthase leading to vasodilation. Moreover, low-level cadmium exposure as determined by blood cadmium levels predict hypertension (increased systolic and diastolic blood pressure) in young children. The effects might reflect secondary effects of cadmium on kidney function. These and other explanations for the findings are discussed. The findings provide further evidence that very low-level heavy metal exposure has detrimental effects on early critical physiological functions. Additional studies are needed to replicate and expand these findings.

Recommendations: Children must be consistently and frequently monitored for low detectable levels of heavy metal exposure. Although not the focus of this study, is should be noted that relatively high levels of prehypertension and hypertension were observed. Physical activity can improve metabolism which may promote toxin clearance in young children while also promoting normal blood pressure and lower body weight. Several physical activity programs currently in use throughout the country have been proposed and should be incorporated into vulnerable

children’s daily school regiments.

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Introduction

Heavy metal is a term that is given to any metallic chemical element that has high density and is toxic in high concentrations (Duruibe, 2007). The most common contaminating heavy metals found in the environment include: arsenic, lead, cadmium, and mercury. These chemical elements are all natural components of the earth's crust and cannot be degraded nor destroyed (ATSDR, 2007). Some heavy metals at minute concentrations are essential to the human body as they participate in metabolism function (NORD, 2006). However, at high concentrations, they too can lead to poisoning.

1.1 Arsenic

Arsenic is a heavy metal naturally found in soil and minerals and is number 33 on the periodic table. Although its chemical form can change, arsenic cannot be destroyed. A major source of arsenic since the 1950s was wood products. Lumber used to build porches, docks, and children's playgrounds was commonly treated with arsenic as a preserving agent (ATSDR, 2007). There were many other possible sources of arsenic in the soil and water.

1.2 Sources in the Environment

Arsenic is mainly found in bedrock, the solid rock below loose soil, which means that it can easily leach into the ground water and come to the surface, for example, through local wells or when coal is mined. Arsenic is also produced by smelting industries as a by-product of the smelting process (NIEHS, 2011) and can get into air through blown dust. It has also been suggested that a major industrial source of contaminating particulates and heavy metals including arsenic is cement production. Cement production produces these contaminants through fossil fuel combustion and raw material processing (Zhao, 2015).

Arsenic contaminated crops are also a source of arsenic exposure. Irrigation water that is

contaminated causes the absorption of arsenic by crops and increases the human exposure by consumption (WHO, 2012). Arsenic can be found in grains, fruits, and vegetables due to the absorption from soil and water. Most crops do not absorb high levels except for rice which can uptake arsenic from water and soil more easily than any other grain (FDA, 2014). Because arsenic is found in soil, minute amounts of arsenic can be found in foods like fruits and other produce. Studies show that the two forms of arsenic have been found in apple juice (FDA, 2013) and children are the major consumers of apple juice.

The US Environmental Protection Agency (EPA) has set an arsenic maximum contaminant level for drinking water. The standard for drinking water is 10 parts per billion (EPA, 2006). Although these regulations are followed in public water systems, in small villages where potable water is not received it may not be recognized or tested.

1.3 Sources of Exposure

Human exposure to arsenic can be through drinking water, food, dust, and air. Arsenic can either be inhaled or ingested. In rare cases it may be absorbed through breaks in the skin. Arsenic is classified as a carcinogen at high and lower doses; long term exposure to low levels of arsenic can also lead to cancer (NLM, 2013). Even below high or lethal doses, arsenic can cause lung, bladder, and skin cancer. It is also well known that higher doses of arsenic can cause skin lesions, bruising, muscle cramping, stomachaches (ACS, 2014), and eventually can become fatal. Arsenic has been associated with the occurrence of many other diseases as well. Some of these include: respiratory diseases, peripheral neuropathy, and liver fibrosis among others.

There are two forms of arsenic, organic and inorganic. Organic arsenic is common in seafood and is relatively harmless to humans. Inorganic arsenic however is highly toxic and is the form that must be avoided by humans. With regard to human exposure, the organic form of

arsenic usually comes from seafood and has low toxicity. Inorganic arsenic is found in the environment. Once absorbed, inorganic arsenic mimics phosphate ions in the human body. Inside cells, arsenic replaces phosphate ions that are needed to yield energy from sugar. The replacement of phosphate ions by arsenic causes cellular respiration to cease. Because of this process, at highest levels of exposure, arsenic begins to destroy major organs like the heart, brain, and nerves. At high levels arsenic becomes carcinogenic by interfering with glucocorticoid metabolism. Glucocorticoids regulate blood sugar and are tumor suppressors. These processes suggest one explanation as to why arsenic can cause cancer or increase the risk for diabetes (Kaltreider, 2001).

1.4 Arsenic Absorption Pathways

When the human body becomes exposed to the inorganic form of arsenic, the body metabolizes it by attaching carbon atoms to make it into its organic form. This process is known as methylation and is thought of as a natural detoxification process in humans, but it is also seen that arsenic silences tumor suppressor genes by methylation (Darthmouth Toxic Metals, 2012). When arsenic is absorbed by the human body, several other processes can occur. Arsenic is reduced to its metabolite form arsenite and become biomethylazed to form monomethylarsonic acid and dimethylarsinic which are the metabolites excreted by the kidneys found in the urine (Thompson, 1993). Most of the arsenic that is absorbed by the human body leaves the body in urine. The arsenic that does remain is usually stored in the bones, tissues or brain and can eventually cause serious damage (EHANS, 2002) including changes in cognitive function.

1.5 Child Exposure

Arsenic exposure has very different effects in children than in adults. The reason for this is because the developing body is not fully able to successfully excrete arsenic. As a result,

children are less efficient in detoxifying and excreting pollutants in general, and the health risks are higher in children.

Children are also more vulnerable to heavy metal contaminants due to their high hand-to-mouth activities. It is estimated that children insert their hand into their mouth at an average of 9.5 times per hour (Lanphear, 2005). Because of their height to weight ratio, children also ingest and inhale more dust, thus making them more vulnerable and giving them higher blood arsenic levels than adults (Steingraber, 2011).

In communities in which the drinking water is high in arsenic concentration, arsenic has been associated with fetal mortality, low birth weight, and decreased cognitive function (NRC, 2014). The arsenic exposure effects can continue through adulthood and can cause lung or cardiovascular disease or cancer (Naujokas, 2013). Low levels of arsenic exposure can also have other severe consequences such as infant infections and decreased IQ (Wasseman, 2014). Some studies have suggested that infant formula contains low levels of arsenic (FDA, 2014). One study suggesting that mother's breast milk may carry arsenic (Bjorklund, 2012) however in a study conducted by Carignan (2015), it was found that infants who were breastfed had overall lower levels of arsenic exposure taken by a urine sample than those who were fed formula exclusively.

1.6 Children are More Vulnerable to Exposure

A growing child's brain is still in development. The blood-brain barrier is not fully formed and can be more porous, making the child prone to neurological toxicity (Steingraber, 2011). In a study by Rocha-Amador (2007) it was explained that exposure to arsenic is known to cause neurological effects particularly a reduction in cognitive capacities in children. In rural towns in Mexico, the influence of arsenic exposure on IQ scores in children was examined. In a small town outside of San Luis Potosi, 80% of children had a urine arsenic levels above the

1.2µg/dL allowable limit (EPA, 2006). An inverse relationship was found between arsenic in urine and IQ scores; as arsenic level increased, IQ scores decreased (Rocha-Amador, 2007).

Arsenic exposure between early infancy and the first year of life is especially dangerous. Rodier (1995) described the effect of arsenic on brain development. The basic structure of the human brain develops before birth, but other structures continue to develop during the postnatal period. In fact, arsenic exposure during this early time period interferes with the formation of several neurological structures such as synaptic connections, receptors, and transmitter systems. The synaptic connections formed during development are critical because they are responsible for signaling further development. Substances such as toxins block or increase signals by mimicking neurotransmitters and in this way interfere with the development of the central nervous system (CNS). The blood-brain barrier begins to develop prenatally and becomes complete six months after birth. Toxic chemicals enter the developing brain relatively freely

Arsenic has specific effects on brain cells. For example, arsenic can increase oxidative stress specifically in brain tissue. Oxidative stress occurs when free radicals damage major components of a cell such as the membrane, proteins, or genetic material by an oxidizing method. When free radicals accumulate, they can lead to cell death (ALS, 2015). Also, the effects of oxidative stress can be exaggerated in some children. Antioxidants, which protect a child from oxidative stress can be lacking in the diets of children, particularly from lower income neighborhoods. A low consumption of antioxidants results in increased oxidative stress, and a lack of specific nutrients such zinc, calcium, and iron (Hamadani, 2011) can result in poor arsenic detoxification.

Although lead is not the heavy metal of focus in the current study, a lead absorption study may be relevant for understanding the risk of arsenic exposure in young children, because

arsenic is also absorbed in the gastrointestinal tract (Rossman, 2007). A study done by McCauley (2011) found associations between children's breakfast regularity and blood lead level. This was studied because fasting in adults was shown to increase lead absorption in the gastrointestinal tract. Children who ate breakfast on a regular basis had lower lead levels than children who do not eat breakfast regularly. It might be assumed that children with irregular eating habits and food insecurity have higher arsenic absorption.

Arsenic interacts with hormones, thus causing different effects by gender in children. Two main hormones that play a vital role in brain development are estrogen and thyroid. Arsenic acts as an endocrine disrupter by interfering with normal hormone function causing learning/memory and cognitive function impairments. In one study, female rats were more susceptible to arsenic-induced neurotoxicity than male rats with regard to effects on the dopaminergic system (Hamadani, 2011). The dopaminergic system is the collection of neurons that uses primarily the neurotransmitter dopamine. Dopamine is important due to its critical role in the CNS function (NIH, 2012).

Similarly, another study by Hamadani (2011) found differential effects of arsenic in boys and girls. In this study, boys showed no adverse effects of arsenic exposure on IQ as compared to girls. Girls' arsenic exposure influenced IQ scores at age five following exposures at the prenatal and/or early childhood periods.

There are several different types of medical tests that can be used to determine whether someone has been exposed to arsenic. These include blood, urine, hair, and fingernail tests that can measure if the person has had exposure to levels that are above the average. The most common test used in most studies is the urine test. While urine test can be used to determine arsenic exposure within the last several days, it cannot tell if the individual has had exposure in

the past. Hair and fingernail tests are used to determine exposure over the past 6-12 months. Although both tests can detect high levels of arsenic, they cannot detect low-level exposures (ATSDR, 2007). Because arsenic can have adverse effects not just at high levels of exposure but also at low levels, blood tests are used to determine low level exposure that occurred in the previous weeks (Hall, 2006).

1.7 Blood Pressure

Maintaining regular blood flow is crucial to the wellbeing of the human body. The blood flow through blood vessels travels by a pressure gradient always from higher (pumping action of heart) to lower (travel to smaller vessels) pressure areas. When the heart beats, it creates blood flow. Pressure begins to form when regular blood flow inside the human body becomes opposed by resistance. Hypertension is the term given to high blood pressure while hypotension refers to low blood pressure. There are two components to a blood pressure, systolic and diastolic blood pressures. Systolic blood pressure is the pressure that is applied by the blood when the left ventricle contracts in order to expel blood into the aorta. The average systolic blood pressure in healthy adults is 120 mmHg. Diastolic pressure refers to the pressure that is at its lowest point caused by the aorta's closure and continuance of blood flow into other vessels. Diastolic blood pressure usually averages approximately between 70 and 80 mmHg in healthy adults (AHA, 2015). The same principles and tests for blood pressure in adults are applied to children. Because children are still in development, however, there is not one standard blood pressure measurement that can be compared across all children of all ages. Blood pressure changes as the child grows. To determine whether a child’s blood pressure measurement is within normal limits, sex, age, and height must be taken into account (Blood Pressure Tables for Children and Adolescents by NIH, 2007).

High blood pressure is a risk factor for cardiovascular disease. It has been shown that high blood pressure during childhood increases the likelihood of developing hypertension during adulthood (Muntner, 2004). Even though this same study showed that hypertension among children has increased over the decades partly because the prevalence of childhood obesity has increased, many other factors could contribute to this finding. Medical conditions such as genetics (family history of high blood pressure), and sleep apnea (WebMD, 2015) can contribute to high blood pressure in children. High blood pressure in children carries the same risk factors as those found in adults. It can be due to being overweight, poor nutrition, and lack of exercise (Mayo Clinic, 2012).

1.8 The Role of Arsenic in Hypertension

Hypertension is a problem worldwide in both children and adults and there are many known risk factors that can contribute to this problem. Even though there are voluntary (salt intake) and involuntary (genetic) risk factors that may lead to hypertension, there are also environmental factors that may increase the risk.

Inorganic arsenic is thought to be a contributing factor to hypertension risk in adults. In many parts of the world, such as Bangladesh and Mexico, people become exposed to amounts over the maximum contaminant level, primarily in their consumption of arsenic contaminated water, in fact the source of most arsenic exposures (Abhyankar, 2012). In Taiwan, hypertension and heart disease have also been associated with the consumption of arsenic (Guha, 2012). In a study done in West Bengal, India by Guha (2012) two groups of adults were compared (exposed and unexposed) and a high association was found between hypertension and high exposure levels of arsenic. Their exposure was due to contaminated drinking water. In a study done in China by Zhang (2012), exposure to low level arsenic in the population's drinking water also was

associated with abnormal blood pressure. In this study, 49.3% of the population had hypertension and there was a significant linear association between blood pressure and blood arsenic levels. Even at low levels, arsenic had an impact on body's blood flow.

1.9 Blood Pressure among Children

While no studies have yet examined the specific association of child arsenic exposure and blood pressure, arsenic is known to have direct effects on physiological functions in children that impact blood pressure. For example, a study done in Taiwan (Su, 2011) examined associations between arsenic in urine and children's BMI, obesity and insulin levels. It was found that children who were obese retained a higher level of arsenic than the non-obese children. This was also true for insulin levels in children; obese children with arsenic exposure had higher insulin levels than normal weight children.

Abnormal blood pressure in children is dangerous because it can lead to many other cardiovascular diseases. For example, adults with hypertension have a two-fold greater likelihood of mortality (Kannel, 1989). Systolic hypertension is a risk factor for myocardial infarction. High blood pressure is a major cause of atherosclerosis, a disease in which plaque builds up in the arteries. Atherosclerosis is formed by endothelial cells activated by factors such as lipoprotein or reactive oxygen species. Arsenic plays a role in this process by activating endothelial cells and increasing inflammatory cell production, reactive oxygen species, and uptake of lipids (Lemaire, 2015). Endothelial cells have the ability to induce vasodilation and vasoconstriction. When adipocytes increase the sympathetic tone, the risk of hypertension increases.

1.10 Blood Pressure and Renal Function

The kidneys play a critical role in maintaining blood pressure homeostasis mostly by

controlling blood volume. When blood volume increases, blood pressure increases and when blood volume decreases it causes a decrease in blood pressure. Two types of mechanisms in the kidneys regulate blood pressure, an indirect mechanism and a direct mechanism.

The direct kidney mechanism triggers greater fluid filtration when there is an increase in blood volume. In this case, the kidneys cannot filtrate and reabsorb fluid fast enough for the increased blood volume and too much fluid leaves the body in urine, resulting in lower blood pressure and lower blood volume. In response to this loss, water is conserved and returned to the bloodstream causing then an increase in blood volume and thus pressure.

The indirect mechanism, also known as the renin-angiotensin mechanism, involves different factors. When needed, kidneys release the hormone rennin, which in turn causes the production of angiotensin II. Angiotensin II increases blood pressure by causing vasoconstriction, by stimulating aldosterone which increases blood volume, and by promoting the release of ADH which also conserves water (Marieb, 2010).

Due to the role of the kidneys in blood pressure regulation, over 50% of patients with chronic kidney disease (CKD) suffer from hypertension. Hypertension is more prevalent in adult CKD patients than in children, but also occurs in a majority of children with CKD (VanDeVoorde, 2011). Having abnormal kidney development in childhood, for example, reduced nephron number, may have triggered CKD which in turn can lead to future cardiovascular disease. Due to their waste removal function, the kidneys become a target organ for many toxic chemicals including heavy metals (Zheng, 2014). Children are at especially increased risk due to their immature kidney development and the susceptibility of developing kidneys to toxic agents.

A study conducted in Bangladesh investigated the association between arsenic exposed

adults and renal function. A negative association was found between urinary arsenic and estimated glomerular filtration rate (kidney function) (Peters, 2014). In other words, as blood arsenic level increased glomerular filtration rate decreased. Reduced glomerular filtration rate is a classic early sign of kidney disease.

Although the effects of arsenic at high concentrations on kidney function are a concern, a relationship between markers of kidney disease and lower blood arsenic levels have also been shown (Skroder, 2015). A study by Chen (2013) in Bangladesh and Hong (2004) in China found positive association between arsenic and abnormal urinary protein levels ("albuminuria") supporting the association between arsenic and the development of CKD. Exposure to other heavy metals like cadmium have also been associated with negative effects on kidney function (de Burbure, 2006).

Abnormal blood pressure has also been associated with neurocognitive deficits. A study by Lande (2003) showed that children with high blood pressure had significantly reduced performance on several cognitive tests, including IQ tests and tests of executive functioning. In another study, children with elevated urine protein (proteinuria) had lower scores on IQ scale (Hooper, 2011).

1.11 Arsenic in Southwestern US

There is special risk of child arsenic exposure in the southwest. The southwest United States has high concentrations of naturally occurring arsenic in ground water that exceed 10ppb when compared to the rest of the national average (Spencer, 2003). Naturally occurring arsenic can be released into the environment through the erosion of rocks and soil. Most of the arsenic concentrations in the southwest occur in ground water and can easily mobilize to other areas. Low-income communities on the U.S. - Mexico border in both Texas and New Mexico are most

affected by contamination from heavy metals. Much of the pollution that is in areas neighboring El Paso, Texas is from a closed-down smelter that operated for over a century (NMSU, 2013). Arsenic based pesticides are also a major source of arsenic in the environment. A widely used pesticide were arsenic compounds like lead arsenate and calcium arsenate. These pesticides were used throughout the 20th century. It was not until the 1980s that arsenic based pesticides were banned in the United States. Pesticides residues however are can continue to contaminate crops because pesticides remain in the soil (Blum, 2012). Pesticides banned in the U.S. continue to be used in other countries including Mexico. Because of the close proximity to the U.S., border region environments can be affected by their use. People residing in the U.S. can also travel to Mexico and bring these products back into this country.

"Colonias" (a community lacking basic infrastructure including city water) are also found along the US-Mexico region and have special risks for arsenic exposure from privately owned wells or improvised above-ground water storage containers. The water coming from these wells or storage containers is not monitored for heavy metals (or other contaminants) and regulations used for public water system do not apply. An environmental health impact assessment study conducted in the west Texas communities sampled in the current child study found arsenic above the maximum contaminant level in approximately half of the water samples collected from residents' homes (Hargrove, 2015). The findings from this environmental study were the impetus for the studies here described.

1.12 Healthy People 2020

Healthy People 2020 is a program that sets science-based goals and objectives within a 10 year target period, and was developed with the intention of promoting health and the prevention of disease. The goals of the current study address environmental health and the

prevention of cardiovascular risk factors in children. The respective objectives are to reduce the proportion of persons of all ages with hypertension and to reduce exposure to arsenic in the population measured by blood concentrations. While this study will not attempt to reduce either outcome, it will examine the association between arsenic exposure and blood pressure in children, and thereby contribute to identifying a possible cause of early cardiovascular disruption in vulnerable children.

1.13 Study Aims & Hypothesis

The current literature included many studies focusing on the effects of arsenic exposure on blood pressure among adults. There are virtually no studies regarding these possible effects in children. As previously stated, children are at much higher risk as adults for arsenic exposure. Because children are still undergoing development, for many reasons as described above, arsenic exposure in children can have a greater impact on their health. It is critical to begin examining the possible effects of arsenic on child health indicators, and specifically, the associations between arsenic exposure and blood pressure in children. This study aimed to investigate whether an association exists between arsenic and systolic or diastolic blood pressure among children. It was hypothesized that a relationship exists between arsenic level and blood pressure. Because no previous studies have examined these associations, no direction of association was predicted.

Methods and Materials

2.1 IRB Approval

Institutional Review Board (IRB) approval was obtained before any of the data collection took place. Parents signed an informed consent form before the study took place. Children signed an assent form before testing. Research team members conducting the study completed all required training on the handling of human subjects, conduct of human subject research, protection of confidentiality and anonymity, protection of data and child research best practices.

2.2 Sample Population

The population for this study was from two far west Texas rural communities within approximately 20 miles north downtown El Paso city center neighboring El Paso, TX. Many of the households in these two communities are low-income and in many cases lacking basic infrastructure. According to the US 2010 Census (American Fact Finder), the most recent statistics currently available, community one had a total population (including adults and children) of 1,971. There were a total of 974 females and 997 males. Community one had a total of 536 households with 271households having children under the age of 18. Ninety-four percent of the population were of Hispanic or Latino descent. In 2010, community two had a total population of 4,188. There were a total of 2,096 males and 2,096 females (American Fact Finder). Community two had a total of 1,165 households with 527 households having children under the age of 18. This community's population was ninety-seven percent Hispanic or Latino.

2.3 Study Participants

Parents and children from one elementary school from each community were recruited to participate in the study. Participating children were between ages 4 and 11 (grade levels were pre-kindergarten through fifth grade). During parent-teacher conferences, parents were recruited

and provided informed consent if they wanted their child or children to participate in the study.

Incentives of $10 per participating child were offered to families. At the time of recruitment, if parents completed questionnaires about their child in addition to informed consent, the parent was given half of the incentive at the time of enrollment (a five-dollar Walmart gift card) and the other half after the child completed participation in the study. If parents did not complete the questionnaires at the time of informed consent, the full incentive of ten dollars was given to the parents after the child completed participation in the study. Parents were provided with the results of testings when incentives were given out (after the study). Results were presented in informational brochures that included education about ways to protect children from heavy metal exposure.

All testing took place during school hours to ensure that all children had equal opportunity to participate. On the testing days, a parent liaison at each school went to gym classes at the start of each session, called for the participating children, and brought them in groups of approximately eight children. Children in each group were tested during the 45 minute class period. When all of the children in the group had completed testing, they were returned as a group to the gym class.

When the children arrived at the testing room, an ID number was given to each child and the study was explained to them. Once they heard the description of the study, and were told that their parents had agreed to their participation, each child was given an assent form to sign. At each school, across all testing sessions no more than three children refused to participate over the course of the study. After the testing session was over, children were able to choose a total of three small "thank-you" prizes (e.g., mini-ball, animal eraser, colored pencil) and each child was given a notification form to take home to the parent advising the parent that their child had been

tested on that day.

2.4 Sample Size

This study was part of a pilot study and the goal of recruitment was to enroll as many children as possible. In the first cohort including children from both schools (April 2014), 124 students were tested. In the second cohort including children from both schools (November 2014), an additional 138 students were tested. The final testing session (April 2015) included "time 2" testing for all of the previously enrolled and tested children and included 204 students.

2.5 Study Design

This was an observational longitudinal study using convenience sampling. No manipulation or randomization was done. Child arsenic levels (in micrograms per deciliter, μg/dL) were measured and were used to predict child systolic and diastolic blood pressure values, controlling for BMI, sex, age and school. Depending on the outcome of the primary analyses, secondary analyses examining the contribution of other factors (for example, BMI, or waist-to-hip ratio) may be examined.

2.6 Data Collection

There were a total of three testing phases conducted at both schools. These occurred in April 2014, November 2015, and April 2015. In the first two testing phases, independent cohorts were recruited and tested. During the last testing phase (April 2015) all of the children initially tested in either April 2014 or November 2014 were re-tested.

Anthropometric measurements

Each child began each testing session with anthropometric measurements. The child's waist, hips, weight and height were measured and recorded in centimeters. Waist and hips were measured with a standard tape measure; for hips, measurements were taken over the clothes

around the broadest point of the buttocks. For waist measurements, children were measured approximately at the level of the navel or at the smallest part of the waist.

Blood pressure was measured using an electronic sphygmomanometer (Model: CONTEC08A, People's Republic of China) for children. The child sat on a chair and the cuff was placed on their upper left arm. Both systolic and diastolic measurements were automatically calculated and recorded.

Blood samples

Blood samples were collected after the anthropometric measurements. Blood samples were collected by a finger stick with an average total collection of 50 microliters for each child. Blood was collected into sterile EDTA micro-vial capillary tubes. The samples were kept at room temperature for about three hours, and then stored at 4°C until tubes were transported in ice to the analytic laboratory.

Blood analysis was done by the chemistry laboratory at the New Mexico State University, Las Cruces. Blood samples were analyzed using Inductive-Coupled Plasma Mass Spectrometry (ICP-MS) and tested for arsenic, lead, cadmium and iron in whole blood. All heavy metals were recorded in micrograms per deciliter.

2.7 Data or Statistical Analysis Plan

All data were entered by research laboratory members and were checked for accuracy and outliers. Descriptive statistics were calculated for child and family demographic characteristics, blood arsenic levels and blood pressure (systolic and diastolic) measures. For the primary analyses, SAS will be used and generalized linear models (PROC GLIMMIX) with school included as a random effect will be calculated. Depending on the outcome of the primary analyses examining whether child blood arsenic predicts systolic and/or diastolic blood pressure,

controlling for gender and age, secondary analyses might be conducted to examine additional influences of arsenic on other physiological measures (e.g., BMI, waist-to-hip ratio).

Results

This was a longitudinal study in which children were tested two times. The first testing was conducted in April 2014 (n = 124) and November 2014 (n = 138) and the second (and last) re-test of all children was completed in April 2015 (n = 204). Fifty-seven of the children tested in 2014 were not available for the second testing. The results included only children who completed two testings.

3.1 Clinical and Demographic Characteristics of the Samples

Table 1 and Table 2 show the clinical and demographic characteristics of the sample population by school, sex, and time (1 or 2). At time one, the sample included 108 males (mean age 7.96) and 96 females (mean age 7.92). School 1 had a total of 54 males (mean age 8.02) and 33 females (mean age 8.18). School 2 had a total of 54 males (mean age 7.91) and 63 females (mean age 7.77).

As shown in Tables 1 and 2, the majority of mothers self-identified themselves as Hispanic (98.8% for male children and 100% for female children). Father's ethnicity was also similar to mother's ethnicity, with the majority being Hispanic (95.1% of fathers of male children and 96.9% for female children).

As for education, the majority of mothers had completed high school (58.3% of mothers of male children and 55.2% for female children). Father's education was somewhat similar to mother's education level; the majority had completed high school (68.9% of fathers of male children and 87.1% for female children). The mean family size was approximately 5 for both genders in both schools and families reported a mean annual income of $22,756.5 for male children and $21,807.7 for female children. According to the U.S. census (2014), the poverty threshold for family of five is $28,695. Thus, many families' income levels fell below the

poverty threshold.

The body mass index (BMI) for study participants was calculated using the child's weight and height. At time one, the mean BMI for males was 18.1 and for females was 17.9. The weight average for males was 66.5 pounds and for females was 65.3 pounds. The height average for males was 4.18 feet and for females was 4.15 feet for females, all initially measured in centimeters but later converted to inches.

At time two, the mean BMI for males was 18.4 and 18.8 for females. The weight average for males was 71.74 pounds and 71.2 pounds for females. The height average for males was 51.5 inches and 51.4 inches for females. The mean BMI percentile range was approximately 60 for both genders (at time one and two) and was within the U.S. 5^{th}-85^{th} percentile range, meaning that children were at a "normal" or "healthy weight" (CDC, 2015).

Systolic and diastolic blood pressures were automatically measured using an electronic sphygmomanometer (Model: CONTEC08A, People's Republic of China). At time one, the average systolic blood pressure for males was 108.9 (±13.7) and the mean diastolic blood pressure was 63.92 (±11.4). For females, the mean systolic blood pressure was 108.2 (±11.5) and the mean diastolic blood pressure was 63.9 (±10.1). At time one, 40.6% of females and 35.2% of males had blood pressures that reached criteria for prehypertension (blood pressure measurement at the 90^{th}-95^{th} percentile) or hypertension (blood pressure measurement above the 95^{th} percentile) (NIH, 2007).

At time two, the average systolic blood pressure for males was 111.4 (±10.5) and the mean diastolic blood pressure was 64.6 (±9.1). For females, the mean systolic blood pressure was 107.9 (±10.2) and the mean diastolic blood pressure was 62.6 (±10.6). At time two 32.3% of females and 43.5% of males had blood pressures that reached criteria for prehypertension

(blood pressure measurement at the 90th-95th percentile) or hypertension (blood pressure measurement above the 95th percentile) (NIH, 2007).

Mean blood arsenic levels at time one were higher than at time two. At time one, the mean arsenic blood level was .97(±.49) for males and .89 (±.44) for females. School two had slightly higher levels than school one (Figure 1 and 2). In school one, the mean arsenic blood level was .93(±.58) for males and .70 (±.55) for females. School two had an arsenic blood level in males of 1.01(±.39) and .98 (±.34) in females. Blood results in time one had higher arsenic blood levels in children compared to time two. In time two, the mean arsenic blood levels in males was .44(±.27) and .42 (±.22) in females. Although mean arsenic blood level did not exceed the 1.2 µg/dL allowable limit in children, some children did have blood arsenic levels above 1.2 µg/dL (Hall, 2006).

3.2 Primary Analyses: Predicting Child Blood Pressure from Blood Arsenic Levels

The first regressions examined the extent to which child blood Arsenic level predicted systolic and diastolic blood pressure, controlling for BMI, sex and age, with school included as a random effect.

Table 3 summarizes the Type III Fixed Effects and parameter estimates for associations between arsenic blood level and child's systolic blood pressure controlling for BMI, sex, age, and school (random effect). Arsenic blood level in children predicted an association between arsenic and systolic blood pressure (F=8.19, p=.0047). For every one µg/dL of arsenic, child systolic blood pressure decreased by 3.99 mmHg. Children's BMI was also a predictor of systolic blood pressure; for every one unit of BMI increase, systolic blood pressure increased 1.06 mmHg (F=45.23, p=.001). The main effects of sex (F=2.96, p=.09), age (F=2.53, p=.11), and time (F=1.52, p=.22) were not significant.

Table 4 summarizes the Type III Fixed Effects and parameter estimates for associations between arsenic blood level and child's diastolic blood pressure controlling for BMI, sex, age, and school (random effect). In this model, children's diastolic blood pressure was predicted by age (F=4.94, p=.03) and time (F=3.65, p=.05). For every one year increase, child diastolic blood pressure increased .712 mmHg. Children's BMI was also a predictor of diastolic blood pressure; for every one unit of BMI increase, diastolic blood pressure increased .613 mmHg (F=17.32, p=.001). The main effects of sex (F=1.02, p=.31) was not significant.

3.3 Secondary Analyses: Examining Effects of Other Heavy Metals (Lead and Cadmium) on Child Blood Pressure

Arsenic accounted for a significant amount of variance in child systolic and diastolic blood pressure. To examine whether this effect was unique to Arsenic, or might be similar in other heavy metals, additional regression models were tested to examine the extent to which lead and/or cadmium might also predict child systolic and/or diastolic blood pressure.

Type III Fixed Effects and parameter estimates for associations between lead and child systolic and diastolic blood pressure are shown in Tables 5 and 6. The association between lead and both systolic and diastolic blood pressure were not significant.

Type III Fixed Effects and parameter estimates for associations between cadmium and child systolic and diastolic blood pressure are shown in Tables 7 and 8. As the tables indicate, the association between cadmium and blood pressure was significant. Cadmium blood level in children predicted an association between cadmium and systolic blood pressure (F=5.45, p=.0205). For every one µg/dL of cadmium, child systolic blood pressure increased by 19.45 mmHg (*p=.0205)*. Children's BMI was also a predictor of systolic blood pressure; for every one unit of BMI increase, systolic blood pressure increases 1.08 mmHg (F=45.66, p=.001).

Cadmium blood level was also associated with diastolic blood pressure (F=8.24, p=.00445). For every one µg/dL of cadmium, child diastolic pressure increased 22.02 mmHg (p=.0045). Children's BMI was a predictor of diastolic blood pressure; for every one unit increase of BMI diastolic pressure increased .617 mmHg (p=.001). Age was also a predictor for diastolic blood pressure; for every one year increase, diastolic pressure increased by .77 mmHg (p=.0164).

To ensure that assumptions for regression analyses were met, a correlation matrix was calculated to examine possible multicollinearity among predictor variables including BMI, age, and arsenic (Figure 3-4). No high correlations (> .80) were found between predictor variables.

3.4 Findings Summary

There was a difference in blood arsenic level in children from school one and two; school two had higher levels compared to the other. Mean blood arsenic levels at time one were also higher than at time two. From the generalized linear model, arsenic blood level was significantly associated with systolic blood pressure (F=8.19, p=.0047). From the secondary analysis, cadmium blood level in children predicted an association between cadmium and systolic blood pressure (F=5.45, p=.0205) but lead did not show any association. From the calculated correlation matrix, no high correlation were found between predictor variables BMI, age, and arsenic level.

Table 1

Demographic Characteristics of Sample by School and Sex (N=204)

	Time 1						Time 2					
	School 1		School 2		Total		School 1		School 2		Total	
	Male (n=54)	Female (n=33)	Male (n=54)	Female (n=63)	Males	Female	Male	Female	Male	Female	Males	Female
Age	M=8.02 SD=1.78	M=8.18 SD=2.02	M=7.91 SD=1.94	M=7.77 SD=1.8	M=7.96 SD=1.85	M=7.92 SD=1.88	M=12.62 SD=20.41	M=8.68 SD=2.00	M=8.58 SD=1.94	M=8.43 SD=1.82	M=10.6 SD=14.6	M=8.5 SD=1.9
As (µg/dL)	M=.93 SD=.58	M=.70 SD=.55	M=1.01 SD=.39	M=.98 SD=.34	M=.97 SD=.49	M=.89 SD=.44	M=.36 SD=.18	M=.39 SD=.19	M=.5187 SD=.32	M=.44 SD=.24	M=.44 SD=.27	M=.42 SD=.22
Pb (µg/dL)	M=.85 SD=.98	M=.72 SD=.64	M=1.08 SD=.80	M=.99 SD=.83	M=.96 SD=.89	M=.89 SD=.78	M=.84 SD=.61	M=.86 SD=.73	M=1.05 SD=.65	M=.84 SD=.45	M=.95 SD=.63	M=.85 SD=.56
Cd (µg/dL)	M=.052 SD=.057	M=.052 SD=.032	M=.079 SD=.093	M=.083 SD=.086	M=.066 SD=.078	M=.072 SD=.074	M=.048 SD=.045	M=.032 SD=.018	M=.063 SD=.055	M=.048 SD=.047	M=.055 SD=.051	M=.042 SD=.039
Systolic BP	M=106.1 SD=11.4	M=109.5 SD=12.74	M=111.7 SD=15.25	M=107.51 SD=10.86	M=108.9 SD=13.7	M=108.2 SD=11.5	M=112.22 SD=10.50	M=108.88 SD=8.86	M=110.54 SD=10.50	M=107.45 SD=10.95	M=111.4 SD=10.5	M=107.9 SD=10.2
Diastolic BP	M=61.39 SD=9.2	M=65.48 SD=13.29	M=66.44 SD=12.78	M=63.19 SD=7.95	M=63.92 SD=11.4	M=63.9 SD=10.1	M=65.20 SD=9.00	M=63.67 SD=10.03	M=63.93 SD=9.21	M=61.97 SD=10.85	M=64.6 SD=9.1	M=62.6 SD=10.6
Hypertension*	31.5%	36.4%	37%	42.9%	35.2%	40.6%	44.4%	36.4%	42.6%	30.6%	43.5%	32.3%
Height (in.)	M=50.1 SD=4.9	M=50.19 SD=5.75	M=50.03 SD=5.26	M=49.56 SD=5.51	M=50.1 SD=5.04	M=49.8 SD=5.6	M=51.52 SD=4.86	M=51.38 SD=5.95	M=51.51 SD=5.23	M=51.35 SD=5.54	M=51.5 SD=5.0	M=51.4 SD=5.7
Weight (lbs.)	M=66.6 SD=23.6	M=63.36 SD=22.3	M=66.37 SD=25.4	M=66.30 SD=27.16	M=66.5 SD=24.4	M=65.3 SD=25.5	M=72.25 SD=25.5	M=67.83 SD=24.91	M=71.21 SD=26.6	M=73.02 SD=30.66	M=71.7 SD=26.9	M=71.2 SD=28.8

		6	0				5		2			
BMI	M=18.1 SD=3.8	M=17.14 SD=3.12	M=18.01 SD=4.18	M=18.25 SD=4.17	M=18.1 SD=3.9	M=17.9 SD=3.9	M=18.61 SD=4.08	M=17.47 SD=3.29	M=18.25 SD=4.09	M=18.70 SD=4.52	M=18.4 SD=4.1	M=18.8 SD=4.2
BMI %	M=62.98 SD=30.16	M=57.03 SD=28.49	M=59.60 SD=33.42	M=63.32 SD=31.36	M=61.29 SD=31.72	M=61.16 SD=30.4	M=68 SD=29.54	M=63.36 SD=28.93	M=64.94 SD=31.35	M=64.95 SD=32.90	M=66.8 SD=30.4	M=64.4 SD=31.4

* Hypertension or prehypertension, defined as blood pressure measurement at the 90-95th percentile and above the 95th percentile respectively.

Table 2

Clinical Characteristics of Sample by School and Sex (N=204)

	School 1		**School 2**		**Total**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
Household Size	M=5.25 SD=1.48	M=5.43 SD=1.50	M=5.16 SD=1.49	M=5.23 SD=1.52	M=5.20 SD=1.478	M=5.29 SD=1.507
Income	M=24291.43 SD=6949.71	M=21957.14 SD=63.36	M=21221.4 SD=15420.57	M=21756.7 SD=17162.06	M=22756.5 SD=21850.5	M=21807.7 SD=16584.3
Mothers Education High School Graduate	64.1% (39/204)	66.6% (21/204)	53.3% (45/204)	50% (46/204)	58.3% (84/204)	55.2% (67/204)
Fathers Education High School Graduate (n=204)	67.5% (37/204)	75.0% (20/204)	69.8% (43/204)	81.0% (42/204)	68.9% (80/204)	87.1% (62/204)
Mothers Ethnicity Hispanic (n=204)	97.4% (38/204)	100% (21/204)	100% (45/204)	100% (46/204)	98.8% (83/204)	100% (67/204)
Fathers Ethnicity Hispanic (n=204)	97.3% (37/204)	100% (21/204)	93.2% (44/204)	95.5% (44/204)	95.1% (81/204)	96.9% (65/204)

Table 3

Type III Fixed Effects and Parameter Estimates for Associations between As Blood Level and Child's Systolic Blood Pressure at Two Time Points Controlling for BMI, Sex, Age, and School (random effect)

Type III fixed effect				*Solutions for fixed effects*					
	F	*p*			Est	SE	DF	T value	*p*
Full Model				*Intercept*	86.29	3.51	199	24.58	<.0001
BMI	45.23	<.0001		BMI	1.06	.16	196	6.73	<.0001
SEX	2.96	.09		SEX Male	2.02	1.17	196	1.72	.0870
AGE	2.53	.11		Sex Female	0	-	-	-	-
TIME	1.52	.22		Age	.55	.34	196	1.59	.1134
AS	8.19	.0047		Time 1	1.41	1.15	196	1.23	.2197
				Time 2	0	-	-	-	-
				As	-3.99	1.39	196	-2.86	.0047
				SEX Least Squares Means					
				Male	110.08	.81	196	136.07	<.0001
				Female	108.07	.85	196	127.45	<.0001

Table 4

Type III Fixed Effects and Parameter Estimates for Associations between As Blood Level and Child's Diastolic Blood Pressure at Two Time Points Controlling for BMI, Sex, age, and School (random effect)

Type III fixed effect				*Solutions for fixed effects*					
	F	*p*			Est	SE	DF	T value	*p*
Full Model				*Intercept*	46.85	3.27	199	14.33	<.0001
BMI	17.32	<.0001		BMI	.61	.15	196	4.16	<.0001
SEX	1.02	.31		SEX Male	1.10	1.09	196	1.01	.314
AGE	4.94	.02		Sex Female	0	-	-	-	-
TIME	3.65	.06		Age	.71	.32	196	2.22	.03
AS	3.21	.07		Time 1	2.04	1.06	196	1.91	.06
				Time 2	0	-	-	-	-
				As	-2.33	1.30	196	-1.79	.07
				SEX Least Squares Means					
				Male	64.37	.75	196	85.44	<.0001
				Female	63.26	.78	196	80.13	<.0001

Table 5

Type III Fixed Effects and Parameter Estimates for Associations between Lead Blood Level and Child's Systolic Blood Pressure at Two Time Points Controlling for BMI, Sex, age, and School (random effect)

Type III fixed effect				*Solutions for fixed effects*					
	F	*p*			Est	SE	DF	T value	*p*
Full Model				*Intercept*	84.5	3.55	199	23.80	<.0001
BMI	44.76	<.0001		BMI	1.07	.159	196	6.69	<.0001
SEX	2.58	.1100		SEX Male	1.89	1.18	196	1.61	.110
AGE	3.30	.0709		Sex Female	0	-	-	-	-
TIME	.226	.6094		Age	.636	.345	196	1.82	.071
PB	1.11	.2934		Time 1	-.485	.948	196	-0.51	.609
				Time 2	0	-	-	-	-
				PB	-.779	.737	196	-1.05	.293
				SEX Least Squares Means					
				Male	110.03	.8134	196	135.26	<.0001
				Female	108.13	.8525	196	126.85	<.0001

Table 6

Type III Fixed Effects and Parameter Estimates for Associations between Lead Blood Level and Child's Diastolic Blood Pressure at Two Time Points Controlling for BMI, Sex, age, and School (random effect)

Type III fixed effect				*Solutions for fixed effects*					
	F	*p*			Est	SE	DF	T value	*p*
Full Model				*Intercept*	45.02	3.290	199	13.69	<.0001
BMI	17.92	<.0001		BMI	.624	.148	196	4.23	<.0001
SEX	.81	.3700		SEX Male	.981	1.091	196	.90	.3700
AGE	5.92	.0159		Sex Female	0	-	-	-	-
TIME	1.13	.2894		Age	.777	.319	196	2.43	.0159
PB	.03	.8630		Time 1	.932	.878	196	1.06	.2894
				Time 2	0	-	-	-	-
				PB	.118	.685	196	.17	.8630
				SEX Least Squares Means					
				Male	64.31	.753	196	85.41	<.0001
				Female	63.33	.789	196	80.25	<.0001

Table 7

Type III Fixed Effects and Parameter Estimates for Associations between Cadmium Blood Level and Child's Systolic Blood Pressure at Two Time Points Controlling for BMI, Sex, age, and School (random effect)

Type III fixed effect				*Solutions for fixed effects*					
	F	*p*			Est	SE	DF	T value	*p*
Full Model				*Intercept*	82.63	3.41	199	24.23	<.0001
BMI	45.66	<.0001		BMI	1.08	.159	196	6.76	<.0001
SEX	2.18	.1417		SEX Male	1.74	1.18	196	1.48	.142
AGE	3.55	.0610		Sex Female	0	-	-	-	-
TIME	.83	.3625		Age	.649	.345	196	1.88	.061
CD	5.45	.0205		Time 1	-.868	.951	196	-.91	.363
				Time 2	0	-	-	-	-
				CD	19.45	8.327	196	2.34	.021
				SEX Least Squares Means					
				Male	109.96	.8152	196	134.88	<.0001
				Female	108.21	.8542	196	126.68	<.0001

Table 8

Type III Fixed Effects and Parameter Estimates for Associations between Cadmium Blood Level and Child's Diastolic Blood Pressure at Two Time Points Controlling for BMI, Sex, age, and School (random effect)

Type III fixed effect				*Solutions for fixed effects*					
	F	*p*			Est	SE	DF	T value	*p*
Full Model				*Intercept*	44.27	3.15	199	14.06	<.0001
BMI	17.62	<.0001		BMI	.617	.147	196	4.20	<.0001
SEX	.68	.411		SEX Male	.899	1.09	196	.82	.411
AGE	5.86	.016		Sex Female	0	-	-	-	-
TIME	.32	.570		Age	.771	.318	196	2.42	.016
CD	8.24	.0045		Time 1	.497	.874	196	.57	.570
				Time 2	0	-	-	-	-
				CD	22.02	7.67	196	2.87	.0045
				SEX Least Squares Means					
				Male	64.27	.753	196	85.39	<.0001
				Female	63.37	.789	196	80.35	<.0001

Figure 1

Arsenic Blood Level by Sex and School, Time 1

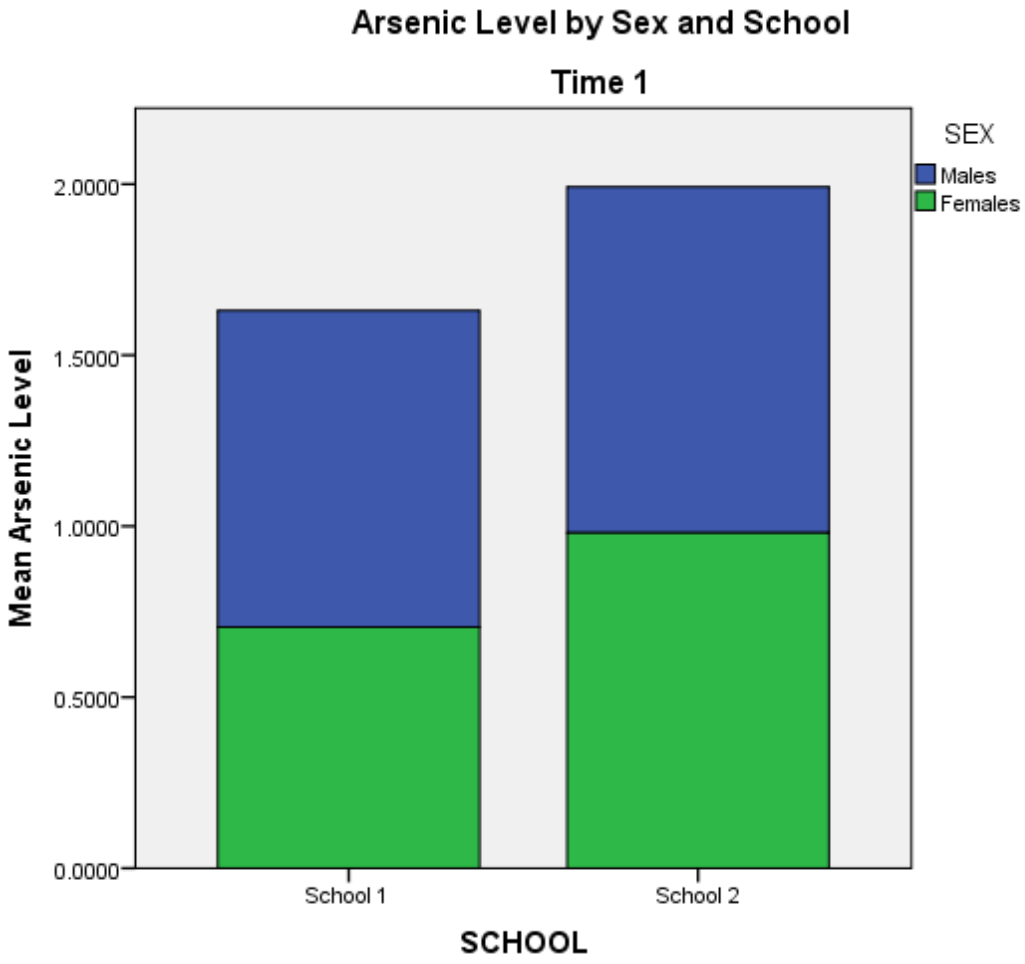


Figure 2

Arsenic Level by Sex and School, Time 2

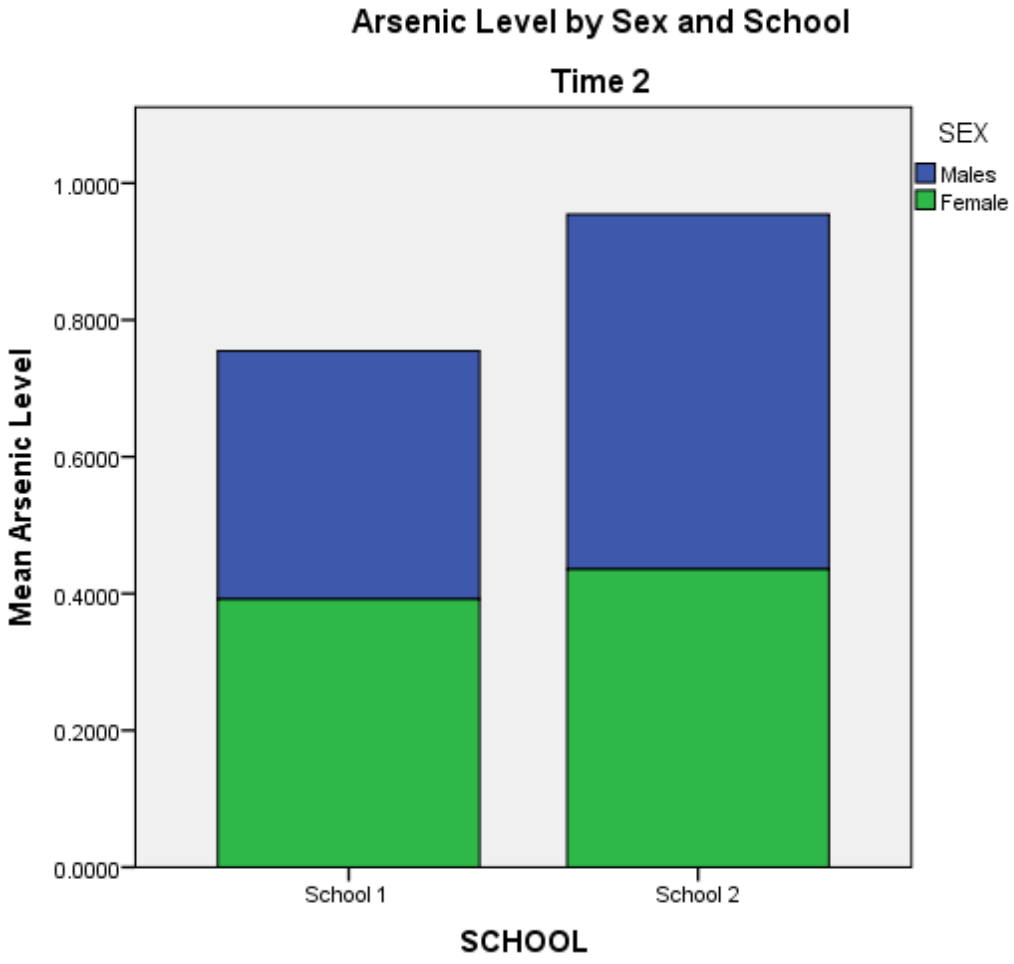


Figure 3

Correlation Matrix between Continuous Variables Time 1, Age, BMI and Arsenic Level

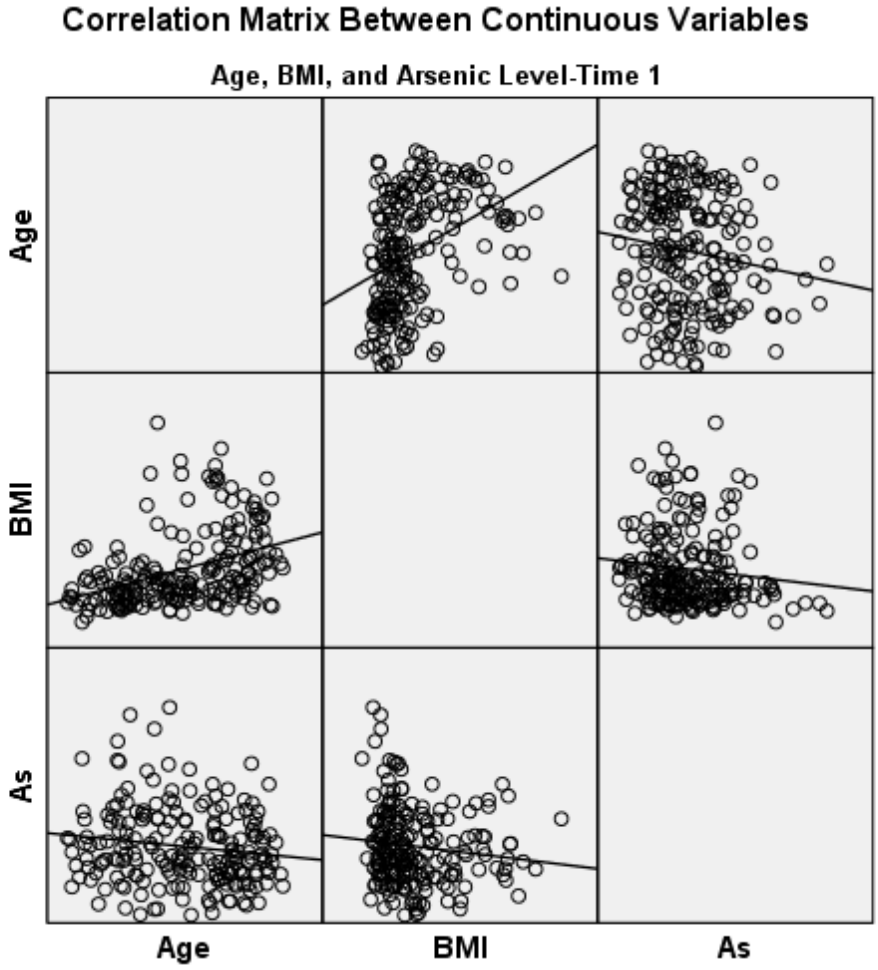
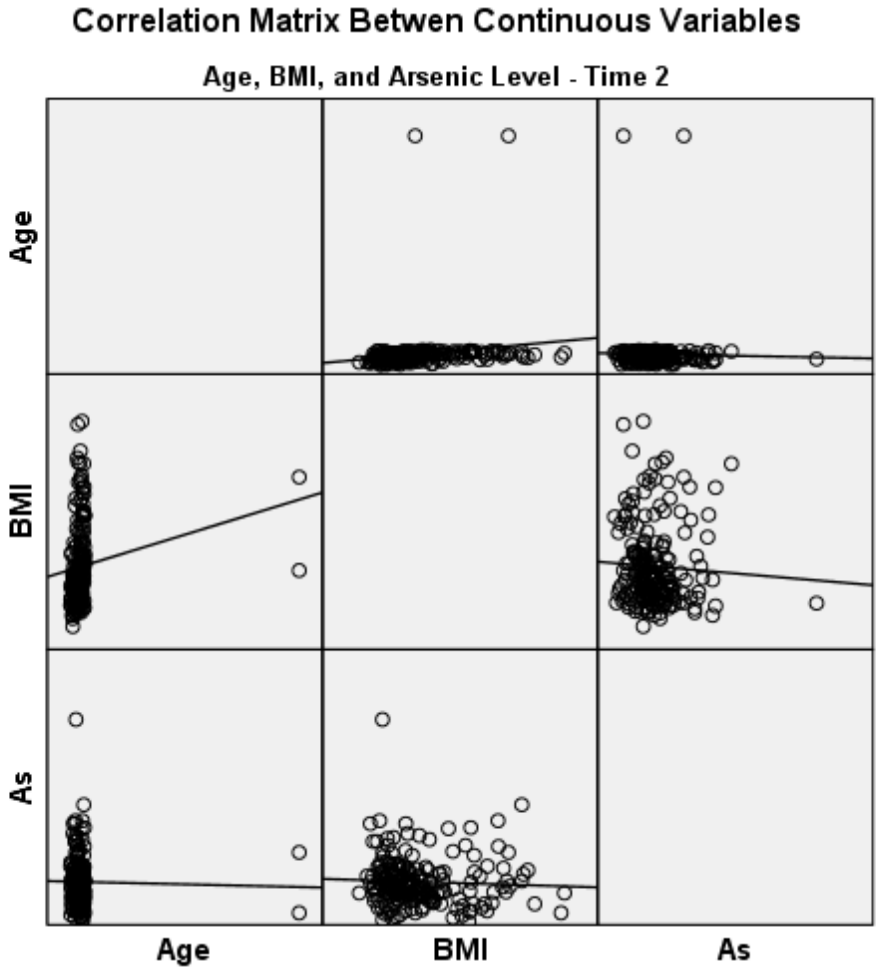


Figure 4

Correlation Matrix between Continuous Variables Time 2, Age, BMI, and Arsenic Level



Discussion

4.1 Low-Level Arsenic and Blood Pressure in Children

Previous adult studies had suggested that arsenic exposure altered blood pressure. No studies had examined possible effects in children. The current study was conducted to examine whether child arsenic levels predicted differences in blood pressure among children ages 4 to 11. The results suggested that arsenic had a significant effect on blood pressure such that as child blood arsenic values increased systolic blood decreased. While in adult studies (Abhyankar, 2012; Guba, 2012; Zhang, 2012) arsenic had a positive association with blood pressure in adults, in other words, as arsenic increased blood pressure increased, in the current study arsenic had an inverse relationship blood pressure in children; for every one μg/dL of arsenic systolic pressure decreased by 3.99 mmHg.

4.2 Arsenic Exposure and Hypotension

As suggested above, different from results of adult studies, in this first child study we found an inverse association between arsenic blood levels and systolic blood pressure. Because of this unexpected result, the literature was searched for possible mechanisms involved in arsenic exposure that could be expected to produce a decrease in blood pressure. In fact, several mechanisms of action suggested by other studies might explain this result.

The endothelium is the tissue that forms a lining of single cells in organs of the body especially in blood vessels, the heart, and the lymphatic vessels. The endothelium is responsible for regulation of thrombosis and platelet adherence, it modulates vascular tone and blood flow, and the endothelium regulates the immune response (Sumpio, 2002). By their ability to cause vasoconstriction and vasodilation, endothelial cells are able to control blood pressure.

The endothelium produces several substances that play a role in the homeostasis of blood

vessels. The most important chemical compound produced is nitric oxide (NO). NO produces and regulates vasodilation and the inflammatory response when activated by reactive oxygen species (ROS). Tousolis (2012) explained nitric oxides role in the endothelium. When a toxic substance or other unwanted factors come into contact with or affect the endothelium, endothelial cells become inflamed and in turn cause the release of nitric oxide which in turn causes dilation and thereby prevents major changes in system function.

When a person suffers from endothelial dysfunction, the natural process of nitric oxide is not achieved; blood pressure regularity is not achieved and will lead to vasoconstriction and eventually causes an increase in blood pressure. When the endothelial dysfunction is severe, it causes the production of nitric oxide to stop. The stop of nitric oxide production is accomplished through the inhibition of its synthesis. Thus, the findings of the current study suggest that the decrease in blood pressure observed in children may be from the disruption of endothelial cells, release of NO and subsequent dilation with lowering of blood pressure. The outcome of hypertension in adults may reflect a long-term breakdown of the process that results from chronic arsenic exposure.

The hypertension found in adults with arsenic exposure has been associated with atherosclerosis. A study by Lemaiere (2015), investigated arsenic's role in atherosclerosis formation. This disease occurs following chronic endothelial cell activation and the presence of reactive oxygen species. Arsenic takes part in this process by participating in endothelial cell activation. Arsenic increases inflammatory molecule production, reactive oxygen species, and uptake of lipids all which have been linked to atherosclerosis. When the endothelial cells become activated, they express adhesion molecules such as vascular adhesion molecule 1 (VCAM-1) that allow the adhesion of cell into the vascular wall which in turn lead to plaque

formation. It was found that arsenic facilitates the adhesion of monocytes to endothelial cells however it does not enhance platelet activation. It was also found that arsenic induces mononuclear adhesion to VCAM-1. This provides an explanation as to why arsenic in adults can lead to hypertension. The inflammation of endothelial cells (by arsenic) disturbs natural processes that in a non-exposed person would cause vasodilation and inhibition of plaque formation and subsequent vasoconstriction in the arteries.

Other studies regarding the endothelium suggested other possible mechanisms by which arsenic may play a role in hypotension. In a study by Liao (2001) the connexin protein 43 (Cx43) caused hypotension in mice. Cx43 is present in all mammals. Connexins, or gap junctions, play an important role in cellular function coordination. Gap junctions also play a vital role in vasoconstriction and vasodilation. Communication through junctions seem to play a role in cardiovascular function. The way this protein was studied was through a knockout strategy where a particular gene was made inoperative in order to observe the roles of these connexins in vascular function. This was achieved by using a recombination system that eventually led to the formation of mouse line that "knocked" out Cx43. In studies previous to Liao's study, Cx43 was associated with hypertension. In Liao's study however, the Cx43 knockout was associated with hypotension. The authors hypothesized that hypotension in the mice was caused by an elevation of nitric oxide synthesis. Due to the elevated NO concentration in plasma, it was thought that the high NO concentration was a result of interference of nitric oxide synthase. Even though this study focused on CX43 and not on a heavy metal like arsenic, it may be useful for hypothesizing a potential mechanism in which the endothelium can lead to a decrease in blood pressure.

Other studies provide support for disruption by arsenic of NO production and also suggest the complexity of the mechanisms involved. For example, an increase in NO production

has been seen in various cells from different studies due to arsenite (arsenic containing an oxygen ion) exposure. These include hamster ovary cells, neonatal rat brain cells, bovine aorta endothelial cells, human fetal brain cells, and umbilical vein endothelial cells in humans (Kao, 2003). From a review of all the finding in these studies, NO production can increase or decrease depending on the cell type in which arsenite is acting on, the molecule species of or arsenic, and the dose or arsenic (Gurr, 2003). The increase of NO production in some human cell types, can alter normal physiological functions such as blood pressure that can be associated to a decrease in blood pressure.

Other mechanisms of hypotension following arsenic exposure have also been suggested. According to the CDC (2010), hypotension may result from gastrointestinal effects of arsenic by ingestion. Hypotension and fluid loss appears to be caused by gastrointestinal lesions caused by arsenic exposure increasing the permeability of small blood vessels. It is also stated that hypotension and vasodilation may be the result of acute arsenic poisoning due to capillary leakage. Most children in our study had arsenic exposure less than the allowable limit (1.2 µg/dL).

Another study by Ramachandran (2006) investigated angiotensinergic stimulation, mechanism involved in blood pressure homeostasis, of the vascular endothelium in mice. The binding reduction of angiotensin II to vascular tissue is said to reduce hypertension, atherosclerosis, and heart failure (Dzau, 2001) due to endothelial cell and angiotensin 1 receptor stimulation that produces vasodilator substances. Mice were engineered to express ATR1 only in endothelial cells. The added angiotensinergic stimulation in endothelial cells resulted in hypotension. The results from this study (Ramachandran, 2006) suggested that angiotensin II activated by Ec-atr1 caused vasodilation. Although this study did not involve any toxin, such as

arsenic, it nonetheless provided another clue as to why arsenic caused lower blood pressure in children in our study. Besides NO, angiotensin II might be disrupted by arsenic and as a consequence cause a decrease in blood pressure. Thus, the decrease in blood pressure in this study may be due to alteration or modifications in NO; most likely arsenic triggers No synthase, therefore causing over production of NO.

The link between early low blood pressure and the higher blood pressures observed in arsenic exposed adults (see above) is not obvious. Additional longitudinal studies are needed to determine whether early chronic low-level arsenic exposure and low blood pressure in children, predispose the system to high blood pressure during adulthood.

4.3 Other Low-Level Heavy Metals and Child Blood Pressure

For comparison, regression models were also calculated to determine whether other commonly present heavy metals, i.e. lead or cadmium, might also predict child blood pressure. The reason for these additional analyses was to determine whether the effects of low-level arsenic were specific to arsenic, or in fact reflected more general effects of low-level heavy metals in children's circulatory systems.

4.4 Low-Level Lead Exposure and Child Blood Pressure

The lead levels in children in this study were uniformly low (see Tables 5 and 6) and none exceeded the current acceptable limit of 5 μg/dL. We examined the possible effects of low-level lead exposure on blood pressure and there were no significant associations. In fact, very few studies have examined the effects in children, and the findings from adult studies are mixed. For example, in an older study by Sparrow (1984), tap water was examined in the homes of 246 adult males and their blood pressure measurement was taken. Lead concentration in water exceeded the U.S. drinking water limit. No significant association was found between lead and

systolic blood pressure or between lead and diastolic blood pressure. Other studies of adults suggested otherwise however. In a study of 254 adult males and 271 adult females blood lead level was significantly associated with blood pressure (Apostoli, 1990). A newer study by the same author had the same findings (Apostoli, 2005). The study consisted of two groups of adults that were tested. One group had 303 adult participants that had occupational lead exposure between 10-80 µg/dL and 206 adults in the general population with lead concentration between .5-9 µg/dL. Both groups had a positive correlation between blood lead levels and systolic and diastolic pressures.

4.5 Low-Level Cadmium and Child Blood Pressure

Similar to lead, cadmium levels in children tested for this study were very low and none exceeded the current "acceptable" limit of 0.5µg/dL as established by the CDC (Mayo Clinic, 2012). When the low cadmium levels were used to predict blood pressure in children, controlling for BMI, sex and age, a significant association was found. However the association was positive, that is, in the opposite direction as the effect observed from arsenic on child blood pressure (p=.0205). For every one µg/dL of cadmium systolic blood pressure increased by 19.45 mmHg and diastolic increase of 22.02 mmHg. In the United States, people become exposed to cadmium by food and smoking; and vegetables and tobacco leaves contain high levels of cadmium from the soil. In this study, parents filled out a medical history form in which it asked if someone in the household smoked and the amount of time this occurred. Overall smoking was very infrequent among our families and we did not analyze this information for the purposes of the study. For future studies however, it would be beneficial to analyze the association of smoking among family members and cadmium levels in children; children might be inhaling second hand smoke.

Like arsenic, cadmium can be inhaled or ingested but not absorbed through the skin. It is also excreted by the kidney through urine. Cadmium is classified as a carcinogen and has been linked to lung cancer, kidney disease, and causing bones to become fragile. Cadmium is classified as a carcinogen and there are several mechanisms by which cadmium influences body function. One is through the induction of apoptosis. In rats cadmium has been shown to induce apoptosis in various organs through a mitochondria dependent pathway (Kondog, 2002). Another process is through the inhibition of DNA repair (Waisenburg, 2003). Like arsenic, oxidative stress also plays a role in cadmium toxicity. Cadmium has been shown to deplete the protein glutathione and eventually leads to the production of reactive oxygen species, which is connected to chronic cadmium nephrotoxicity (Liu, 2009). Similar effects of cadmium exposure seen in adults are also present in children although children are at a higher risk because children are known to absorb more toxins than adults (see Introduction, page 3 above). Arsenic is known to cause cognitive and behavioral problems; cadmium has not been yet linked to these outcomes but perhaps only because the studies have not yet been conducted.

Because of the association of cadmium and systolic blood pressure and the high prevalence of prehypertension and hypertension stages in children in this study, it may be important to investigate cadmium's role in renal function. As stated previously (page 9 above), the kidneys play a critical role in the regulation of blood pressure. Cadmium has been associated with cardiovascular disease including stroke, heart failure, and myocardial infarction (Peters, 2010), though results are mixed. A study in China (Chen, 2013) examined a population of adults living near a cadmium smelter with men blood cadmium levels between 3.84 µg/dL for women and 3.32 µg/dL for men. It was found that both systolic and diastolic blood pressures increased as cadmium blood concentration increased. The prevalence of hypertension was also correlated

with increasing cadmium concentration. Children's placement in prehypertension or hypertension stages in our study might be due to cadmium levels in the blood rather than arsenic.

Cadmium can have a direct effect on blood pressure due to its effects in kidney. In fact, cadmium is said to be nephrotoxic meaning it has the ability to cause kidney tubular damage. The kidney is the target organ of cadmium ingestion. Cadmium is retained in the kidney and has a half-life of 10-30 years (Jarup, 1983). Long term exposure to low or high level exposures have been seen to lead to kidney failure causing glomerular damage due to a decreased glomerular filtration rate (Jarup, 2009).

A study in Sweden studied cadmium exposure in a nonsmoking population of adults. This study compared currently non-smoking adults with and without a history of smoking. Ex-smokers who had quit five years before the start of the study had higher cadmium levels than those with no history of smoking. The parameters of kidney function were also investigated. B_2-microglobulin-creatine clearance (used to determine whether glomeruli or renal tubules were damaged and would be expected to show early renal damage) was related to urinary cadmium (Olsson, 2002).

Another study by Swaddiwudhipoing (2014) also investigated this association but in Thai children. This study saw a positive association between urinary cadmium and B_2-microglobulin but not with blood pressure. Perhaps the renal effects present have not been established long enough to observe an effect in blood pressure although this association has been seen in adults (Swaddiwudhipoing, 2010) but not in children (Cao, 2009). In a study of Bangladeshi pre-school aged children found that chronic exposure to cadmium damaged kidney function; an increase of .5 µg/dL in cadmium was associated with a decrease in glomerular filtration rate by .2SD (Skroder, 2015). In this same study, an association among boys was found between

cadmium and blood pressure.

4.6 Mercury Exposure and Child Blood Pressure

Given the significant findings for arsenic and cadmium with regard to child blood pressure, for future studies, it will also be important to investigate the possible effects of mercury on child blood pressure. Mercury can exist in three forms, elemental, inorganic, and organic. Mercury is biomethylyzed to from methyl mercury which is found in fish at high concentrations. The human body does not have an excretion mechanism for mercury, meaning that mercury accumulates during the person's life time (Solonen, 1995). Various studies have suggested that mercury increased free radical production, oxidative stress, and reduced immune function among others (Clarkson, 1997; Rungby, 1992; Insug, 1997). Mercury is also known to cause certain cardiovascular effects. One study found a significant relationship between coronary heart disease, myocardial infarction, and renal disease, and mercury exposure (Boffeta, 2001). It is also been associated with hypertension; a European study showed significant increase in systolic blood pressure associated with mercury exposure among mercury miners (Kobal, 2004).

4.7 Possible Activities to Reduce Child High Blood Pressure in our Study Population

An unexpected finding of this study was that a high percentage of children with blood pressure readings indicating "prehypertension" or "hypertension." Prehypertension is defined as having a blood pressure measurement at the 90^{th}-95^{th} percentile. Hypertension is defined as having as having blood pressure measurement above the 95^{th} percentile (NIH, 2007). The percentile ranking for each child was determined according to the child's gender, age, and height. At time one, 35.2% of males and 40.6% of females had blood pressure readings consistent with either prehypertension or hypertension. At time two, 43.5% of males and 32.3% of females were at these stages.

High blood pressure in children is usually assumed to be caused by conditions such as kidney disease, heart defects, or hormonal disorder. However, the most common high blood pressure factors among children, similar to adults, are overweight and obesity (Riley, 2012). Obesity combined with low-level cadmium exposure could be a particularly "toxic" combination. Obesity in children has increased over the past year especially among ethnic minorities and low income children. In an article published in 2006 by Kumanyika, studies the differences in obesity in children by ethnicity and socioeconomic status. Although it is not as recent, it gathers information from the 1970s to 2002. An increase of obesity prevalence over the years is seen which can still provide useful information that can be used to develop ideas in order to reduce obesity and blood pressure in children. It's stated that obesity among African American and Mexican American children has increased about 10-12% when compared to white children. Despite the differences by ethnicity, low income children are at a greater risk for obesity. The mean average household income for the children in the study was about $22,756.5 indicating that these children live in poverty according to the U.S. poverty threshold for a family of five.

A study by the national heart, lung and blood institute found that children obesity levels decrease as parental education and income increase (McNutt, 1997). While most parents had completed high school in our study, other parents self-reported to have less education, as little as only elementary education. Ethnic minorities and low income minorities have been reported to spend far more hours watching television compared to the other youth (Roberts, 1999). Due to this high amount of television, the Kaiser foundation found that food advertising affects children's food preferences that lead them to consume less nutritional food (1999). Food availability may also be a problem in low income communities; low income communities have

fewer convenience stores that have fresh and affordable groceries. These statistics may represent the communities in the study. Education can play a big role in child's weight. Perhaps by educating the parents on smarter, healthier choices will make an impact on the child's health and reduce their BMI overall. This could be achieved by schools offering a class in which parents learn about nutrition, importance of physical activity, and the dangers of being overweight.

Throughout the school day, children are sitting down most of the time. Activity breaks throughout the school day may help children increase physical activity. Having short little breaks may also help in reducing weight but most importantly relieve stress that can sometimes be associated with high blood pressure. Several activities have been put into practice and positive results have been seen in states throughout the U.S. Some of these would be helpful to children in this study. One program is the *Take 10* in which physical activities are incorporated as part of the academic curriculum for mathematics, language arts, science and social studies (Stewart, 2004). These activities take 10 minutes long and do not hinder with classroom learning time as it is part of the classroom lesson plan. An example of an activity is one where children are learning the benefits of different food groups. The teacher calls out a food group and asks a student to give an example and as the teacher is calling out the benefits of the food have students do the activity. For example, when learning about grains, students will run in place because grains are carbohydrates and they provide energy needed to do various activities.

Activities like these can have a great impact on the child's health. Besides increasing physical activity throughout the day, *Take 10 can* improve on-task behavior; evaluation of this program showed a 20% improvement in on-task behavior among the least on-task children (Whitt-Glover, 2013). Other programs similar to *Take 10* have been seen to improve some health measures. *ABC for Fitness,* program that has similar strategies as *Take 10,* had significantly

smaller decrease in BMIs when compared to the control group.

In the current study, many children had high body mass index (calculated for gender, height, and weight) providing another possible prediction for the high percentage of hypertension in children. In time one, males had a mean BMI of 61.29 and females had a mean BMI of 61.16. In time two, males had mean of 66.8 and females a mean of 64.4. Getting children to reduce their weight and ultimately their blood pressure will be beneficial to their health in the future. There are several intervention or programs that have been put into place to reduce this problem in children that could also be beneficial to our study sample. It is possible that exercise programs could have two beneficial effects. By increasing metabolism through exercise, fat stores could be reduced and toxin excretion increased.

An intervention by McMurray (2002) in North Carolina for young adolescents (middle schools children) was done in the efforts of decreasing blood pressure and body fat. The schools selected were in rural communities because it has been shown that rural youth is more at risk for obesity than urban youth (Harrell, 1998). It was designed to be carried out in eight weeks; 30 minute exercises for three days a week. Exercises included mini soccer, aerobic tag, and endurance circuit among others. The program consisted of four schools; a control school, one with education only, one with physical activity only, and the other with both physical activity and education. Results showed that an increase in physical activity was able to reduce blood pressure that usually increased during this age. The group with the most improvement was the group that received both education and physical activity.

This study implied that physical activity was an important part of reducing blood pressure in young adolescents and also in younger children. One of the factors that support this link is the effect on the renin-angiotensin-aldosterone system. Exercise is able to lower plasma renin levels

which influences both blood pressure and blood volume. Most physical education classes in elementary school provide between 6 to 10 minutes of aerobic exercises in the 40 minute class (Luepker, 1999). This type of program could be a good fit for this rural population with apparently large numbers of children with prehypertension and hypertension.

Another intervention had similar tactics to reduce cardiovascular disease risk factors in elementary-school children (Harrell, 1995). Twelve schools were recruited including a control school. For a total of eight weeks, regular classroom and physical education teachers and physical were educated to teach students healthy choices (importance of physical exercise and healthy foods etc.) twice a week along a physical activity three times a week.

Results showed a decrease in several factors including total serum cholesterol and reduced body fat when compared to the control group. Children also had increased healthy heart knowledge. Diastolic blood pressure did not increase in intervention group as it did in the control group. A classroom-based intervention as such is adequate because all children benefit from the intervention rather than only the children that are in more need of the aid.

Limitations

5.1 Convenience Sampling

This study used convenience sampling and thus children were not systematically sampled throughout the two communities. At the same time, the studies were designed to provide equal opportunity to all children for participation. Recruitment was conducted during mandatory parent-teacher nights at the schools, all testing was conducted during the school day, and children who were sick or absent on testing days were given additional opportunities to participate.

5.2 Sample Size

While the overall sample size for this study was relatively large (N=204) the study included children ranging in age from approximately 4 to 11 years. Age was controlled in all models, but because this is a very broad age range we can question whether we were able to completely characterize differences at different ages. Future studies would benefit from using one year cohorts followed longitudinally.

5.3 Blood Pressure

The American Heart Association recommends to measure blood pressure twice with one minute between readings and the average of both measurements be taken. This is especially important if a child has relatively high or low blood pressure. Blood pressures reported in this study reflected one-time measurement only. For future studies, blood pressure could be measured three times and an average taken.

5.4 Demographic Information

Some parents did not provide demographic information. It was every parents' right to deny collection of this information. At the same time, the demographic characteristics of families living in these communities has been observed to be homogeneous and we believe that

the demographic characteristic described are an accurate depiction of the communities included in this study.

Conclusion

The current study found a difference of blood arsenic level in children between schools; school two had slightly higher levels compared to school one. Mean blood arsenic level at time one were also higher than at time two. From the primary analysis, blood arsenic level was significantly associated with systolic blood pressure ($F=8.19$, $p=.0047$) in the opposite direction that it was expected. For every one µg/dL of arsenic, child systolic blood pressure decreased by 3.99 mmHg. From the secondary analysis, cadmium showed a positive association with blood pressure. For every one µg/dL of cadmium, child systolic blood pressure increased by 19.45 mmHg n. The mean blood cadmium level in children did not exceed the allowable limit of 0.5µg/d.

Although the mean blood arsenic level did not exceed the acceptable limit set by the EPA, prevention steps for arsenic and other heavy metal exposure should be considered for children living in these two communities. Many children had criteria for prehypertension and hypertension. Many children had BMIs over the 85th percentile, meaning that they were overweight or obese. Even if high BMIs are a cause of high blood pressure in these children, heavy metal exposure, especially to cadmium due to its positive association with systolic blood pressure, should be a concern for exposure prevention. In school activities should be incorporated to help children reduce obesity and blood pressure in children from these two elementary schools. Parental nutrition education would also help children reduce body fat and therefore reduce blood pressure.

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Curriculum Vita

Tania A. Mayorga was born in California and moved to El Paso, Texas at the age of 13. She is the second of three siblings and the second to attend college in her family. During her undergraduate career at the University of Texas at El Paso (UTEP), Tania Mayorga was a member of the Medical Professions Organization, the National Society of Collegiate Scholars, Alpha Lamda Delta, and Alpha Lamda Delta-Executive Council. For one year and a half (Spring 2012-Spring 2013), she volunteered as a research assistant in the Department of Health Sciences under the supervision of Dr. Dominguez where she learned and assisted with extensive laboratory protocols. During the summer of 2012, Tania Mayorga took part in the Medical School Matriculation Program in the University of Texas Medical Branch in Galveston, Texas. She graduated in 2013 with a Bachelor's of Science degree in Biomedical Sciences with a minor in Chemistry. Right after completing her undergraduate work, Tania Mayorga was accepted into the Masters of Public Health degree program at UTEP in fall 2013. Within these two and a half years, Tania was part of the research laboratory team under the supervision of Dr. Sobin where took part in the Child Health Screening Project at UTEP. In 2015, Tania Mayorga was also an EnHIP Scholar led by the National Library of Medicine (NLM) where she developed a tutorial on NLM services for undergraduate UTEP students. During the summer of 2015, Tania Mayorga interned with El Paso First-Health Plans.

Tania Mayorga plans to attend medical school in the future. She plans on using her public health sciences and medical training to improve social and health environment in resource-challenged communities along the U.S. Mexico border.

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