AIR POLLUTION AND REPEATED ULTRASOUND MEASUREMENTS OF FETAL GROWTH IN MEXICO CITY

by

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DEDICATION

I dedicate this dissertation to those giants whose shoulders I proudly stand upon--my deceased grandmothers: (Andrea “Andy” Hill and Bessie S. Perry) and great grandmothers (Ruth E. Short, Gertrude Hill, Maggie Blackwell, Mary E. Smarr, and Narsisus Fullenwider). They left behind legacies of sacrifice, independence, zeal for knowledge, and above all, faith in God; all of which helped to develop the woman, researcher, and author responsible for the work presented in the subsequent pages of this document.

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LIST OF ABBREVIATIONS

AC	Abdominal circumference
AIUM	American Institute of Ultrasound Medicine
BPD	Biparietal diameter
BTEX	Benzene, toluene, ethylbenzene and xylenes
CO	Carbon monoxide
FL	Femur length
HC	Head circumference
IUGR	Intrauterine growth restriction
LBW	Low birth weight
NO_2	Nitrogen dioxide
O_3	Ozone
PAHs	Polycyclic aromatic hydrocarbons
Pb	Lead
PM	Particulate matter
PM_{10}	Particulate matter less than 10 micrometers in aerodynamic diameter
$PM_{2.5}$	Particulate matter less than 2.5 micrometers in aerodynamic diameter
RI	Rohrer's ponderal index
SGA	Small for gestational age
SO_2	Sulfur dioxide
WHO	World Health Organization

ABSTRACT

The possible adverse effect of ambient air pollution on various birth outcomes (e.g., weight, length of gestation) is a global public health concern. However, understanding of prenatal exposure to air pollutants and the process of intrauterine growth is limited. This dissertation addressed research gaps in this area by evaluating maternal air pollution exposure and fetal growth using a novel methodological approach.

Overall, the objective of this dissertation was to assess exposure to ozone (O_3) and particulate matter less than 2.5 micrometers in aerodynamic diameter ($PM_{2.5}$) during the first trimester and growth trajectories of four fetal anthropometric parameters [head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL)], within a Mexico City cohort of pregnant women. First, a systematic review of the epidemiological literature on maternal exposures to air pollutants and fetal growth, as assessed with data from ultrasound examination of fetal anthropometric parameters, revealed scant and limited research exploring *in utero* assessment of fetal growth related to prenatal air pollution exposure. Secondly, uncertainty related to the use of repeated ultrasound measurements of fetal parameters performed by multiple clinicians (the inter-observer variability) was found to be minimal, with intraclass correlation coefficients (ICCs) ≥ 0.995. Lastly, we explored differences in fetal anthropometric growth trajectories, estimated with fractional polynomial mixed-effects prediction models, with increased maternal first trimester air pollution exposure, estimated by

spatial interpolation models of exposure assessment. Increased maternal exposure to air pollution in the first trimester was negatively associated with the growth of fetal anthropometric parameters at various periods of gestation; point estimates of effect varied by air pollutant and fetal parameter.

Reductions of fetal parameter growth trajectories associated with increased air pollution exposure lend support to the continued review and enforcement of existing air pollution standards, and efforts to reduce exposure to pollution, especially among vulnerable populations.

CHAPTER I
INTRODUCTION

1.1 Intrauterine Growth Restriction

Prevalence rates of intrauterine growth restriction (IUGR) widely differ across geographic populations, and often within national statistics, depending on the chosen definition of IUGR. Nonetheless, IUGR is the second leading cause of perinatal mortality and is estimated to occur in 5% of the general obstetric population (Peleg et al. 1998). In developing countries, approximately 20 million babies are diagnosed as growth restricted infants (Imdad et al. 2011). In some studies, excluding congenital anomalies, IUGR has been implicated as a causal factor in 50% of stillbirths (Figueras and Gardosi 2011). An estimated 72% of unexplained deaths have also been associated with fetal growth restriction (Mandruzzato et al. 2008).

Statistics on fetal growth restriction are often nested in national and global rates of perinatal mortality and various adverse birth outcomes such as low birth weight, preterm and stillbirth. According to the World Health Organization (WHO), 2.6 million stillbirths occurred worldwide in 2009, a decrease of only 1.1% in comparison to the 3 million reported in 1995. Just over 4% of these reported stillbirths could have been averted with the detection and management of fetal growth restriction (World Health Organization 2011). The WHO analysis also highlighted that 98% of these occurrences happened in low and middle income countries. Similar statistics exist explaining the

contribution of IUGR to incidence of other adverse pregnancy outcomes such as premature delivery.

In addition to contributing to neonatal death, morbidity risks may depend on the duration of growth restriction. Longer periods of slowed fetal growth *in utero* have been associated with increased perinatal morbidity (Illa et al. 2009). Recent reviews suggest that this same principle can be applied to the findings of a case control study that examined IUGR and cerebral palsy. Risk of cerebral palsy increased with growth restriction occurring closer to term but not with IUGR during early pregnancy (Figueras and Gardosi 2011).

1.1.1 Distinction between Common Indicators of Fetal Growth Restriction in Population Studies

Globally, the assessment of low birth weight (LBW), defined as weighing less than 2500 grams at birth, has become a widely accepted indicator of poor fetal health (Balcazar and Haas 1991; Kannan et al. 2006; Lee B.E. 2003; Kramer 1987). Both preterm birth and IUGR are etiologies of LBW. Preterm infants are those born before 37 weeks of gestation and IUGR refers to any process that is capable of limiting intrinsic fetal growth potential *in utero*. Therefore it is important to note that being classified as IUGR is not the same as being preterm and vice versa (Balcazar and Haas 1991; Kannan et al. 2006; Gardosi et al. 2011; Haas et al. 1987; Semba and Bloem 2008).

In clinical settings, IUGR is diagnosed with the use of the fetal biometry profile, that is, measurements of size and rate of growth for specific parameters: head circumference (HC), abdominal circumference (AC), femur length (FL), and biparietal

diameter (BPD) (Carrera 2001), and is not commonly assessed in epidemiological studies. Distinctions should be made between these indicators of fetal growth when interpreting results of population studies. For example, in developing countries like Mexico, most cases of LBW infants are typically the result of growth restriction, rather than duration of gestation (preterm births) (Balcazar and Haas 1991; Kramer 1987; Semba and Bloem 2008; Carrera 2001).

Similarly, previous epidemiological studies have classified an infant as having experienced IUGR based on observations made at birth. Infants have also been identified as small for gestational age (SGA), defined as having a birth weight less than the 10^{th} percentile based on sex and gestational age (World Health Organization 2008), which introduces a third common indicator of fetal growth that is often used synonymously with IUGR. Based on this definition on fetal growth restriction, approximately 80-85% of diagnosed infants are actually constitutionally small, and 10-15% are pathologically small and intrauterine growth restricted (Saleem et al. 2011).

In addition to distinguishing between growth indicators, understanding levels of each indicator is also of importance. Since the knowledge and understanding of LBW and small for gestational age (SGA) is more common in epidemiological studies than the clinical concept of IUGR, much of the focus of this dissertation will be placed on IUGR. Several methods have been suggested to classify types of IUGR on the basis of sonographic features. These include examining trends in the curves of fetal parameters separately, as well as the use of fetal parameter ratios, (e.g., HC/AC ratio). In practice, IUGR is classified as proportionate (symmetrical) or disproportionate (asymmetrical) by calculating the ponderal index of Rohrer [(fetal weight / fetal length (crown-rump)^3) x

100]. If the Rohrer Index (RI) is normal (≥ 2.20), IUGR is considered symmetrical; if it is abnormally low, IUGR is considered asymmetrical (Carrera, JM 2001).

Although birth outcome literature is lacking for the Mexico City population, earlier studies examining fetal growth patterns in the Mexico City population found that in cases where growth restriction occurred, the type of growth restriction, proportionate or disproportionate, produced different birth outcomes. Results from an earlier Mexico City study reported that preterm and term infants diagnosed as having proportionate growth restriction exhibited 1.5 and 9.5 times the early neonatal mortality of preterm and term infants with disproportionate growth restriction, respectively (Balcazar and Haas 1991). Another analysis used birth and first 48 hours death records from 10,024 live born infants in Mexico City and 12,786 live born infants in Santa Cruz, Bolivia, to characterize early postnatal mortality rates by different types of IUGR and prematurity. The authors reported that proportionate growth restriction, according to the Rohrer's Index, was found to carry a 1.4-2.01 elevation in risk of neonatal death, and disproportionate growth restriction carried a 2.9-5.7 elevated risk of neonatal death (Haas et al. 1987) .

Knowing and understanding the health risks associated with being growth-restricted is only possible once a working definition of growth-restriction has been established. A current limitation in epidemiological birth outcome literature is the inability to assess IUGR during gestation, resulting in the use of various indicators of the fetal growth process interchangeably, when clinically they differ to some degree. Classifying infants as intrauterine growth-restricted at birth may result in biased effect estimates as a result of misclassification for two major reasons. 1) Clinical literature

specifies that being growth-restricted and SGA are not synonymous. Intrauterine growth restriction is the assessment of actual changes in fetal parameter measurements collected during gestation, where SGA is a statistical concept that identifies overall growth restriction by comparing neonate birth measurements of weight to population percentiles of weight (Carrera, JM 2001; Kingdom and Baker 2000). Small for gestational age has become a widely accepted proxy for the diagnosis of IUGR, mostly because previous studies were limited to the use of retrospective data, and therefore could only utilize national and population-specific growth curves to assess growth patterns (Semba and Bloem 2008); 2) The use of birth weight as a proxy for fetal growth may not be the best endpoint since recent literature has shown that birth weight poorly reflects IUGR during the first two trimesters (Hemachandra et al. 2006). The use of LBW as an endpoint for IUGR assessment ignores the possibility that injury to growth could occur during one time period, but the fetus could continue to grow and achieve population growth standards by birth (Woodruff et al. 2009; Hemachandra and Klebanoff 2006) This is also supported by an earlier clinical model that examined fetal growth occurring in early and late stages of pregnancy. That model stated that early fetal growth is attributed to hyperplasia (cell proliferation), and later growth to hypertrophy (cell growth). The author then proposed that agents that damage the fetus during the first trimester may reduce the cell population, thereby causing a permanent hindrance to the growth potential, but that the damage caused later in gestation would reduce the cell size but the infant could potentially later catch-up to predicted growth (Winick, M 1974).

1.2 Air Pollution and Fetal Growth Restriction

1.2.1 Criteria Air Pollutants

Although there are several atmospheric toxicants that can negatively affect human health, the focus of this dissertation is on maternal exposures to air pollutants commonly studied in epidemiological assessments. Atmospheric air pollution is a complex mixture of primary and secondary compounds in gaseous and solid physical states. The United States Environmental Protection Agency (US EPA) has identified six "criteria" pollutants of interest for environmental and human health research. These pollutants are particulate matter of two size fractions (less than 10 micrometers in aerodynamic diameter, PM_{10} and less than 2.5 micrometers, $PM_{2.5}$), ozone (O_3), nitrogen dioxide (NO_2), carbon monoxide (CO), sulfur dioxide (SO_2) and lead (Pb) (US EPA 2012) . The focus of this dissertation is on maternal exposures to $PM_{2.5}$ and O_3, as these are two major contributors of air pollution in Mexico City, Mexico that remain with annual concentrations that exceed the previously set health standards (National Institute of Ecology 2011).

1.2.2 Particulate Matter

The most visible form of air pollution, particulate matter (PM), can best be described as airborne particles of varying sizes in diameter. Particulate matter contributes to the atmospheric pollution by natural and anthropogenic sources of emission. The natural sources of particulate matter include dust, pollen, and ash, to name a few. However, the most harmful sources of particulate matter are a result of human activities (Girard 2010a). Anthropogenic sources of particulates include, but are not limited to coal-fired power plants, industrial processes, and transportation emissions. The emission source of PM and its atmospheric chemistry after emission determines the particle composition, which

ultimately is a measure of how harmful exposure to a specific type of PM may be. Depending on the source, particulates may consist of various classes of metals, oxides, and polycyclic hydrocarbons. Exposure to PM is also dependent on the size of the particles. Larger particles (diameters > 10 micrometers) do not travel as far and do not stay suspended in the atmosphere as long as medium-sized particles (diameters 1-10 micrometers) and fine particles (diameters < 1 micrometer). These smaller particles not only stay in the air longer, but once ingested or inhaled they remain in the body longer also (Girard 2010a). In the general population PM exposures have been associated with respiratory, cardiovascular and cerebrovascular mortality (Anderson et al. 2012). However the focus of this dissertation is on fetal health outcomes, more specifically fetal growth restriction. According to a recent review, increases in particulate matter exposures in early pregnancy are associated with risk of being intrauterine growth restricted (according to the SGA definition of IUGR) (Glinianaia et al. 2004).

1.2.3 Ozone

Existing in the stratosphere as a protectant from ultraviolet rays, ozone (O_3) is a harmful, colorless, harsh smelling pollutant in the troposphere. Ozone is a secondary aerosol formed as the result of chemical reactions between nitrogen oxides and volatile organic compounds. High temperatures on sunny days contribute to the production of unhealthy ozone levels (US EPA 2012) . Exposure may irritate the nasal passages and the eye among the general population, and low level exposures may exacerbate previous respiratory and cardiovascular diseases (Girard 2010b). Specific to Mexico City, increased exposure to ozone was associated with increased mortality in the general population with higher increases among the elderly (O'Neill et al., 2004). Maternal

exposure to ozone has been associated with reduced birth rate and an increased risk of IUGR (Salam et al. 2005).

1.2.4 Hypothesized biological mechanisms

Although many of the biological mechanisms explaining the association between air pollution and birth outcomes are not completely understood, during gestation, physiological changes occur that may make the expecting woman more susceptible to air pollution exposures. First, compared to non-pregnant women, pregnant women experience an approximate 50% increase in their alveolar ventilation rate (Hackley et al. 2007). This could cause an increased uptake of inhaled pollutants, allowing for a potential increase in the amount of fine particulates that are inhaled. The $PM_{2.5}$ contains a mixture of metals and harmful constituents that can be introduced into the blood stream (Hackley et al. 2007). Another potential influence of maternal physiology on the possible link between air pollution and fetal health is the fact that 80% of women develop hemodilutional anemia during pregnancy (Hackley et al. 2007; www.Rxmed.com). As a result of the anemia, the volume of blood increases, which means an increase in plasma, and a reduction in the concentration of red blood cells and hemoglobin. Since hemoglobin is the oxygen delivery system to the fetus, and it has a higher binding affinity to carbon monoxide than oxygen, combined with exposure to a pollutant such as carbon monoxide this poses a threat to the amount of oxygen delivered to the fetus, and poses a potential danger for fetal hypoxia (Kingdom and Baker 2000; Hackley et al. 2007; Shah and Balkhair 2011; Veras et al. 2008) .

Recent reviews have highlighted potential biological mechanisms explaining air pollution and adverse fetal outcomes (Shah and Balkhair 2011; Veras et al. 2010). Prenatal

exposure to NO_2, SO_2, and PM can lead to oxidative inflammation in the lungs and the placenta which leads to the systemic release of cytokines which can trigger preterm birth. Nitrogen dioxide has also been suspected to induce lipid peroxidation in the placenta, disrupting fetal development. Polycyclic aromatic hydrocarbons (PAHs) become harmful as a result of metabolic activation that converts the bulky compounds into electrophiles that react with DNA (Hecht, S 2008). Polycyclic aromatic hydrocarbon exposure can lead to the formation of DNA adducts causing cell mutation or cellular death, and an increase in blood viscosity which reduces the amount of blood flow to the placenta and uterus (Shah and Balkhair 2011; Veras et al. 2010; Perera et al. 2005; Perera et al. 1998). Many of these hypothesized relationships have been explained in detail in a recent workshop report (Figure 1; duplicated from (Slama et al. 2008)).

The patho-physiological mechanisms described in the literature have provided a foundation to properly identify and understand the mechanistic process responsible for the reported changes in fetal physiology at birth associated with air pollution. However, evidence quantifying how fetal growth may be associated with exposure to specific pollutants during critical periods of gestation is needed to evaluate these hypothesized mechanisms.

1.3 Previous Assessment of Air Pollution and Birth Outcomes

Previous epidemiological studies have reported associations between ambient and traffic related air pollution and birth outcomes, including LBW preterm delivery, SGA, and IUGR (Glinianaia et al. 2004; Perera et al. 2005; Perera Frederica P. 1998; Bell et al. 2010; Parker et al. 2011; Ritz et al. 2007; Maisonet et al. 2004; Rich et al. 2009). Reviews of the literature have highlighted the heterogeneity between methodologies,

study populations, birth outcomes, and exposure periods of interest among research studies within the last decade (Glinianaia et al. 2004; Shah and Balkhair 2011; Maisonet et al. 2004). These differences could potentially explain the variation in reported associations between maternal exposure to air pollutants and reproductive outcomes.

To briefly summarize by pollutant and exposure period, statistically significant associations have been determined to exist between third trimester 100 $\mu g/m^3$ increments in total suspended particles (TSP), increases in first trimester interquartile range (IQR) TSP and term LBW (Maisonet et al. 2004). Increases in PM_{10} exposure $\geq$ 40 $\mu g/m^3$ and $PM_{2.5}$ increases $\geq$ 37 $\mu g/m^3$ in the first month of pregnancy have been significantly associated with SGA (Maisonet et al. 2004; Dejmek et al. 1999). Intrauterine growth restriction has also been associated with an increase in 10 ppb of NO_2 during the first month of pregnancy (Shah and Balkhair 2011). Third trimester concentrations of O_3 CO, SO_2, and IUGR associations have also been reported in previous literature (Glinianaia et al. 2004; Shah and Balkhair 2011; Maisonet et al. 2004).

Measurement metrics used in previous literature ranged from city-wide averages, nearest monitor, inverse distance weighting, and personal measures. The use of ambient air monitors compared to personal monitors is an issue that has been studied given potential for exposure misclassification (Shah and Balkhair 2011). In most cases, personal exposures are higher than outdoor exposures. However, depending on the pollutant and the sampling season, personal exposures may be significantly associated with ambient pollutant concentrations (Rojas-Bracho et al. 2000; Sarnat et al. 2005). Recent reviews have reported that few studies looking at reproductive outcomes chose to use direct measures of personal exposure to ambient air pollution (Glinianaia et al. 2004;

Shah and Balkhair 2011; Maisonet et al. 2004; Woodruff et al. 2009). Perhaps this is attributed to the potential burden that may be added to the women's condition by wearing the "light weight" backpack for a series of sampling days.

1.4 Dissertation Overview

1.4.1 Chapter Two: Clinical Assessments of IUGR for Epidemiological Studies

Chapter two of this dissertation aims to address a current limitation in the previous literature that assessed maternal exposure to air pollutants and various birth outcomes. A report from a workshop held in 2007 to discuss methodological issues and the current status of epidemiological research pertaining to this topic agreed that a better understanding of the biological mechanisms explaining the reported associations between air pollution and adverse birth outcomes is needed. Currently the primary indicator of fetal health is birth weight and the authors of the review highlight the difficulty in distinguishing between reduced birth weight as a result of actual fetal growth restriction, preterm delivery, or a combination of both etiologies. (Woodruff et al., 2009). The authors of the previous review identified a possible solution to this issue, which was first used by (Slama et al., 2006): ultrasound measurements of specific fetal parameters at birth. While this approach assists with confirmation that a neonate is growth restricted at birth, serial ultrasound measurements of fetal parameters should be used to accurately assess changes in growth patterns *in utero*, thereby accurately identifying growth restricted fetuses. To date, few studies have utilized fetal ultrasound data to analyze pregnancy outcomes associated with maternal exposure to air pollutants, and the literature that does exist also consists of varying methodological approaches. Therefore chapter two of the present dissertation aims to support the use of ultrasound

measurements as an alternative method for assessing fetal growth restriction in our study population by discussing the results of a literature review of epidemiological studies where ultrasound measurements were used to assess fetal health in response to air pollution exposures.

1.4.2 Chapter Three: Interobserver Assessment of Ultrasound Measurements of Fetal Parameters

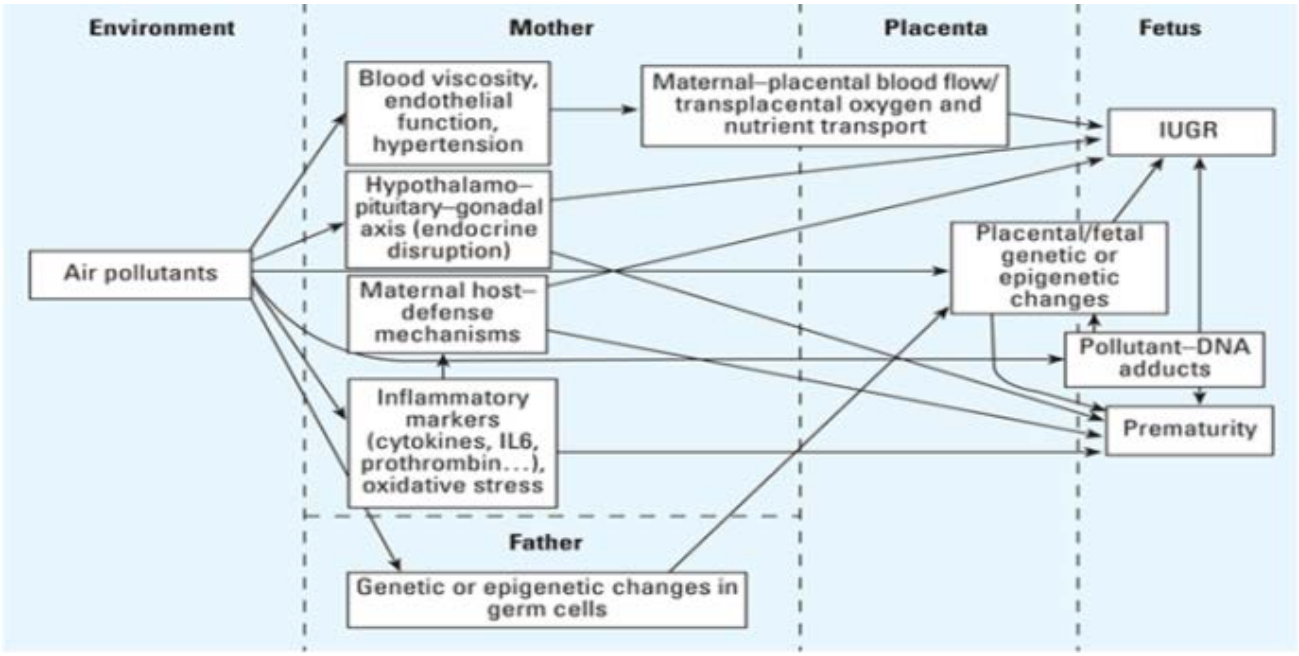
The quality control/assurance of any methodology is a necessary assessment that adds to the understanding of method strengths and limitations. While this dissertation proposes the use of serial ultrasound measurements in epidemiological studies of fetal growth because of the known strengths of this approach, discussed in detail in previous sections, uncertainty regarding the reliability of fetal anthropometric measurements captured by sonography remains a concern in both the clinical and epidemiological setting (Perni et al. 2004; Ritz et al. 2014). A systematic review of the methodology used in studies whose purposes were to create fetal ultrasound reference charts found there to be substantial heterogeneity in fetal biometry methodologies, including the performance of quality assurance measures such as interobserver agreement assessments (Ioannou et al. 2012). Given that measurement error can be both systematic (e.g., related to clinician training, physical/mental state during exam) and random (e.g., biological variation in anthropometric parameters, mechanical variation in equipment), an assessment of measurement error reduces uncertainty pertaining to reliability of fetal biometry. Chapter 3 of this dissertation aims to quantitatively assess the reliability of the collected ultrasound data to be used in the subsequent dissertation analysis.

1.4.3 Chapter Four: First Trimester Exposure to Air Pollution and IUGR during Pregnancy

The link between air pollution and birth outcomes has not been explored as it relates to the densely populated and heavily polluted Mexico City, Mexico (Smarr et al., 2013). Methodological studies (Woodruff et al., 2009) have proposed that the use of spatial and temporal models, proper adjustment for confounding, and the identification of 'critical windows of exposure' may improve exposure assignments in birth outcome studies. This idea lends support to the findings in (O'Neill et al., 2002) that suggest differences in methods used to estimate PM_{10} exposure in Mexico City may be important to consider when assessing related health effects. A more recent analysis (Brauer et al., 2008) compared birth outcomes assessed using exposure metrics estimated by different methods. Specific to Mexico City, an analysis was conducted to explore the differences in odds of being born preterm associated with exposure to air pollutants evaluated by different exposure metrics in the ELEMENT cohort (Rivera-González 2012).

Lastly, chapter five of this dissertation summarizes the overall hypothesis, objectives, and major findings of each research specific aim. Future directions and exploratory aims relevant to maternal exposure to air pollutants and fetal health are explained in this final section of the dissertation.

Figure I-1. Potential mechanisms explaining air pollution IUGR



1.5 REFERENCES

Peleg D, Kennedy CM, Hunter SK. 1998. Intrauterine Growth Restriction: Identification and Management. Am Fam Physician 58(2): 453-460.

Imdad A, Yakoob MY, Siddiqui S, Bhutta ZA. 2011. Screening and triage of intrauterine growth restriction (IUGR) in general population and high risk pregnancies: a systematic review with a focus on reduction of IUGR related stillbirths. BMC public health 11 Suppl 3: S1.

Figueras F, Gardosi J. 2011. Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. American journal of obstetrics and gynecology 204(4): 288-300.

Mandruzzato G, Antsaklis A, Botet F, Chervenak FA, Figueras F, Grunebaum A, et al. 2008. Intrauterine restriction (IUGR). Journal of perinatal medicine 36(4): 277-281.

Organization WH. 2011. 2.6 million babies stillborn in 2009 : New global and country estimates published in Lancet Series.

Illa M, Coloma JL, Eixarch E, Meler E, Iraola A, Gardosi J, et al. 2009. Growth deficit in term small-for-gestational fetuses with normal umbilical artery Doppler is associated with adverse outcome. Journal of perinatal medicine 37(1): 48-52.

Balcazar H, Haas JD. 1991. Retarded Fetal Growth and Early Neonatal Mortality in a Mexico City Population. Bulletin of PAHO.

Kannan S, Misra DP, Dvonch JT, Krishnakumar A. 2006. Exposures to Airborne Particulate Matter and Adverse Perinatal Outcomes: A BiologicallyPlausible Mechanistic Framework for Exploring Potential Effect Modification by Nutrition. Environmental health perspectives 114(11): 1636-1642.

Lee BE , Park H.S., Kim Y.J., Hong Y.C., Kim H, Lee J.T. 2003. Exposure to air pollution during different gestational phases contributes to risks of low birth weight. Human Reproduction 18(3): 638-643.

Kramer MS. 1987. Determinants of low birth weight: methodological assessment and meta-analysis. Bulletin of World Health Organization 65(5): 663-737.

Gardosi J, Figueras F, Clausson B, Francis A. 2011. The customised growth potential: an international research tool to study the epidemiology of fetal growth. Paediatric and perinatal epidemiology 25(1): 2-10.

Haas J, Balcazar H, Caulfield L. 1987. variation in early neonatal mortality for different types of fetal growth retardation. American Journal Of Physical Anthropology 73(467-473).

Semba RD, Bloem MW. 2008. Nutrition and health in developing countries NJ , USA: Humana Press, 63-86.

Carrera J. 2001. Ultrasound and Fetal Growth. New York ; London: Parthenon.

Saleem T, Sajjad N, Fatima S, Habib N, Ali SR, Qadir M. 2011. Intrauterine growth retardation--small events, big consequences. Italian journal of pediatrics 37: 41.

Kingdom J, Baker P. 2000. Definitions of Intrauterine Growth Restriction In: Intrauterine Growth Restriction : Aetiology and Management, (Philip B, ed). London:Springer, 1-4.

Hemachandra AH, Klebanoff MA, Duggan AK, Hardy JB, Furth SL. 2006. The association between intrauterine growth restriction in the full-term infant and high blood pressure at age 7 years: results from the Collaborative Perinatal Project. International journal of epidemiology 35(4): 871-877.

Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. 2009. Methodological issues in studies of air pollution and reproductive health. Environmental research 109(3): 311-320.

Hemachandra AH, Klebanoff MA. 2006. Use of serial ultrasound to identify periods of fetal growth restriction in relation to neonatal anthropometry. American journal of human biology : the official journal of the Human Biology Council 18(6): 791-797.

Winick M. 1974. Nutrition and fetal development. . New York: : Wiley.

EPA US. 2012. National Ambient Air Quality Standards (NAAQS).

Ecologia INd. 2011. Zona Metropolitana del Valle de México. In: Cuarto almanaque de datos y tendencias de la calidad del aire en 20 ciudades mexicanas (2000-2009). Mexico City, Mexico, 410.

Girard JE. 2010a. The Earth's Atmosphere. In: Principles of Environmental Chemistry, Part Second. Sudbury Massachusetts Jones and Bartlett, 40-65.

Anderson JO, Thundiyil JG, Stolbach A. 2012. Clearing the air: a review of the effects of particulate matter air pollution on human health. Journal of medical toxicology : official journal of the American College of Medical Toxicology 8(2): 166-175.

Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D. 2004. Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. Epidemiology 15(1): 36-45.

US EPA. 2012. Ground-level Ozone. Available: http://www.epa.gov/glo/basic.html [accessed January 14, 2014.

Girard JE. 2010b. Chemistry of the Troposphere. In: Principles of Environmental Chemistry Part Second. Sadbury, Massachusetts:Jones and Bartlett, 92-123.

O'Neill MS, Loomis D, Borja-Aburto VH. 2004. Ozone, area social conditions, and mortality in Mexico City. Environmental research 94(3): 234-242.

Salam MT, Millstein J, Li YF, Lurmann FW, Margolis HG, Gilliland FD. 2005. Birth outcomes and prenatal exposure to ozone, carbon monoxide, and particulate matter: results from the Children's Health Study. Environmental health perspectives 113(11): 1638-1644.

Hackley B, Feinstein A, Dixon J. 2007. Air pollution: impact on maternal and perinatal health. Journal of midwifery & women's health 52(5): 435-443.

Med R. illness information. Available: http://www.rxmed.com/b.main/b1.illness/b1.1.illnesses/anemia_during_pregnancy.html.

Shah PS, Balkhair T. 2011. Air pollution and birth outcomes: a systematic review. Environment international 37(2): 498-516.

Veras M, Damaceno-Rodrigues N, Caldini E, Maciel R, Mayhew T, Saldiva P, et al. 2008. Particulate urban air pollution affects the functional morphology of mouse placenta. Biology of reproduction 79(3): 578-584.

Veras M, Caldini E, Dolhnikoff M, Saldiva P. 2010. Air pollution and effects on reproductive-system functions globally with particular emphasis on the Brazilian population. Journal of toxicology and environmental health Part B, Critical reviews 13(1): 1-15.

Hecht SS. 2008. Human Phenanthrene Metabolites as Probes for the Metabolic Activation and Detoxification of Carcinogenic Polycyclic Aromatic Hydrocarbons. 1-22.

Perera FP, Tang D, Rauh V, Lester K, Tsai WY, Tu YH, et al. 2005. Relationships among Polycyclic Aromatic Hydrocarbon–DNA Adducts, Proximity to the World Trade Center, and Effects on Fetal Growth. Environmental health perspectives 113(8): 1062-1067.

Perera Frederica P. WRM, Jedrychowski Wieslaw, Rauh Virginia, Manchester David, Santella Regina M., Ottman Ruth 1998. A Study of the Effects of Environmental Polycyclic Aromatic Hydrocarbons on Birth Outcomes in Poland. American journal of epidemiology 147(3): 309-314.

Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. 2008. Meeting report: atmospheric pollution and human reproduction. Environmental health perspectives 116(6): 791-798.

Bell ML, Belanger K, Ebisu K, Gent JF, Lee HJ, Koutrakis P, et al. 2010. Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources. Epidemiology 21(6): 884-891.

Parker JD, Rich DQ, Glinianaia SV, Leem JH, Wartenberg D, Bell ML, et al. 2011. The international collaboration on air pollution and pregnancy outcomes: initial results. Environmental health perspectives 119(7): 1023-1028.

Ritz B, Wilhelm M, Hoggatt KJ, Ghosh JK. 2007. Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles. American journal of epidemiology 166(9): 1045-1052.

Maisonet M, Correa A, Misra D, Jaakkola JJ. 2004. A review of the literature on the effects of ambient air pollution on fetal growth. Environmental research 95(1): 106-115.

Rich DQ, Demissie K, Lu SE, Kamat L, Wartenberg D, Rhoads GG. 2009. Ambient air pollutant concentrations during pregnancy and the risk of fetal growth restriction. Journal of epidemiology and community health 63(6): 488-496.

Dejmek J, Selevan SG, Benes I, Solansky I, Srim RJ. 1999. Fetal Growth and Maternal Exposure to Particulate Matter during Pregnancy. Environmental health perspectives 107(6): 475-480.

Rojas-Bracho L, Suh HH, Koutrakis P. 2000. Relationships among personal, indoor, and outdoor fine and coarse particle concentrations for individuals with COPD. Journal of Exposure Analysis & Environmental Epidemiology 10(3): 294-306.

Sarnat JA, Brown KW, Schwartz J, Coull BA, Koutrakis P. 2005. Ambient gas concentrations and personal particulate matter exposures: implications for studying the health effects of particles. Epidemiology 16(3): 385-395.

Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. 2009. Methodological issues in studies of air pollution and reproductive health. Environmental research 109(3): 311-320.

Slama RS-T, S ; Thiebaugeorges, O ; Goua, V ; Forhan, A ; Ducot, B ; Annesi-Maesano, I ; Heinrich, J ; Schweitzer, M ; Magnin, G ; Bouyer, J ; Kaminski, M ; Charles, M A. . 2006. Relation Between Atmospheric Pollutants and Head Circumference in Utero and at Birth: a Cohort Study Relying on Ultrasound Imaging During Pregnancy.[Abstract]. Epidemiology 17(6 (Suppl)): S129-S130.

Perni S, Chervenak F, Kalish R, Magherini-Rothe S, Predanic M, Streltzoff J, et al. 2004. Intraobserver and interobserver reproducibility of fetal biometry. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 24(6): 654-658.

Ritz B, Qiu J, Lee PC, Lurmann F, Penfold B, Erin Weiss R, et al. 2014. Prenatal air pollution exposure and ultrasound measures of fetal growth in Los Angeles, California. Environmental research 130: 7-13.

Ioannou C, Talbot K, Ohuma E, Sarris I, Villar J, Conde-Agudelo A, et al. 2012. Systematic review of methodology used in ultrasound studies aimed at creating charts of fetal size. BJOG : an international journal of obstetrics and gynaecology 119(12): 1425-1439.

Smarr MM, Vadillo-Ortega F, Castillo-Castrejon M, O'Neill MS. 2013. The use of ultrasound measurements in environmental epidemiological studies of air pollution and fetal growth. Current Opinion in Pediatrics 25(2): 10.1097/MOP.1090b1013e32835e32831e32874.

O'Neill MS, Loomis D, Torres Meza V, Retama A, Gold D. 2002. Estimating particle exposure in the Mexico City metropolitan area. Journal of exposure analysis and environmental epidemiology 12(2): 145-156.

Brauer M, Lencar C, Tamburic L, Koehoorn M, Demers P, Karr C. 2008. A Cohort Study of Traffic-Related Air Pollution Impacts on Birth Outcomes. Environmental health perspectives 116(5): 680-686.

Rivera-González LO. 2012. Environmental Toxicants And Preterm Birth In Mexico City: A Geo-Spatial Approach For Use In Epidemiology. Ann Arbor, Michigan: University of Michigan.

O'Neill M, Osornio-Vargas A, Buxton MA, Sanchez BN, Rojas-Bracho L, Castillo-Castrejon M, et al. 2012. Air pollution, inflammation and preterm birth in Mexico City: Study design and methods. The Science of the total environment.

CHAPTER II
THE USE OF ULTRASOUND MEASUREMENTS IN ENVIRONMENTAL EPIDEMIOLOGICAL STUDIES OF AIR POLLUTION AND FETAL GROWTH

2.1 Abstract

Recently, several international research groups have suggested that studies about environmental contaminants and adverse pregnancy outcomes should be designed to elucidate potential underlying biological mechanisms. The purpose of this review is to examine the epidemiological studies addressing maternal exposure to air pollutants and fetal growth during gestation as assessed by ultrasound measurements. The six studies discussed in this review found that exposure to certain ambient air pollutants during pregnancy is negatively associated with the growth rates and average attained size of fetal parameters belonging to the growth profile. Fetal parameters may respond to maternal air pollution exposures uniquely, and this response may vary by pollutant and timing of gestational exposure. Current literature suggest that mean changes in head circumference (HC), abdominal circumference (AC), femur length (FL), and biparietal diameter (BPD) are negatively associated with early-pregnancy exposures to ambient and vehicle-related air pollution. The use of more longitudinal studies, employing ultrasound measures to

assess fetal outcomes, may assist with the better understanding of mechanisms responsible for air pollution related pregnancy outcomes.

2.2 Introduction

Maternal exposures to environmental contaminants during pregnancy are a global public health concern. Previous literature has reviewed studies on the relationship between air pollutants and pregnancy outcomes including low birth weight, small for gestational age (SGA), and preterm birth. [1-4] These studies contribute to current understanding of the adverse effects of air pollution exposure during pregnancy.

However, these studies commonly lack an assessment of how these exposures affect fetal growth over the course of the pregnancy, and whether certain times in gestation are particularly critical. Fetal growth outcomes studied include weight and anthropometric measurements of abdominal circumference (AC) or head circumference (HC), collected at the time of birth. However, assessment of fetal health at birth does not fully capture the timing of changes to fetal growth and development during gestation. Birth weight is not an adequate single predictor of fetal health since birth weight varies by factors such as race and gender. [5-7] Also, low birth weight can result from both growth restriction and preterm birth, [8, 9] so determining if growth restriction, preterm birth or a combination played a role is difficult when weight and dimensions of the baby are assessed only at birth.

2.3 Estimation of Maternal Exposure to Air Pollution during Pregnancy

Air pollution is a heterogeneous mixture of particles and gases. People can be exposed to air pollution inside their homes or other buildings (from indoor sources and/or infiltration of outdoor pollutants); in the workplace; outdoors; and commuting. Effects of

air pollution exposure on respiratory and cardiovascular health, and more recently on birth outcomes, are well documented. [10-12*] Many studies obtain daily concentrations of air pollutants from outdoor monitors and match them to the dates of the pregnancy, creating overall pregnancy or trimester-specific exposure estimates. Commonly measured outdoor pollutants include (ozone (O_3), particulate matter less than 10 and 2.5 micrometers in aerodynamic diameter (PM_{10} and $PM_{2.5}$), carbon monoxide (CO), nitrogen dioxide (NO_2), and sulfur dioxide (SO_2)). Other pollutants of concern include volatile organic compounds and specific components of particles (metals, organics). As an alternative or supplement to pollutant concentrations from outdoor monitors, pollutant exposure during pregnancy can be estimated based on personal or indoor monitoring of the study participants. [13] Other studies evaluate 'biomarkers' of pollutant exposure, such as oxidative stress markers in urine or DNA adducts in blood.[14, 15]

Mechanisms hypothesized for how air pollutant exposure may cause preterm birth or reduce fetal growth include oxidative stress, endocrine disruption, pulmonary and placental inflammation, blood viscosity and hemodynamic responses.[10, 16] During gestation, physiological changes may make the expecting woman more susceptible to air pollution exposures. Compared to a non-pregnant woman, pregnant women experience an approximate fifty percent increase in alveolar ventilation rate.[17] This allows for increased uptake of inhaled pollutants, including fine particulates. Some particulate matter constituents can enter the blood stream. Common gestational complications include development of hemodilutional anemia. This could result in decreased oxygen binding capacity. Since carbon monoxide (CO) is a common air pollutant, with higher binding affinity to hemoglobin than oxygen, an increase in CO exposures among

pregnant women could reduce the amount of oxygen delivered to the developing fetus. DNA damage and oxidative stress are other potential mechanisms. Polycyclic aromatic hydrocarbons (PAHs) are a class of compounds related to vehicle emissions that are known carcinogens and DNA damaging agents. Markers of PAH exposure (formation of PAH DNA-adducts in maternal and cord blood) have been studied in relation to adverse birth outcomes. [15, 18]

2.4 Ultrasound Measures of Fetal Growth

Previous epidemiology literature has relied on birth registry data to assess pregnancy outcomes associated with maternal exposure to air pollutants. [3, 4, 19] While this may be useful for evaluating birth weight and preterm birth, a late assessment of growth restriction could potentially introduce bias. Classifying infants as growth-restricted at birth may result in biased effect estimates as a result of misclassification for two major reasons. 1) Clinical literature specifies that being growth-restricted and small for gestational age are not synonymous. [*20, 21] 2) Birth weight as a proxy for fetal growth may not be the best endpoint since recent literature has shown that birth weight poorly reflects IUGR during the first two trimesters and ignores the possibility that growth impairment affecting long-term health could occur during one time period, but the fetus could continue to grow and achieve population growth standards by birth.[5, 22]

Recent reviews suggest that reducing the time between exposure and outcome assessment may result in more accurate classifications of growth restriction *in utero*. [5, 23] Ultrasound technology is a classic clinical methodology that can be used for assessment of fetal growth during pregnancy. Ultrasonography is widely used in prenatal care to estimate gestational age, assess fetal growth, and determine physical abnormalities and

various other vital assessments. In clinical settings, IUGR can be diagnosed with the use of the fetal growth profile, that is, measurements of HC, AC, femur length (FL), and biparietal diameter (BPD) by ultrasound scans.[24]Identifying growth restriction *in utero* could potentially elucidate specific mechanisms explaining growth restriction related to environmental exposures.

2.5 Studies of Air Pollution and IUGR

To identify epidemiology studies addressing the link between air pollution and IUGR, keyword and reference lists searches using PubMed were conducted using the key words "air pollution, fetal growth and ultrasound" This search identified eight studies examining growth restrictions associated with maternal air pollution exposures during gestation. Six studies, summarized in Table 1[25-30] covered two categories of outdoor air pollution; ambient and vehicle-related. Two other studies primarily focused on environmental tobacco smoke (ETS) [*31, 32] and are not discussed here.

2.6 Ambient Air Pollution and IUGR

The first published study to employ ultrasound scans to measure fetal growth in association with maternal exposure to ambient air pollution was set in France in the EDEN study of pre- and early postnatal determinants of the child's development and health. [25] Maternal exposure to atmospheric nitrogen dioxide (NO_2) was examined in relation to changes of *in utero* measurements of HC. Study participants were recruited from two French maternity hospitals at less than 24 weeks of gestation. Individual NO_2 exposures were estimated from time of fertilization to the date of each ultrasound exam for 366 women residing within 2 kilometers from the nearest monitor. Adjusted linear and logistic regression models were used to determine HC reductions and odds of HC

reduction at various time periods. HC measurements decreased 3.8 mm and 3.1 mm between 30-34 weeks and at birth, respectively, comparing the highest tertile of NO_2 exposure (NO_2 > 31.4 μg/m^3) with the lowest.

In another study in France, 271 nonsmoking pregnant women were recruited from two maternity hospitals at < 20 weeks of gestation to examine associations between IUGR and airborne benzene exposures.[27] The benzene exposures were estimated with measures from personal diffusive samplers worn for one week during the 27th gestational week. Fetal parameters measured during gestation and at birth included HC and BPD; BPD being the only parameter measured in each trimester. Adjusted linear regression models showed reductions in mean HC during each stage of pregnancy in association with elevated levels of log-transformed benzene exposures, with the greatest reported reduction of 1.9 mm at third trimester and at birth.

In a Brisbane, Australia cohort, associations between fetal ultrasound measurements during mid–pregnancy and ambient air pollution exposure during the first trimester were evaluated. This retrospective study collected 15,623 ultrasound scans from 14,734 Australian pregnancies.[26] The scans were originally collected to create a population specific growth curve, so eighty-four percent of the pregnancies had one scan, thirteen percent had two scans, and three percent had three ultrasound scans performed during pregnancy. The final analysis included only the scans of women living within two kilometers of pollutant monitors and only scans captured during gestational weeks 13-26; resulting in a varying total of scans (120-510) depending on the pollutant model. Exposure during pregnancy was estimated using air pollution and meteorological data from the Air Services Unit, Queensland Environmental Protection Agency. Utilizing 5

temperature monitors and 18 ambient air monitors, most within a 30 kilometer radius from Brisbane, hourly concentration readings were obtained for O_3; NO_2; SO_2 and PM_{10}. Daily averages were calculated for PM_{10}, NO_2, and SO_2, while an 8-hour average was calculated for O_3 and temperature. Ultrasound and air pollutant data were analyzed in a four stage regression model using generalized estimating equations (GEE). Reductions were reported for all parameters among exposed women, with AC being associated with most pollutants and reporting the highest decreases (-1.67 mm). Statistically significant reductions in HC, BPD, and AC associated with NO_2 exposures were seen only in models restricted to women who spent ≥ 15 hours/day at home.

2.7 Vehicle-Related Air Pollution and IUGR

The INMA (Spanish Children's Health and Environment) study in Sabadell, Spain performed a total of 1,692 ultrasound examinations measuring all four growth parameters for 562 pregnancies. The majority of the scans were one per woman; however, three percent of the women received 3-6 ultrasounds.[28] This study measured NO_2 and BTEX (benzene, toluene, ethylbenzene, *m/p*-xylene and *o*-xylene) as markers of motor vehicle exhaust. Exposure metrics were calculated using geospatial information systems techniques and land regression modeling to account for intra-urban variations in air pollution. The 57 monitoring sites used passive samplers to take one-week measurements, in three campaigns for NO_2 and four campaigns for BTEX. Pollutant averages were calculated as a proxy for annual mean concentrations. Land cover, topography, population density, roads, and distance to local sources of pollution were used as predictor variables in models estimating outdoor air pollution levels associated with home addresses of the study participants. The models were adjusted for the daily

variances in NO_2 concentrations observed at the stationary monitors. Mixed-effect models were used to estimate five windows of fetal exposure: from last menstrual period (LMP) to weeks 12, 20 and 32 of gestation; and average exposures during weeks 12-20, and 20-32. With these windows of exposure and the exposure models, average cumulative exposures were calculated for each woman during pregnancy. BTEX and NO_2 exposures during weeks 1-12 were associated with unadjusted mean reduction in BPD growth between 20-32 weeks: -0.124mm/week and -0.075 mm/week respectively. Results were similar for attained fetal size at 32 weeks of gestation.

The INMA study has several cohorts across Spain, including in Valencia. This cohort recruited 855 women; data from 785 were used examine the association between growth parameters and outdoor NO_2 exposures.[30] The study design was similar to the Sabadell cohort. However, linear-mixed models fit to the data revealed associations between higher NO_2 exposures and reductions in fetal parameter attained size at 32 weeks of 9% for all parameters except FL (6%). Length and HC at birth were reduced by 6% among mothers exposed to NO_2 levels above the median (38 $\mu g/m^3$).

The Generation R cohort in the Netherlands consisted of 8,880 pregnant mothers followed from years 2001 to 2005. The analysis sample consisted of 7,772 women with varying total ultrasound measurements.[29] Fetal FL and HC were the only ultrasound parameters measured in the Netherlands Generation R study. FL was a proxy for total body length and HC represented fetal development. Parameters were measured in each trimester. The number of scans per trimester differed because first trimester measurements were restricted to mothers with a normal (28 days) menstrual cycle and a known LMP. This Dutch study assessed individual exposures to PM_{10} and NO_2 at the

home addresses of expecting mothers, combining continuous monitoring data and dispersion modeling techniques. Continuous outdoor monitor data was collected using standard methods set by the Netherlands Ministry of Infrastructure and Environment. Multiple linear regression and mixed-effect models were used to assess the relationship between fetal parameters and pollution exposures, both cross-sectionally and longitudinally. A statistically significant reduction of 1.74 mm in third trimester HC was associated with the highest quartile PM_{10} exposures. Risk of preterm birth and SGA were also reported.

2.8 Conclusion

Understanding potential mechanisms by which fetal growth parameters may be negatively associated with air pollutant exposures is critical. The six existing studies estimated exposure to air pollution using data from personal and ambient monitors and other information. While some associations between maternal pollution exposure and restricted fetal growth were observed, features of these studies, including examination of different pollutants using different statistical models, hinder comparison of results. Furthermore, the number of studies is limited thus preventing general conclusions at this stage. We next make comments that may help guide design of future research.

The approach for collection and analysis of ultrasound measurements in these epidemiological studies varied, as did the choice of fetal growth parameters examined. Ultrasound images, like most imaging methods, are prone to measurement error, especially when images taken by multiple ultrasound technicians are used without accounting for possible systematic differences in technique between individuals. Evaluating the potential magnitude and existence of such measurement error is possible

with statistical models and by correlation methods. Intra- and inter- reliability assessments should be performed to produce intraclass correlations that measure the agreement of two or more observers measuring the same fetal growth parameters.[33-35] The use of mixed-effect models could also adjust for measurement error, by including an indicator variable for each technician, when reliability is unable to be assessed separately by different technicians measuring the same fetus at the same visit.

A key feature of this research is the hypothesized critical windows of exposure, during which a given pollutant can affect target organs in the developing fetus. Although some authors selected critical windows *a priori*, windows were often simply the periods of gestation where routine ultrasound scans were taken within that population. While this is a logical approach, current literature suggests other methods to statistically study windows of susceptibility based on timing of exposure and outcome variables.[36]

Accurate assessment of fetal growth, i.e. growth rate versus fetal size, is important. When examining changes in fetal growth, repeated ultrasound measurements should be collected for each developing fetus. This may better indicate the rate of change in these parameters and could potentially identify critical windows of exposure. With advanced statistical models, the change in attained size of fetal parameters may also be evaluated at given periods of gestation, so fetal measures should ideally be taken during suspected time windows of particular developmental relevance. The current studies utilizing ultrasound data had repeated measures for three percent or less of their population.

Statistical models used to test associations and which potential confounders were adjusted for varied by study. Common covariates across the studies in Table 1 included maternal age, smoking, pre-pregnancy weight or body mass index, socio-economic variables, gestational age and fetal sex. Some models included nutritional measures. [29, 30] Additional exposure variables included pesticides, paints, noise, temperature and seasonality. [26, 27, 29]

While the studies reviewed here were unable to provide clear linkages between slowed growth in the parameters and specific mechanisms, clinical literature suggests that the reductions in the growth rates of fetal parameters are associated with increased risks of prenatal morbidity and mortality.[37] Further research using repeated ultrasound measures of fetal parameters is needed to assess changes in fetal growth in response to air pollutant exposures.

Table II-1. Epidemiological studies of associations between air pollution exposures during pregnancy and ultrasound measures of fetal parameters

Reference	Study Location	Number of Women	Number of Ultrasound Measurements	Time Period of Ultrasound Measurements	Fetal Parameters Evaluated†	Air Pollutants‡	Air Pollutant Exposure Windows	Summary of Key Results	Additional Covariates*
25	France	366	2 per pregnancy	2nd and 3rd trimester	HC	NO_2	Fertilization - trimester ultrasound exam	Decreases in HC were associated with the highest tertile of NO_2 exposure.	Parity, gestational diabetes, and maternity center
26	Australia	14,734	1-3 per pregnancy	13-26 weeks of gestation	HC, AC, FL, and BPD	O_3, NO_2, SO_2, and PM_{10}	Monthly; first four months of pregnancy	Negative associations between early exposures to pollutants and mean changes in growth parameters during mid-gestation (13-26 weeks)	Concurrent temperature exposures, and seasonality and long-term trend
27	France	271	2-3 per pregnancy	2nd and 3rd trimester	HC and BPD	Benzene	7 day sampling period	Reductions in BPD and HC associated with log transformed exposures of benzene in 2nd and 3rd trimesters.	Urinary cotinine levels,birth order, occupational exposure to paints or pesticides, month of conception, maternal age at end of studies, and center
28	Sabadell, Spain	562	1 per pregnancy	12, 20, and 32 weeks of gestation	HC, AC, and BPD	NO_2 and BTEX	LMP until 12, 20, and 32 weeks; 12-20 and 20-32 week averages	Exposure during weeks 1-12 was negatively associated with BPD growth in weeks 20-32	Season of conception, parity
29	Netherlands	7,772	3 per pregnancy	1st, 2nd and 3rd trimester	HC and FL	NO_2 and PM_{10}	Conception - trimester ultrasound exam	NO_2 and PM_{10} exposures during pregnancy were inversely associated with HC and FL at various stages of gestation.	Parity, folic acid supplementation use, alcohol consumption, paternal height, and road traffic noise exposure
30	Valencia, Spain	785	3 per pregnancy	12, 20, and 32 weeks of gestation	BPD, AC, and FL	NO_2	0–12, 12–20, 20–32, 32-delivery; 0–20 and 0–32 wk avg.	Adverse effects on fetal parameters were higher in association with NO_2 exposure in earlier stages of pregnancy	Maternal gestational weight gain, country of origin, zone of residence, parity, alcohol, caffeine vegetable,fruit and energy intake and season of conception

†Fetal parameters: head circumference (HC), abdominal circumference (AC), femur length (FL), and biparietal diameter (BPD)

‡Air Pollutants: nitrogen dioxide (NO_2), ozone (O_3), sulfur dioxide (SO_2), particulate matter < 10 µm aerodynamic diameter (PM_{10}), and benzene, toluene, ethylbenzene, m/p-xylene, and o-xylene compounds (BTEX)

*Common covariates included maternal age, smoking, SES measures, weight/BMI and fetal sex.

REFERENCES

Ballester F, Estarlich M, Iniguez C *et al.* Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. Environmental health : a global access science source 2010; 9:6.

Estarlich M, Ballester F, Aguilera I *et al.* Residential Exposure to Outdoor Air Pollution during Pregnancy and Anthropometric Measures at Birth in a Multicenter Cohort in Spain. Environmental health perspectives 2011; 119.

Malmqvist E, Rignell-Hydbom A, Tinnerberg H *et al.* Maternal exposure to air pollution and birth outcomes. Environmental health perspectives 2011; 119:553-558.

Rich DQ, Demissie K, Lu SE *et al.* Ambient air pollutant concentrations during pregnancy and the risk of fetal growth restriction. Journal of epidemiology and community health 2009; 63:488-496.

Woodruff TJ, Parker JD, Darrow LA *et al.* Methodological issues in studies of air pollution and reproductive health. Environmental research 2009; 109:311-320.

Ghosh R, Rankin J, Pless-Mulloli T, Glinianaia S. Does the effect of air pollution on pregnancy outcomes differ by gender? A systematic review. Environmental research 2007; 105:400-408.

Jedrychowski W, Perera F, Mrozek-Budzyn D *et al.* Gender differences in fetal growth of newborns exposed prenatally to airborne fine particulate matter. Environmental research 2009; 109:447-456.

Kramer MS. Determinants of low birth weight: methodological assessment and meta-analysis. Bulletin of World Health Organization 1987; 65:663-737.

Semba RD, Bloem MW. Nutrition and health in developing countries In: second. NJ , USA: Humana Press; 2008. pp. 63-86.

Slama R, Darrow L, Parker J *et al.* Meeting report: atmospheric pollution and human reproduction. Environmental health perspectives 2008; 116:791-798.

Shah PS, Balkhair T. Air pollution and birth outcomes: a systematic review. Environment international 2011; 37:498-516.

Parker JD, Rich DQ, Glinianaia SV *et al.* The international collaboration on air pollution and pregnancy outcomes: initial results. Environmental health perspectives 2011; 119:1023-1028.

Nethery E, Teschke K, Brauer M. Predicting personal exposure of pregnant women to traffic-related air pollutants. The Science of the total environment 2008; 395:11-22.

Perera FP, Tang D, Rauh V *et al.* Relationships among Polycyclic Aromatic Hydrocarbon–DNA Adducts, Proximity to the World Trade Center, and Effects on Fetal Growth. Environmental health perspectives 2005; 113:1062-1067.

Tang D, Li T, Liu JJ *et al*. PAH–DNA Adducts in Cord Blood and Fetal and Child Development in a Chinese Cohort. Environmental health perspectives 2006; 114.

Kannan S, Misra DP, Dvonch JT , Krishnakumar A. Exposures to Airborne Particulate Matter and Adverse Perinatal Outcomes: A BiologicallyPlausible Mechanistic Framework for Exploring Potential Effect Modification by Nutrition. Environmental health perspectives 2006; 114:1636-1642.

Hackley B, Feinstein A, Dixon J. Air pollution: impact on maternal and perinatal health. Journal of midwifery & women's health 2007; 52:435-443.

Perera FP, Tang D, Tu YH *et al*. Biomarkers in Maternal and Newborn Blood Indicate Heightened Fetal Susceptibility to Procarcinogenic DNA Damage. Environmental health perspectives 2004; 112.

Bell ML, Belanger K, Ebisu K *et al.* Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources. Epidemiology 2010; 21:884-891.

Gardosi J, Figueras F, Clausson B, Francis A. The customised growth potential: an international research tool to study the epidemiology of fetal growth. Paediatric and perinatal epidemiology 2011; 25:2-10.

Zhang J, Mikolajczyk R, Grewal J *et al.* Prenatal application of the individualized fetal growth reference. American journal of epidemiology 2011; 173:539-543.

Hemachandra AH, Klebanoff MA, Duggan AK *et al.* The association between intrauterine growth restriction in the full-term infant and high blood pressure at age 7 years: results from the Collaborative Perinatal Project. International journal of epidemiology 2006; 35:871-877.

Ritz B, Wilhelm M. Ambient air pollution and adverse birth outcomes: methodologic issues in an emerging field. Basic & clinical pharmacology & toxicology 2008; 102:182-190.

Carrera JM. Ultrasound and Fetal Growth. New York ; London: Parthenon; 2001.

Slama R, Thiebaugeorges O , Goua V *et al.* Relation Between Atmospheric Pollutants and Head Circumference in Utero and at Birth: a Cohort Study Relying on Ultrasound Imaging During Pregnancy.[Abstract]. Epidemiology 2006; 17:S129-S130.

Hansen CA, Barnett AG, Pritchard G. The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. Environmental health perspectives 2008; 116:362-369.

Slama R, Thiebaugeorges O, Goua V *et al*. Maternal personal exposure to airborne benzene and intrauterine growth. Environmental health perspectives 2009; 117:1313-1321.

Aguilera I, Garcia-Esteban R, Iniguez C *et al.* Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA Sabadell cohort. Environmental health perspectives 2010; 118:705-711.

van den Hooven EH, Pierik FH, de Kluizenaar Y *et al.* Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. Environmental health perspectives 2012; 120:150-156.

Iniguez C, Ballester F, Estarlich M *et al.* Prenatal exposure to traffic-related air pollution and fetal growth in a cohort of pregnant women. Occupational and environmental medicine 2012; 69:736-744.

Iniguez C, Ballester F, Amoros R *et al.* Active and passive smoking during pregnancy and ultrasound measures of fetal growth in a cohort of pregnant women. Journal of epidemiology and community health 2012; 66:563-570.

Dejin-Karlsson E, Ostergren PO. Psychosocial factors, lifestyle, and fetal growth: the added value of both pre- and post-natal assessments. European journal of public health 2003; 13:210-217.

Perni SC, Chervenak FA, Kalish RB *et al*. Intraobserver and interobserver reproducibility of fetal biometry. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2004; 24:654-658.

Verburg BO, Mulder PG, Hofman A *et al*. Intra- and interobserver reproducibility study of early fetal growth parameters. Prenatal diagnosis 2008; 28:323-331.

Yang F, Leung KY, Lee YP *et al.* Fetal biometry by an inexperienced operator using two- and three-dimensional ultrasound. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 2010; 35:566-571.

Sanchez BN, Hu H, Litman HJ, Tellez-Rojo MM. Statistical methods to study timing of vulnerability with sparsely sampled data on environmental toxicants. Environmental health perspectives 2011; 119:409-415.

Pedersen NG, Figueras F, Wojdemann KR *et al.* Early fetal size and growth as predictors of adverse outcome. Obstetrics and gynecology 2008; 112:765-771.

CHAPTER III
INTEROBSERVER RELIABILITY OF FETAL ULTRASOUND MEASUREMENTS

3.1. Abstract

We aimed to determine the agreement of ultrasound measurements of fetal parameters captured by three sonographers and visually assess the reliability of the methodology used for making the measurements. In a double-blinded study, sixty-seven fetuses between 15.1 and 40.1 weeks of gestation received triplicate ultrasound examinations by three clinicians. Ultrasound measurements of head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL) were collected. Gestational age and fetal weight were estimated for each fetus by the ultrasound equipment using an internal algorithm. Interclass correlation coefficient (ICCs), Cronbach's Alpha coefficient, and coefficient of variation were calculated for each parameter. Bland Altman plots were used to visually detect bias in measurement methodology. Interobserver variability was small between the three clinicians. Reliability coefficients confirmed correlation among the clinicians' measurements with high ICCs; HC= 0.985, BPD= 0.995, AC= 0.985, FL= 0.996, fetal weight=0.987 and gestational age= 0.965. Cronbach's Alpha coefficients were consistent with the ICC values (HC= 0.993, BPD= 0.996, AC= 0.985, FL=0.990, fetal weight=0.972 and gestational age=

0.983). Mean differences for the four fetal parameters were close to zero (-0.056 to 0.069 cm).This analysis suggests that the ultrasound measurements made by different clinicians in a Mexico City clinic are comparable, and the reliability observed is quantitatively similar to that seen in other clinical settings in which such analyses were done. We conclude that technician bias will not play an important role in statistical analyses of fetal growth and development using these parameters.

3.2 Introduction

Fetal ultrasonography is commonly used by clinicians to determine gestational age, assess the growth of the developing fetus, and assist with diagnosing medical conditions (microcephaly, Down Syndrome, etc.) *in utero* (Perni et al. 2004; Yang et al. 2010). However, in spite of the extensive obstetric use of the ultrasounds, few studies have quantified the inter- and intra-observer reliability of sonographic data collected on fetal parameters for epidemiological studies (Sarris et al. 2012). A systematic review of the methodology used in studies whose purposes were to create fetal ultrasound reference charts found substantial heterogeneity in fetal biometry methodologies, including the performance of quality assurance measures such as interobserver agreement assessments (Ioannou et al. 2012).

With ultrasound measurements being increasingly used in epidemiological studies to better understand the relationship between maternal exposures to environmental toxicants and fetal health, more studies assessing the inter- and intra-observer reliability of fetal measurements captured by ultrasound are needed (Smarr et al. 2013). Ultrasound technology is naturally prone to measurement error and the use of this methodology in longitudinal studies would potentially entail repeated measurements of fetal

anthropometry by different people. This may introduce interobserver variability and measurement error, and require control for technician in statistical analyses for an epidemiological study, to improve statistical inference on associations of interest.

The aim of the present study was to determine the agreement between fetal biometry data collected by multiple clinicians in Mexico City, for the purpose of ascertaining the reliability of the measurements for use in epidemiologic studies.

3.3 Methods

3.3.1 Interobserver Reliability Assessment

We conducted a prospective, clinical study on women recruited as part of a study of air pollution and adverse birth outcomes in Mexico City (O'Neill et al. 2012). Inclusion criteria for the parent study were that the women be in the first trimester of pregnancy at the time of recruitment, 18 years of age or older, residents of Mexico City, non-smokers, and without pregnancy complications. For this reliability assessment, 67 women received ultrasound evaluations by three clinicians at the Hospital Materno Infantil Inguaran (Inguaran Maternal Infant Hospital). Institutional Review Board (IRB) approval was received from all participating institutions (the University of Michigan, the Inguaran Hospital, and the Universidad Nacional Autonoma de Mexico (National Autonomous University of Mexico).

Intraobserver reliability was previously assessed for the three participating clinicians as a part of standard training, following the guidelines in (Carrera 2001) and was not feasible to be assessed for measurements taken for this analysis. For each pregnant woman, we assessed interobserver reliability of measurements of four fetal

parameters: head circumference (HC), abdominal circumference (AC), biparietal diameter (BPD) and femur length (FL). Each woman received an examination by the three clinicians on the same day, who were assigned a number (1-3). Ultrasonography was performed using the Aloka SSD-1000, Hitachi Aloka Medical, Ltd.

Fetal measurements were taken according to the guidelines of the American Institute of Ultrasound in Medicine (AIUM) (AIUM 2007). Clinicians were provided a procedural protocol that described the measurement guidelines to be followed when capturing each fetal parameter measurement. BPD and HC were to be measured from axial images through the fetal brain at the level of the thalami. The BPD measurement was to be taken from the outer edge of the proximal skull to the inner edge of the distal skull, whereas HC measured the outer perimeter of the skull. The ellipsoid method was used to assess organ volume, using digital caliper placement instead of manual tracing. AC was measured on an axial plane at the level of the bifurcation of the main portal vein into the left and right branches and the stomach. This was to help reduce distortion of the circular shape of the abdomen; again manual tracing was avoided by using the calipers to designate measurement locations. Finally, FL was measured by including only the femoral diaphysis length.

Fetal biometry was collected by each clinician in a private exam room. After the first clinician's measurements were recorded on the fetal ultrasound form, the ultrasound screen was cleared before the second clinician entered the room; the same procedure was followed for the third clinician. No strict order of which clinician performed the exam was followed; in other words, clinician 1 did not always give the first exam and clinician 3 the last. However, ultrasound exams were required to be performed on the same day.

3.3.2 Statistical Methods

Since the study design was double-blinded, which reduces observer and analyst bias, biometry data were entered into a database with assigned identification numbers (ID's) for each clinician. The database was provided to the project data manager, at which point it was verified that personal identifiers were not contained in the data, but only participant and clinician ID's and dates of examinations. Univariate analyses were performed to obtain descriptive statistics for each fetal parameter. Dependent t-tests were used to compare the mean differences of fetal parameter measurements for each pair of clinicians. Significant differences would identify potential systematic, between-clinician, biases in fetal measurements.

Reliability was assessed with the use of four methods. First, to assess the similarity among all three observers' measurements, the inter-class correlation coefficients (ICCs) were calculated using mixed models. The ICC is defined as $\frac{\sigma^2_b}{\sigma^2_b + \sigma^2_w}$, where σ^2_b is the between-clinician variability and σ^2_w is the within-clinician (random) variability. In previously published agreement assessments, an ICC value greater than 0.75 was adopted as reflecting reliable agreement. A value in that range would indicate that at most, 25% of the variation in the ultrasound measures is due to differences between clinicians. Cronbach's Alpha coefficient, a measure of internal consistency, was used to assess the variance related to fetal differences compared with differences among the clinicians making the measurements on the same fetus (Lyons 1997). The coefficient of variation (CV) was calculated for each clinician's set of four fetal parameter measurements to assess the dispersion within one clinician's measurements of the same

parameter. The CV, expressed as a percentage, is the ratio of the standard deviation (SD) of the measurement error for the given parameter and the mean value of that parameter. The CV was calculated using linear regression models to produce the adjusted residual variance; smaller CV values mean less dispersion in a clinician's measurements of a given parameter.

Finally, visual assessment of ultrasound measurements agreement between each clinician pair was confirmed with the use of identity plots of unadjusted parameter measurements. Bland-Altman plots were used to visually assess repeatability of the ultrasound measurements used to assess the fetal growth since the method should remain consistent for each mother being examined. All variables were standardized to have mean 0 and SD 1. For each measurement, the differences and means were calculated between each combination of two clinicians. Differences are plotted on the y-axis against measurement means on the x-axis. Using the standardized measurements in the Bland-Altman plots, a mean difference of 0 would mean perfect agreement between measurements taken by two clinicians. Positive difference values indicate that the second clinician's measurement was smaller than that of the first clinician. Negative difference values indicate that the second clinician's measurement was larger than that of the first clinician. This provides a visual assessment of measurement consistency and potential measurement bias. Scatter plots of unadjusted parameter measurements made across gestation by each clinician on the same fetus, as well as the difference in standardized measurements between each clinician pair against gestational age, were also used to visually compare differences in parameter measurements collected by three clinicians on

the same fetus. Statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC).

3.3.3. Sensitivity Analyses

Sensitivity analyses were performed to account for extraneous variation that could be introduced to between-clinician variation as a result of having a wide distribution of gestational ages at which ultrasound measurements were collected. Therefore all statistical analyses previously described were re-applied to data that had been grouped using two concepts. The first analysis was performed by grouping the data based on trimesters. Data collected between 15.1 and 26.9 weeks of gestation were grouped as 2nd trimester measurements and data collected between 27.0 and 40.1 weeks were grouped as 3rd trimester measurements. The second method used to group ultrasound measurements was based on the distribution of gestational age. With the median gestational age being 36.6 weeks, all measurements collected less than the median gestational age were grouped together, leaving all measurements collected at gestational ages greater than or equal to the median to be grouped together.

3.4 Results

The total number of measurements performed on each participant differed across fetal parameters and by clinician. Table 1 offers summary statistics: the total number of ultrasound measurements collected by each clinician, and the mean, minimum and maximum values for each parameter, by clinician. Fetal measurements were collected during gestational periods of 15.1 to 40.1 weeks. On average, fetal measurements made by the three clinicians were similar across the various parameters of interest. Mean HC

(cm) measurements for clinicians 1-3 were 31.18, 31.39, and 31.19, respectively; similar closeness in the magnitude of the mean biometry data across clinicians was observed for all fetal parameters.

The mean differences between each clinician's ultrasound measurements are presented in Table 2. Mean differences between clinician pairs were not statistically significant for most parameters, with the exception of BPD. The mean difference of BPD measurements between clinicians 1 and 2 was 0.036 cm, p=0.003. Similar differences were observed for BPD measurements between clinicians 1 and 3 and clinicians 2 and 3: 0.067 cm; p<0.001 and 0.037 cm; p=0.021, respectively. The coefficient of variation was not consistently lower for all measurements recorded by any one clinician (Table 2). Overall, clinician 1 had the smallest dispersion in HC and FL measurements (CV_{HC}= 3.600%, CV_{FL}=6.503%), compared to measurements made by the other two clinicians. Clinician 2 has the smallest CV values for BPD and AC measurements (CV_{BPD}= 4.212%, CV_{AC}= 3.922%). Variance in ultrasound measurements performed by a single clinician was similar in magnitude across all fetal parameters. However, measurements made by clinician 3 had more variation compared to the other two clinicians.

As shown in Table 3, the values for ICC and Cronbach's Alpha reliability coefficients were very high for all parameters. The ICCs for parameter measurements across all gestational ages were: HC= 0.985, BPD= 0.995, AC= 0.985, FL= 0.996, fetal weight= 0.987 and gestational age= 0.965. Cronbach's Alpha coefficients of unadjusted values were consistent with the ICC values (HC= 0.993, BPD= 0.996, AC= 0.985, FL=0.990, fetal weight=0.972 and gestational age= 0.983).

Among those measurements captured during the second trimester (gestational age 15.10-26.9 weeks), between clinician variation for each fetal anthropometric parameter was small, as assessed by ICCs: (HC= 1.000, BPD= 0.996, AC= 0.999, FL= 1.000, fetal weight= 0.997 and gestational age= 0.988). Alpha coefficient's were all greater than the accepted value of 0.70 but were smaller than the values reported for all measurements; α's: (HC= 0.906, BPD= 0.936, AC= 0.777, FL= 0.915, fetal weight= 0.899 and gestational age= 0.882). For measurements collected in the 3rd trimester, ICCs were smaller and Alphas were larger than values reported for measurements collected in the second trimester: (ICCs: HC= 0.946, BPD= 0.983, AC= 0.963, FL= 1.000, fetal weight= 0.977 and gestational age= 0.895; α's: HC= 0.990, BPD= 0.999, AC= 0.984, FL= 1.000, fetal weight= 0.986 and gestational age= 0.976).

Measurements were also analyzed by those captured below and above the median gestational age (36.6 weeks). For the measurements made at less than 36.6 weeks, interobserver variation for each fetal anthropometric parameter was similar to the estimated variation of all measurements collected during gestation, as assessed by ICCs: (HC= 0.981, BPD= 0.997, AC= 0.986, FL= 0.995, fetal weight= 0.988 and gestational age= 0.996). Alpha coefficient's of unadjusted values were also similar to values previously reported for all measurements; α's: (HC= 0.999, BPD= 1.000, AC= 0.995, FL= 0.998, fetal weight= 0.985 and gestational age= 0.986). For those fetal measurements collected at 36.6 weeks or higher, ICCs and Alphas were smaller than values reported for measurements collected at earlier time periods: (ICCs: HC= 0.961, BPD= 0.954, AC= 0.975, FL= 0.991, fetal weight= 0.957 and gestational age= 0.842;

α's: HC= 0.957, BPD= 0.918, AC= 0.979, FL= 1.000, fetal weight= 0.957 and gestational age= 0.941).

Measurement agreement between all of the clinicians was visually confirmed for all fetal parameters as seen in figures 1-10. Unadjusted values show that with the exception of one or two measurements, the clinicians' measurements were similar across all parameters on measurements performed on the same fetus. The Bland Altman agreement plots show that most of the measurements made between any pair of clinicians were clustered around the mean difference. Mean differences were all close to zero, ranging from -0.056 to 0.069 cm for the four fetal parameters typically used in a growth profile (HC, BPD, AC, and FL). Bland-Altman plots showed that fetal measurements made by clinicians 1 and 2 tended to vary less. Other graphs show that there is a lack of systematic bias between clinician measurements of fetal parameters.

3.5 Discussion

Fetal ultrasound measurements are clinically the gold standard for estimating gestational age and monitoring fetal health and development throughout pregnancy. Therefore it is important to know the agreement of measurements and repeatability of measurement methods when multiple persons are performing ultrasound examinations on a single mother-fetal pair. The objective of this study was to assess the reliability of fetal parameters used in the clinical growth profile (HC, AC, BPD, and FL), in addition to gestational age calculated with the use of the fetal parameter measurements. We found the fetal ultrasound measurements made by multiple clinicians using the same ultrasound equipment to be similar in terms of agreement and method repeatability.

For the most part, mean differences in parameter measurements were not statistically different between various combinations of clinician pairs. However, small, statistically significant differences were estimated for the measurement mean of BPD between all clinician pairs, ranging from 0.030 to 0.067 centimeters. We calculated the standard measurement error associated with each fetal parameter and determined these differences in BPD measurements between clinicians to be within the allowable range of measurement error. Clinically, these differences are not generally regarding as substantive when using these fetal parameters to estimate gestational age or diagnose adverse fetal disorders. Anatomical parameters as indicators of poor health, growth and development are observed for changes in measurement values that are ± 2 SD of the mean population value (Carrera 2001) or fall below the 10^{th} percentile (Hughey 2005). Also, given that we had previously calculated between clinician variance of parameter measurements, we were able to estimate the amount of standard measurement error for each parameter. For BPD we found that measurement error should range be less than 0.08 cm and this could explain the significant difference between parameter measurements.

The fetal measurements in our analysis were captured at between 15.1 and 40.1 weeks of gestation, a period almost identical to the 15 to 40 week range used by Perni and colleagues (Perni SC, Chervenak FA et al. 2004). This is consistent with the literature which suggests fetal growth parameters are best captured from 12 to 42 weeks. Other studies restricted their biometry data to early pregnancy (9-14 weeks) (Verburg et al., 2008) or measurements prior to a fetus reaching full term status, i.e., 37 weeks (Yang et al., 2010). Another notable difference is that most previous reliability assessments only

included differences between two observers; we had three clinicians recording fetal measurements on the same 67 participants.

We found that the variation in the anthropometric measurements resulting from between clinician differences ranged from 0.4 to 1.5 % for the four fetal parameters: HC (ICC = 0.985); AC (ICC = 0.985); BPD (ICC = 0.995); and FL (ICC = 0.996). These findings are consistent with other studies that reported high ICCs for sonographic measurements of these fetal parameters, as (Perni et al., 2004) reported ICCs of 0.994, 0.980, 0.995 and 0.990 for HC, AC, BPD and FL, respectively. The same similarities exist between the alpha coefficients reported by (Perni et al., 2004) and those estimated in our analysis. Measurement agreement for these parameters reported in (Yang et al, 2010) also showed low between clinician variability, but their study differed in ultrasound technology used to capture the measurements. The analysis performed by Yang et al involved the use of two and three dimensional imaging tools.

Given the broad range of gestational ages at which fetal parameter measurements were collected, compared to other similar studies, we also performed sensitivity analyses to evaluate whether ICCs varied in magnitude when calculated from measurements made at different time windows of gestational age, that is, in earlier or later pregnancy. However we still find ICCs to be greater than 0.90 for all parameters, with the exception of gestational age which was always greater than 0.80 in both sensitivity analyses that we performed. It is also important to note that for one of the analyses, dividing measurements collected by trimester, the small number of measurements available for each of the parameters may decrease statistical power and therefore should be interpreted cautiously. We also emphasize that ICCs were still high when we divided the data into

two groups based on the median gestational age at which measurements were collected. However, we believe that the gestational age was in fact influential in the estimation of Alpha Coefficients. Compared to the values reported for all measurements made by each of the clinicians, not accounting for gestational age, Alphas were inflated and mostly driven by the measurements that were closer together and made before 36.6 weeks of gestation. Alphas estimated for parameters measured after this time period were still much higher than the 0.70 accepted value, but were smaller than the values reported for measurements collected at earlier time periods. A possible explanation for this difference could be explained by the linear growth of these parameters that usually occurs prior to 36.6 weeks of gestation. Therefore, with less complex changes in these parameters at these time periods, measurements may be easier to measure for most clinicians.

Intraclass correlation coefficients were not calculated to assess within-clinician variance in measurements as a result of time constraints that were placed on the availability of the ultrasound equipment at the clinic. However, the clinicians had been previously certified with regard to their intraclass correlation coefficients on fetal ultrasound, and all three were within the guidelines.

The CVs, which account for each clinician's measurement dispersion compared to the average parameter measurement, ranged from 3.60% – 7.98% across all four fetal parameters and 3 clinicians. These percentages are similar to those reported by Verburg et al., who estimated CVs for all four parameters measured by two clinicians to range from 1.4% to 5.9%. One possible explanation for the slightly higher values of CV that we estimated is the fact that we were unable to account for possible differences in measurements as a result of fetal variability. CVs were not estimated on repeated

measures by the same clinician for the same fetus, but used all measurements of a specific parameter made by a clinician across the various fetuses.

We did, however, use mixed effect models to calculate the interobserver ICCs, and the covariance parameters of those models account for random error as a result of fetal differences. Also, variation is expected to increase as parameter size increases, and we examined fetuses from 15-41 weeks of gestation, whereas the Verburg study chose an earlier time frame (9-14 weeks).

Visually, the Bland Altman plots for BPD and FL measurements showed less bias than the other two parameters in terms of the dispersion of the data points around the mean difference line. This was comparable to the results of studies previously mentioned. These results are also consistent with clinical literature reporting that the variability in ultrasound measures of BPD and FL is much smaller than for the HC and AC parameters (Carrera 2001). Biologically, BPD and FL have slower growth rates during gestation when compared to HC and AC. The fact that these parameters do not change *in utero* as drastically as the other parameters generally results in less associated measurement error. FL and BPD measurements are also easier to capture given their bony structures, compared to the measurement error that is associated with capturing the true shape of the AC and HC parameters.

In summary, the reliability of the ultrasound measurements in our study is consistent with the reliability reported in previous studies. Given the lack of published growth curves or fetal reference data for the Mexico City population, the results of this analysis could serve as quality assurance tests for the future construction of biometry

reference literature for this population. These findings also lend support to the use of fetal ultrasound measurements in epidemiological research studies of fetal growth and development.

Table III- 1. The summary statistics for fetal parameters measured by ultrasound by three clinicians in Mexico City

Fetal Parameter	Clinician	N	Mean ± SD	Minimum	Maximum
Head Circumference (cm)	1	67	31.18 ± 4.31	11.20	35.00
Head Circumference (cm)	2	59	31.39 ± 4.33	11.30	40.00
Head Circumference (cm)	3	60	31.19 ± 4.43	11.20	35.40
Abdominal Circumference (cm)	1	67	30.77 ± 5.03	10.30	35.70
Abdominal Circumference (cm)	2	57	31.14 ± 4.91	10.40	36.00
Abdominal Circumference (cm)	3	60	30.82 ± 5.09	10.30	35.60
Femur Length (cm)	1	67	6.70 ± 1.14	1.67	7.72
Femur Length (cm)	2	62	6.77 ± 1.10	1.70	7.70
Femur Length (cm)	3	60	6.71 ± 1.14	1.65	7.60
Biparietal Diameter (cm)	1	67	8.44 ± 1.18	2.92	9.58
Biparietal Diameter (cm)	2	59	8.46 ± 1.15	2.80	9.50
Biparietal Diameter (cm)	3	60	8.39 ± 1.19	2.70	9.30
Gestational Age (weeks)	1	67	35.26 ± 4.98	15.10	40.10
Gestational Age (weeks)	2	59	35.37 ± 4.77	15.60	39.60
Gestational Age (weeks)	3	60	35.63 ± 4.76	16.00	40.00

Table III- 2. Reliability measures and mean difference for the four fetal biometric parameters measured by three clinicians

Reliability Variables	**Fetal Growth Parameters**			
	HC (cm)	**BPD (cm)**	**AC (cm)**	**FL (cm)**
Interobserver ICC	0.985	0.995	0.985	0.996
Alpha Coefficient	0.995	0.999	0.995	0.999
Coefficent of Variation:				
Clinician 1	3.600	4.287	4.149	6.503
Clinician 2	5.198	4.212	3.922	6.855
Clinician 3	5.174	5.726	6.729	7.983
Mean Difference $_{\text{Clinician 1 - Clinician2}}$	-0.034	0.036*	-0.007	0.002
Mean Difference $_{\text{Clinician 1 - Clinician3}}$	0.013	0.067**	-0.048	-0.004
Mean Difference $_{\text{Clinician 2 - Clinician3}}$	0.069	0.0367*	-0.056	0.008

***p<0.05 , ** p<.0001**

Table III-3. Reliability values for fetal measurements collected by multiple clinicians by trimester

	All			2nd Trimester(15.1-27 weeks)†			3rd Trimester(≥ 27 weeks)‡		
Fetal Parameter	**Interobserver ICC**	**Standardized Alpha Coefficient ***	**Alpha Coefficient**	**Interobserver ICC**	**Standardized Alpha Coefficient***	**Alpha Coefficient**	**Interobserver ICC**	**Standardized Alpha Coefficient***	**Alpha Coefficient**
Head Circumference (cm)	0.985	0.995	0.993	1.000	0.999	0.906	0.946	0.979	0.990
Abdominal Cirucmference (cm)	0.985	0.995	0.985	0.999	1.000	0.777	0.963	0.984	0.984
Biparietal Diameter (cm)	0.995	0.999	0.996	0.996	0.999	0.936	0.983	0.994	0.999
Femur Length (cm)	0.996	0.999	0.990	1.000	1.000	0.915	0.986	0.996	1.000
Fetal Weight (g)	0.987	0.995	0.972	0.997	0.998	0.899	0.977	0.990	0.986
Gestational Age (weeks)	0.965	0.988	0.983	0.992	1.000	0.882	0.895	0.960	0.976

* Alpha coefficient for standardized values, other values reported are for raw values

†number of parameter observations for 2nd trimester: HC (12), AC (11), BPD (12), FL (15), GA (12) and FW (12)

‡number of parameter observations for 3rd trimester: HC (174), AC (173), BPD (174), FL (174), FW (174) and GA (174)

Table III-4. Reliability values for fetal measurements collected by multiple clinicians before and after the median gestational age

	All			Gestational Age< 36.6 Weeks			Gestational Age≥ 36.6 Weeks		
Fetal Parameter	**Interobserver ICC**	**Standardized Alpha Coefficient***	**Alpha Coefficient**	**Interobserver ICC**	**Standardized Alpha Coefficient***	**Alpha Coefficient**	**Interobserver ICC**	**Standardized Alpha Coefficient***	**Alpha Coefficient**
Head Circumference (cm)	0.985	0.995	0.993	0.981	0.994	0.999	0.961	0.962	0.957
Abdominal Cirucmference (cm)	0.985	0.995	0.985	0.986	0.994	0.995	0.975	0.976	0.979
Biparietal Diameter (cm)	0.995	0.999	0.996	0.997	0.999	1.000	0.954	0.963	0.918
Femur Length (cm)	0.996	0.999	0.990	0.995	0.999	0.998	0.991	0.997	1.000
Fetal Weight (g)	0.987	0.995	0.972	0.988	0.996	0.985	0.957	0.966	0.957
Gestational Age (weeks)	0.965	0.988	0.983	0.996	0.984	0.986	0.842	0.935	0.941

* Alpha coefficient for standardized values, other values reported are for raw values

†number of parameter observations for gestational age < 36.6 weeks: HC (92), AC (91), BPD (92), FL (92), FW (92) and GA (92)

‡number of parameter observations for gestational age ≥ 36.6 weeks: HC (94), AC (93), BPD (94), FL (94), FW (94) and GA (94)

Figure III-1a. Identity plot of head circumference measurements for clinicians 1 and 2

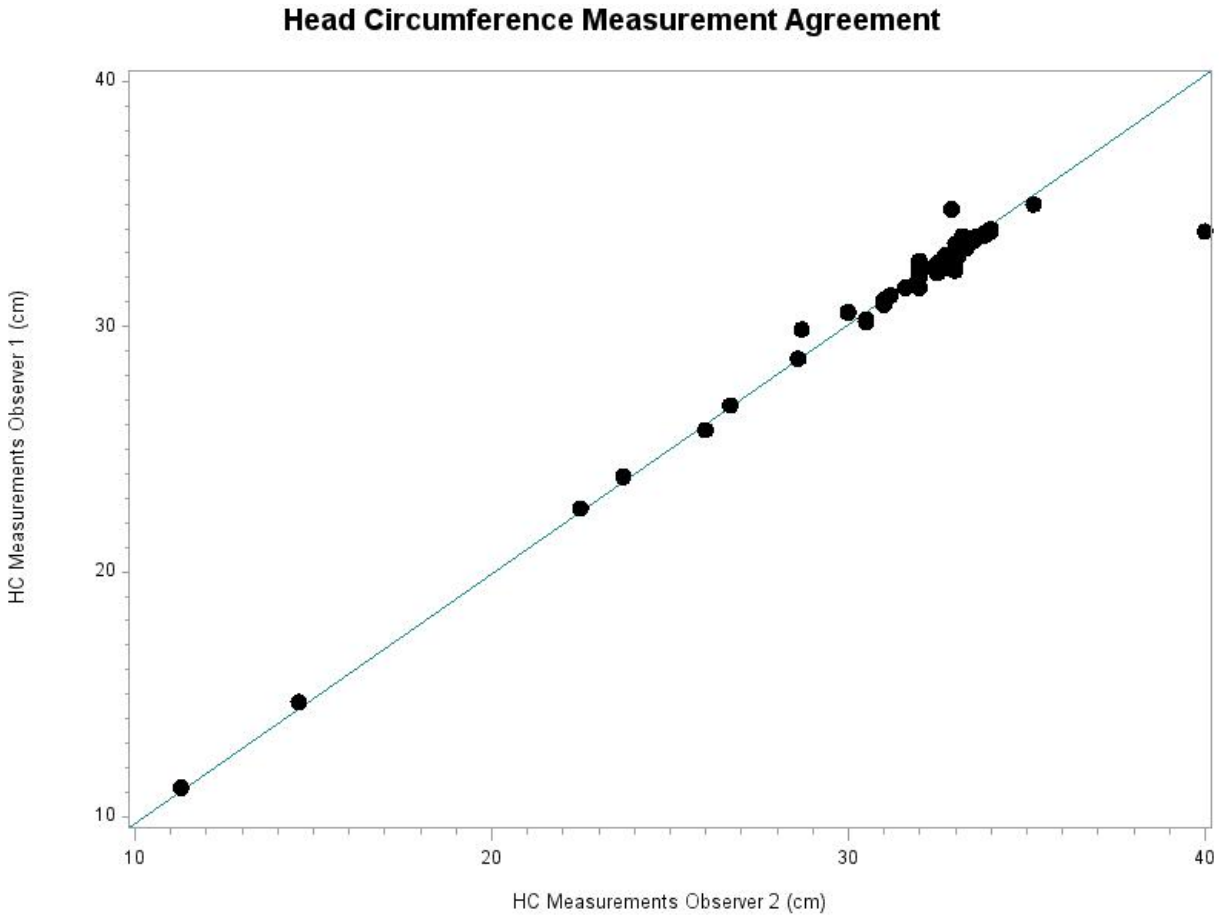


Figure III-1b. Identity plot of head circumference measurements for clinicians 1 and 3

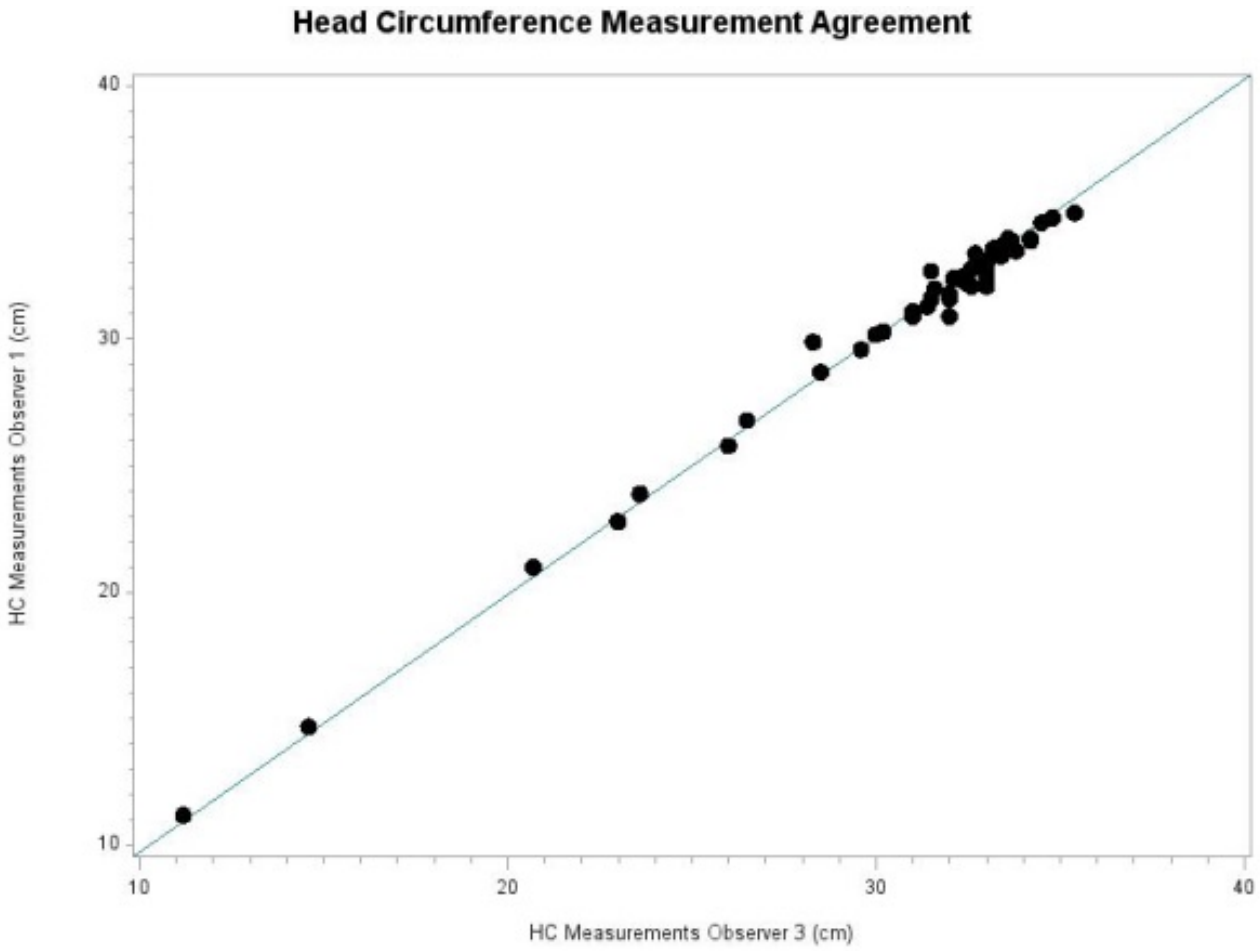


Figure III-1c. Identity plot of head circumference measurements for clinicians 2 and 3

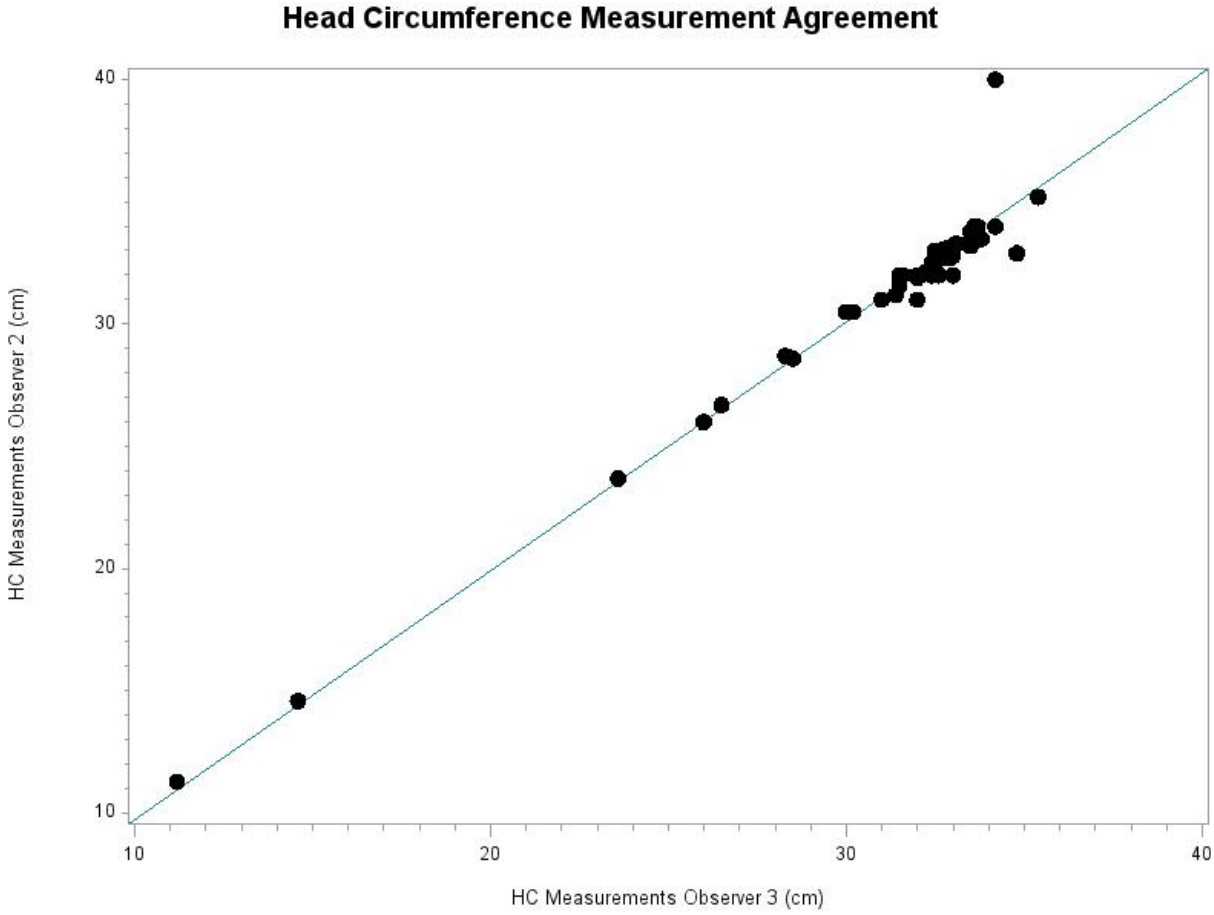


Figure III-2a. Identity plot of abdominal circumference measurements for clinicians 1 and 2

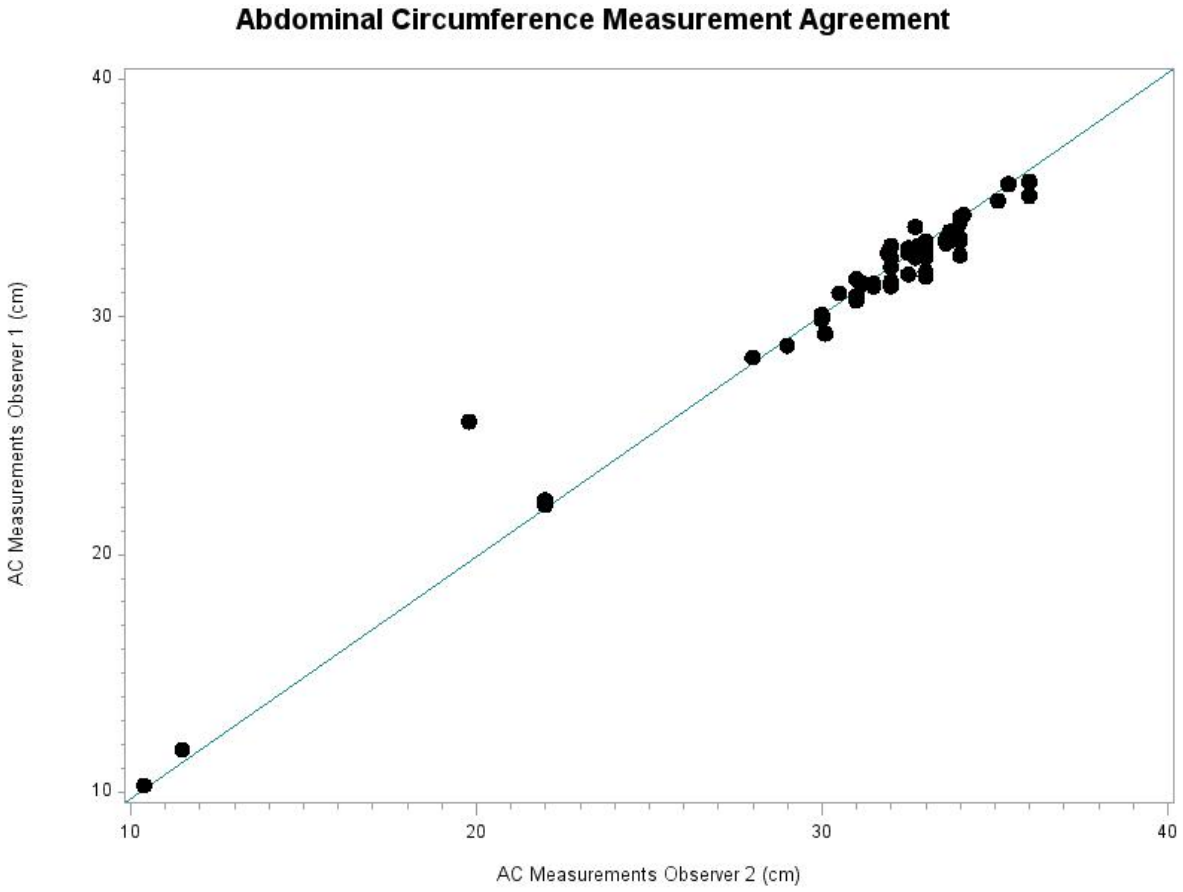


Figure III-2b: Identity plot of abdominal circumference measurements for clinicians 1 and3

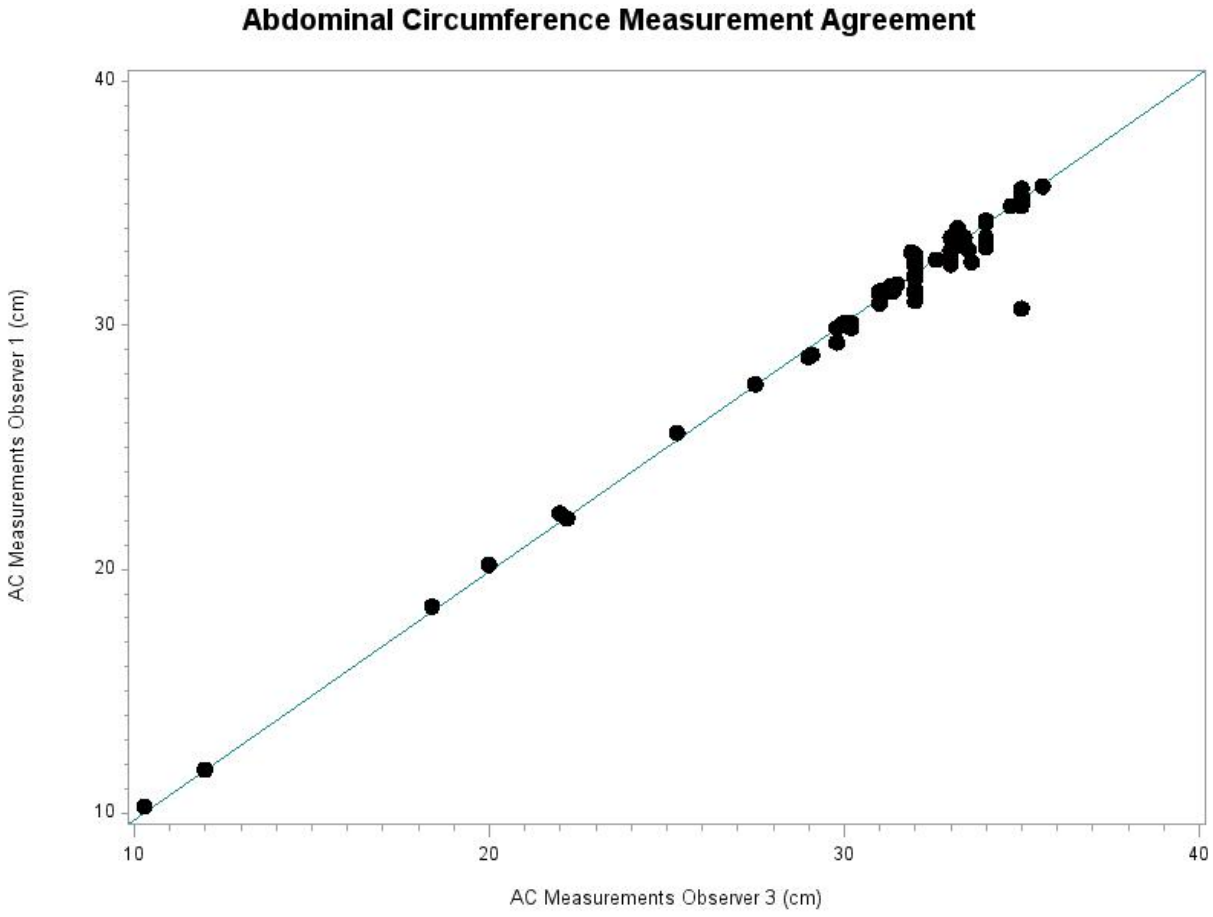


Figure III-2c: Identity plot of abdominal circumference measurements for clinicians 2 and 3

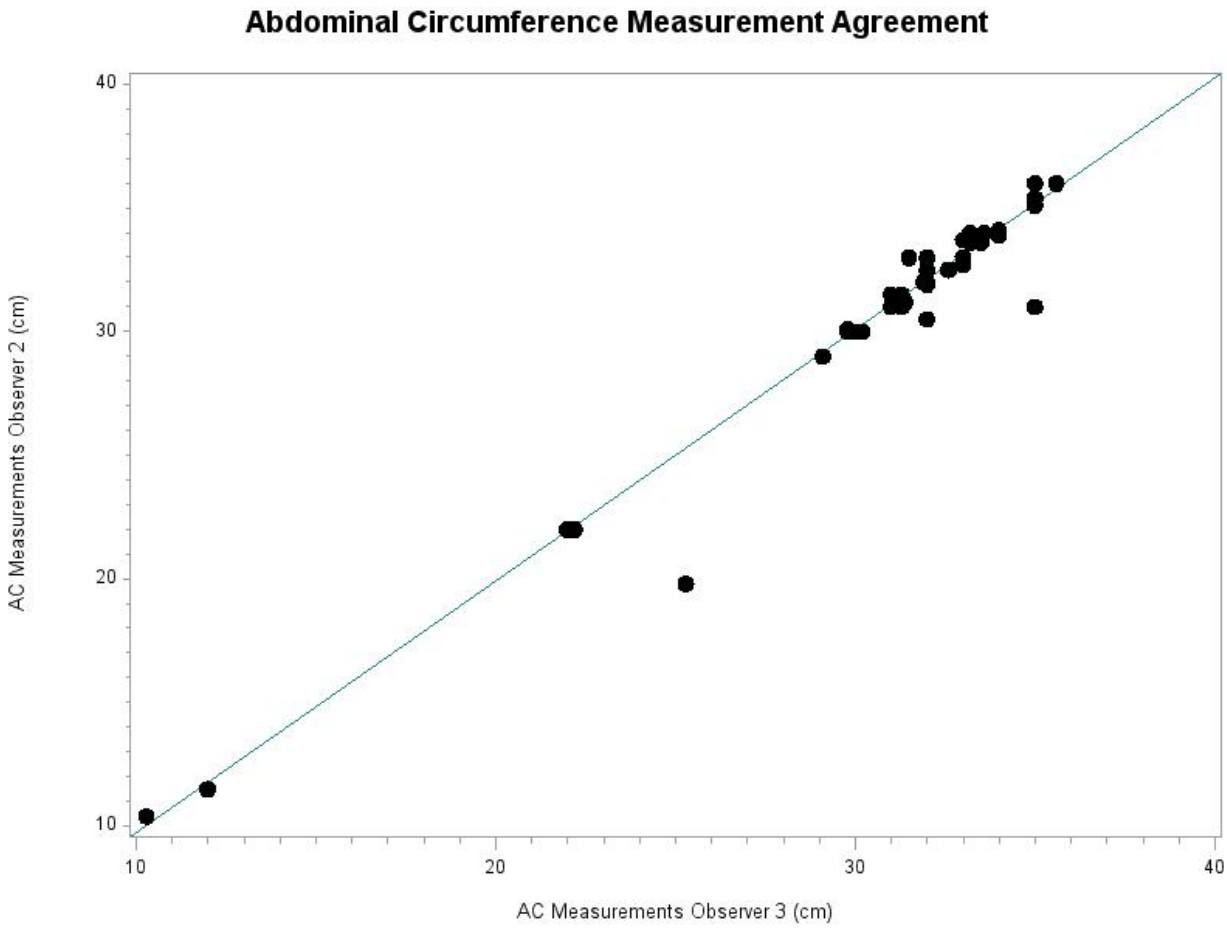


Figure III-3a: Identity plot of biparietal diameter measurements for clinicians 1 and 2

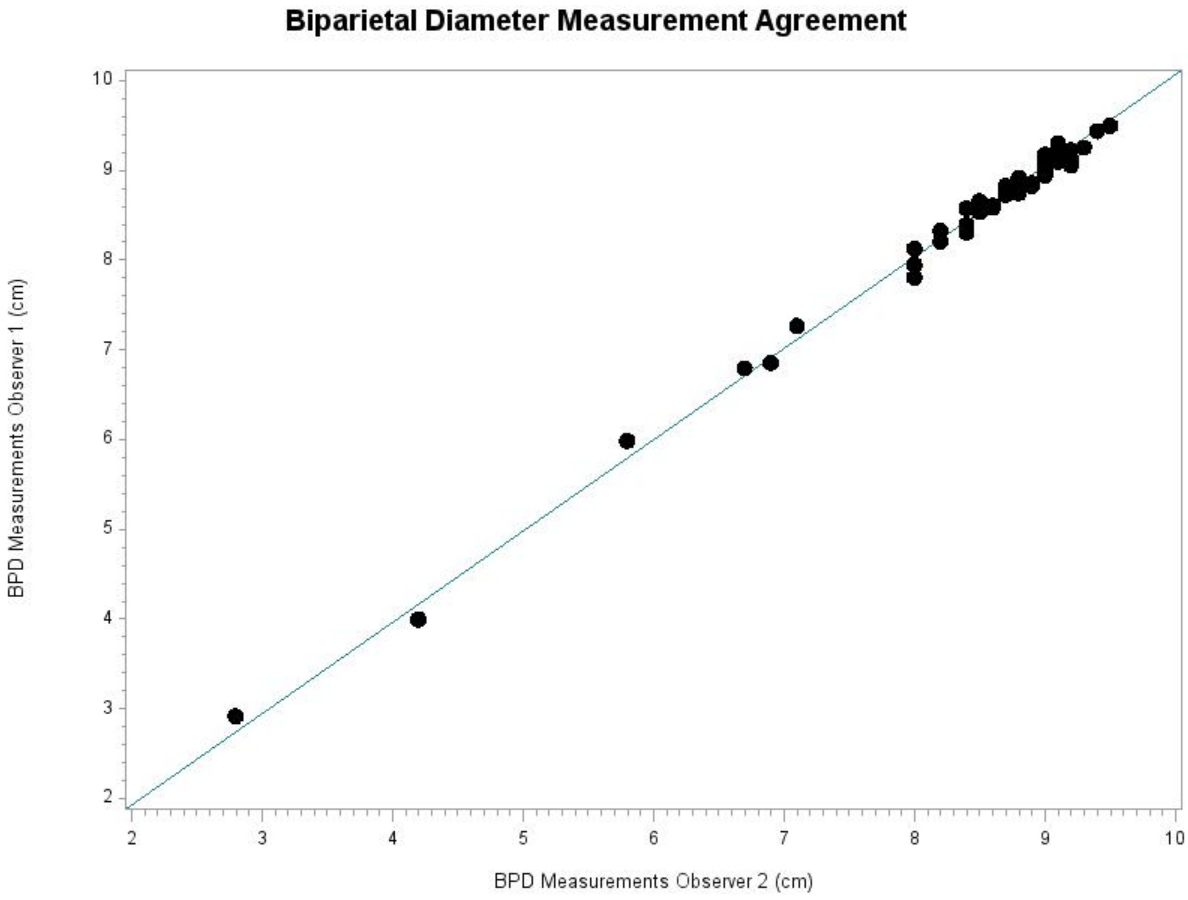


Figure III-3b: Identity plot of biparietal diameter measurements for clinicians 1 and 3

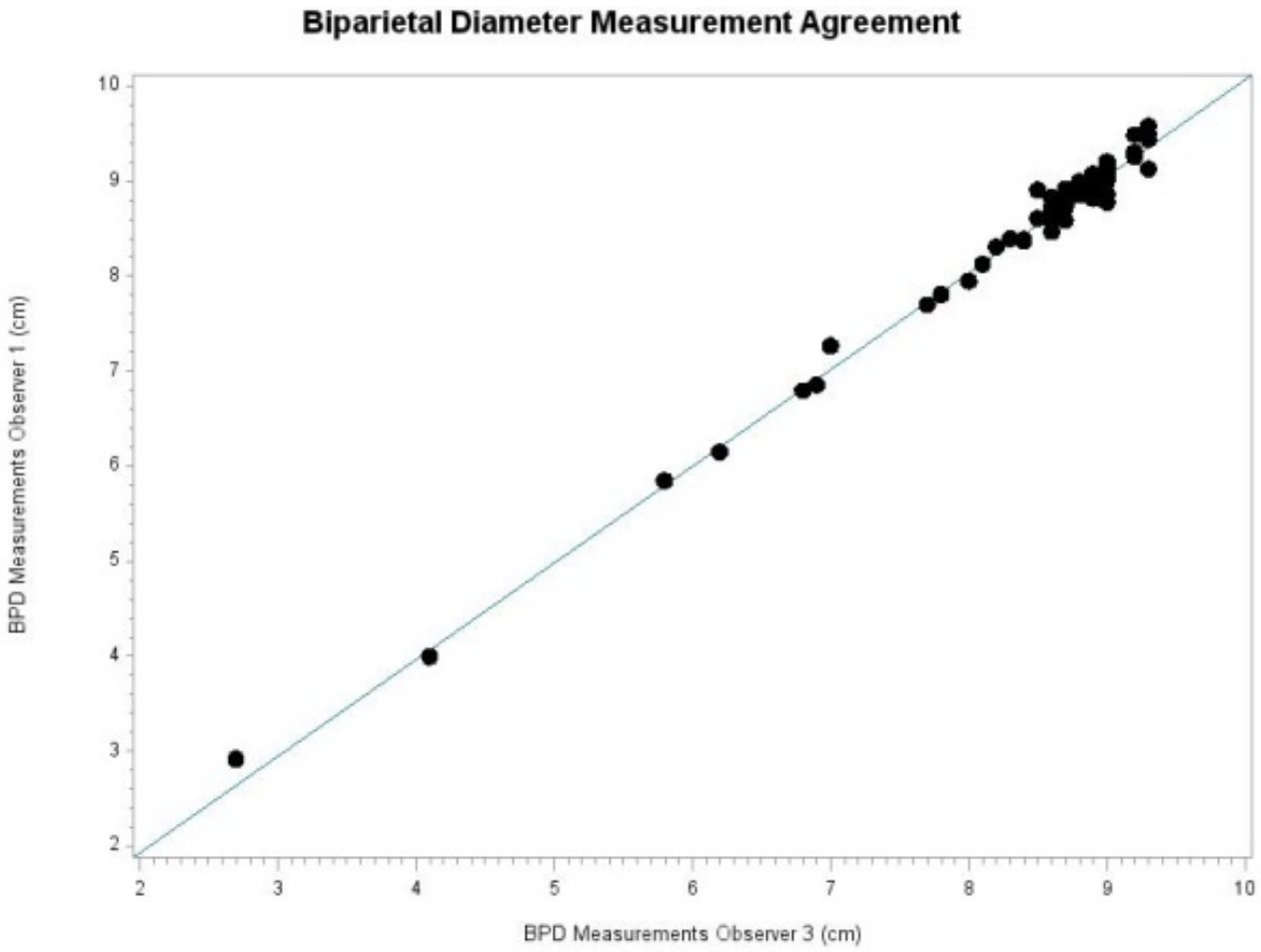


Figure III-3c: Identity plot of biparietal diameter measurements for clinicians 2 and 3

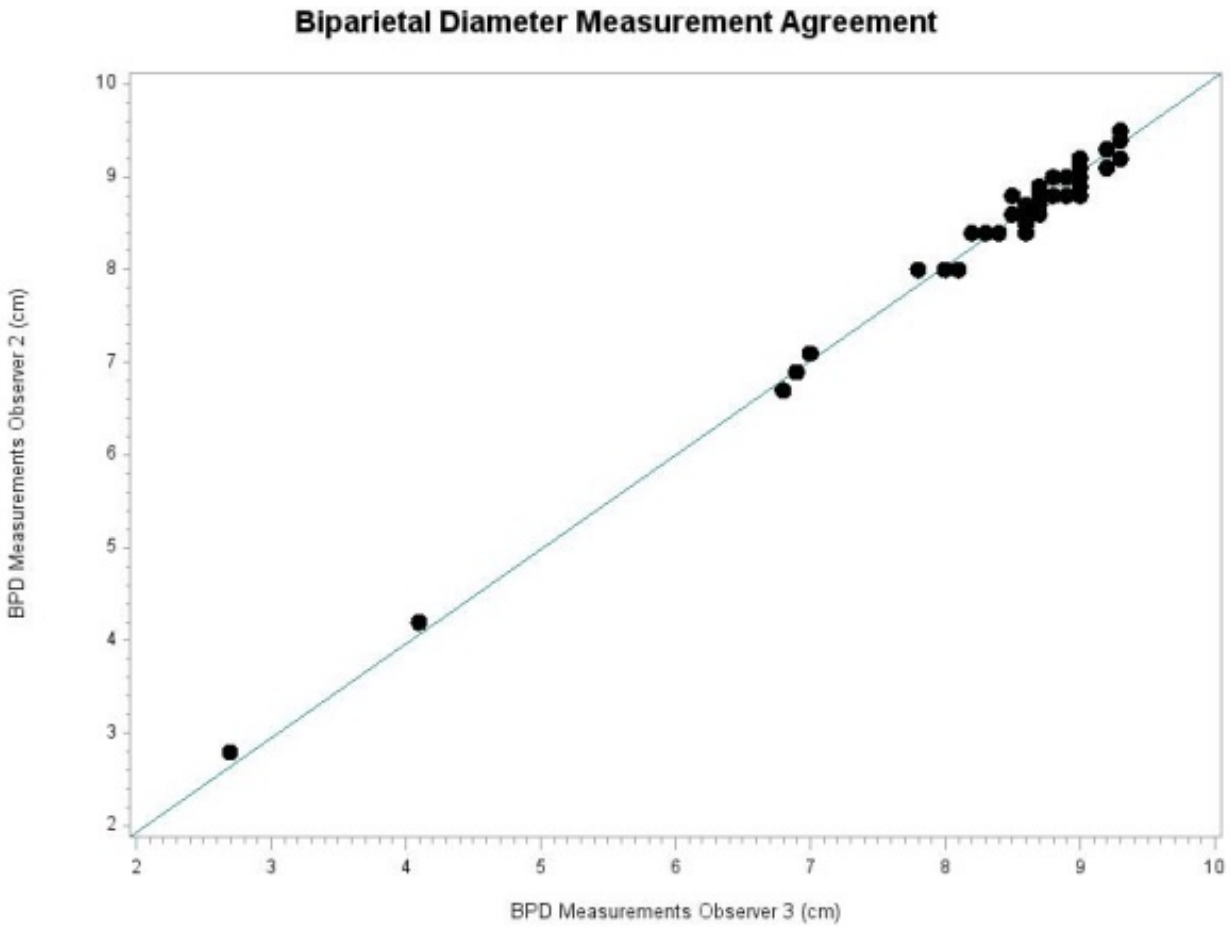


Figure III-4a: Identity plot of femur length measurements for clinicians 1 and 2

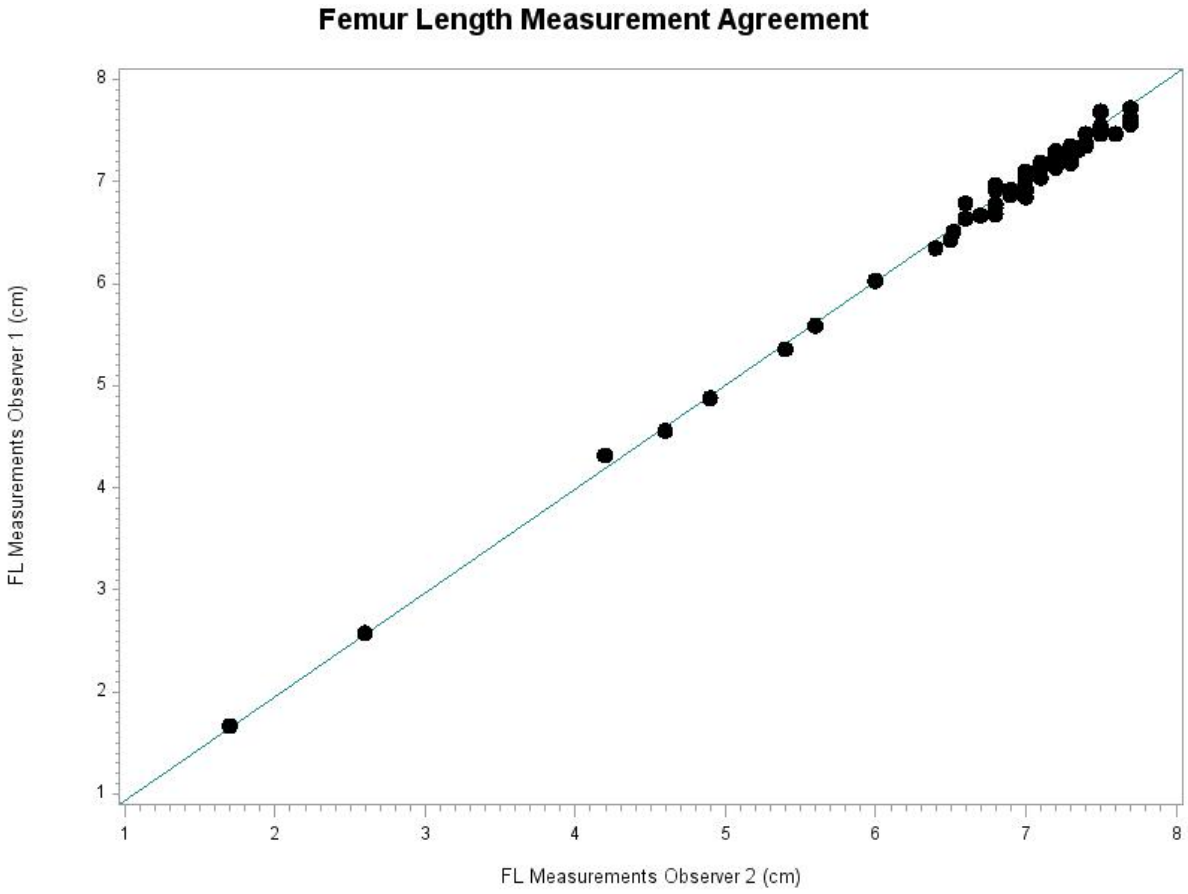


Figure III-4b: Identity plot of femur length measurements for clinicians 1 and 3

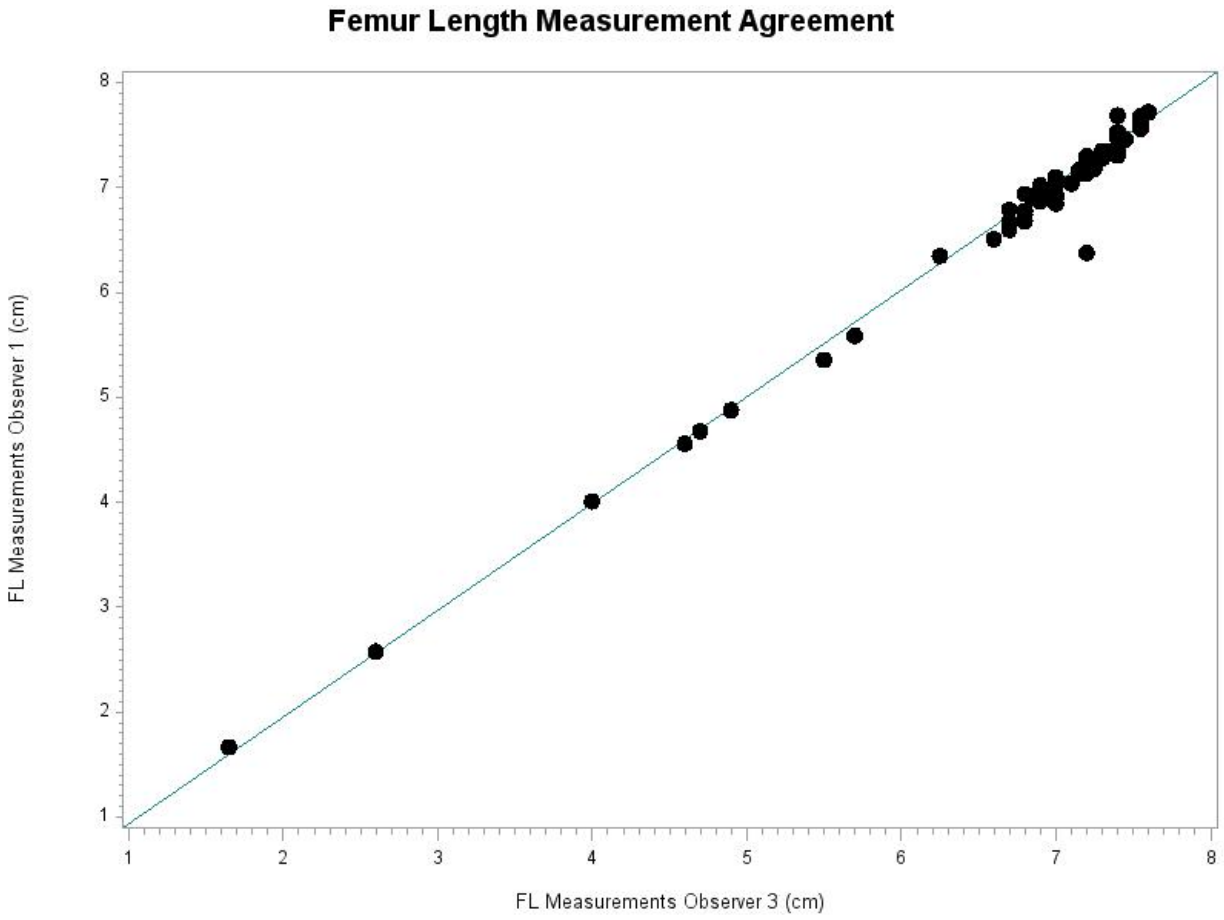


Figure III-4c: Identity plot of femur length measurements for clinicians 2 and 3

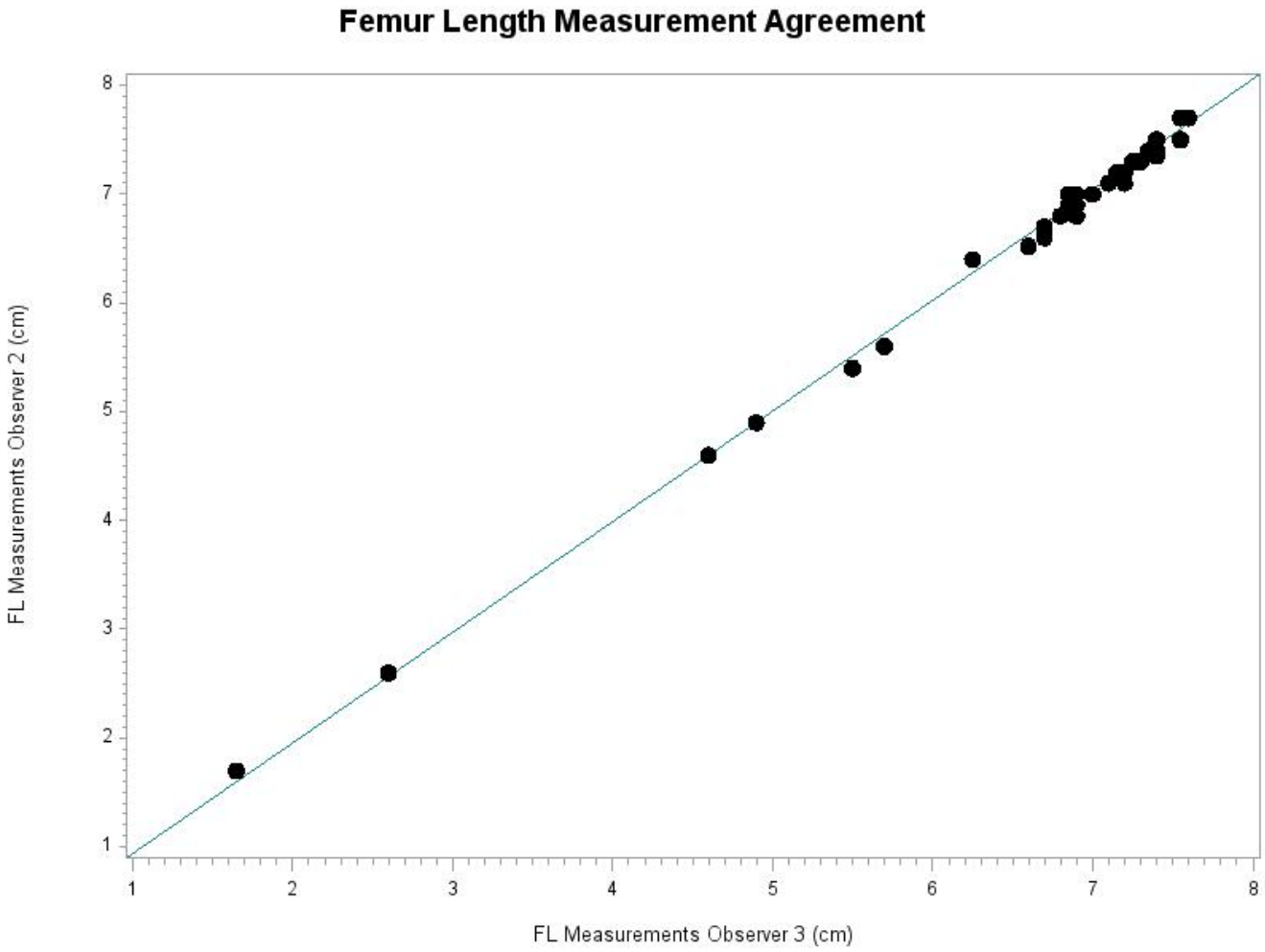


Figure III-5a: Bland Altman plots of head circumference measurements for clinicians 1 and 2

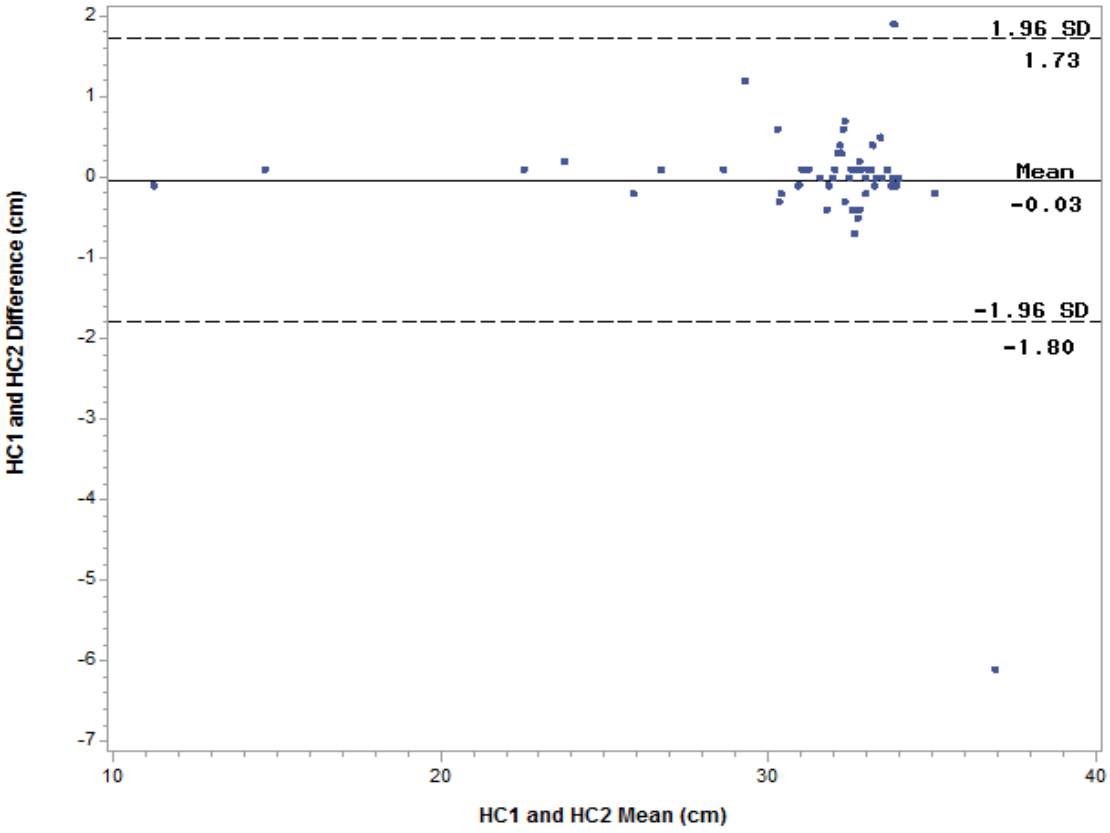


Figure III-5b: Bland Altman plots of head circumference measurements for clinicians 1 and 3

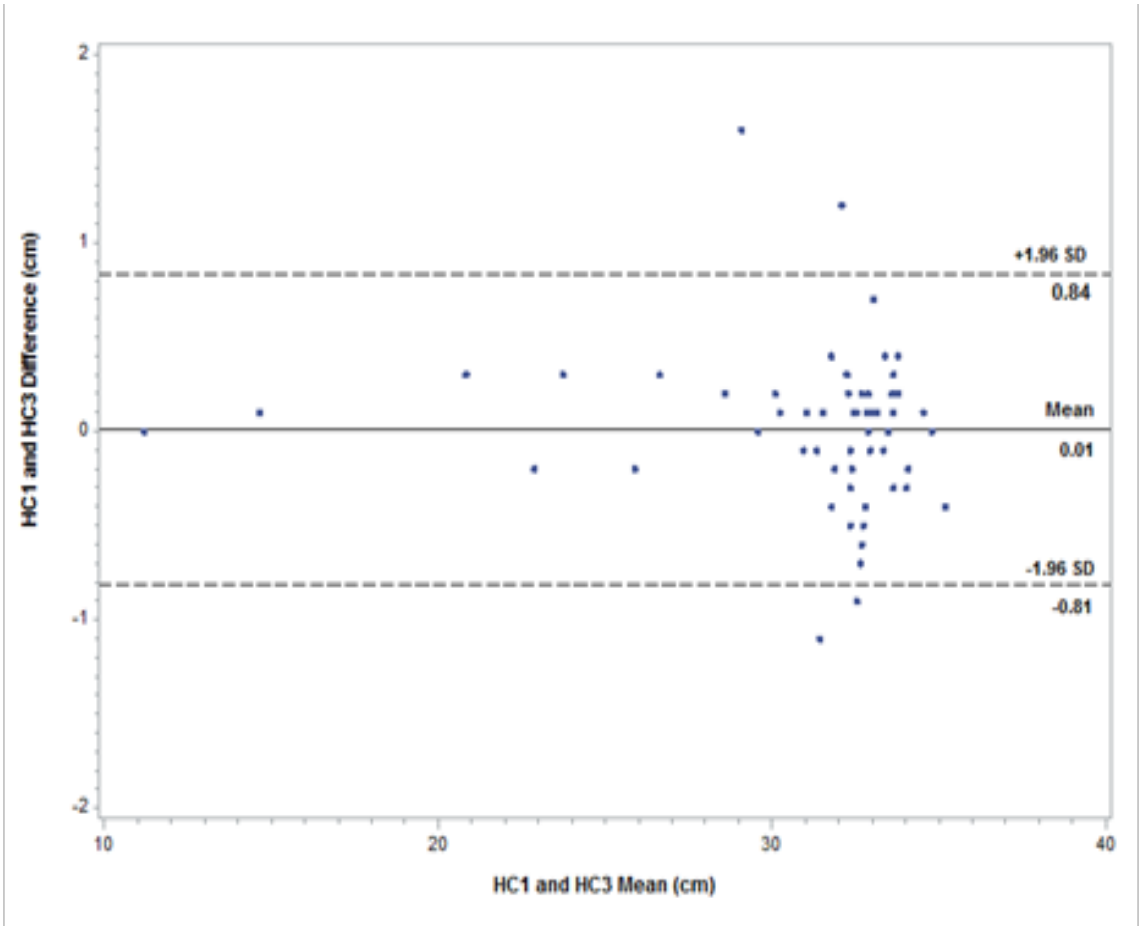


Figure III-5c: Bland Altman plots of head circumference measurements for clinicians 2 and 3

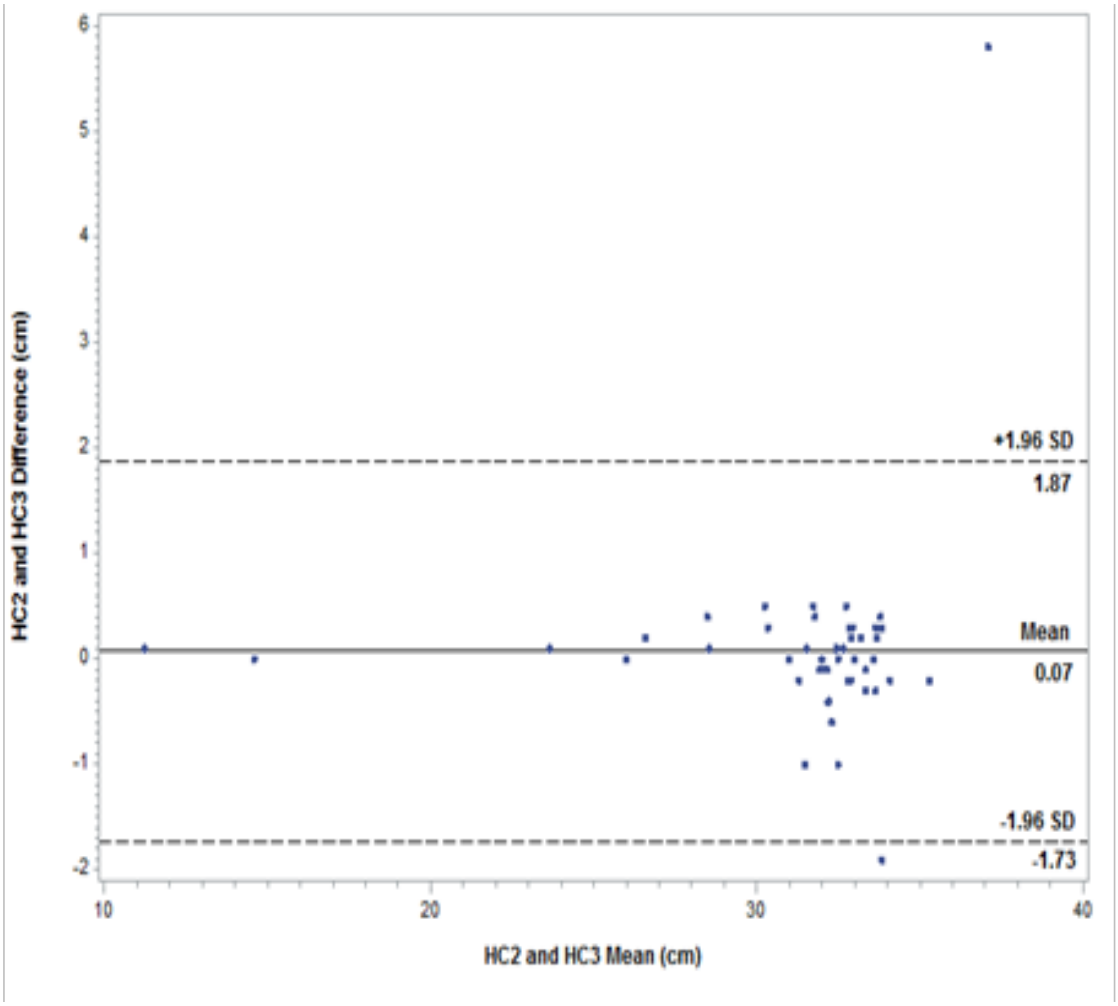


Figure III-6a: Bland Altman plots of abdominal circumference measurements for clinicians 1 and 2

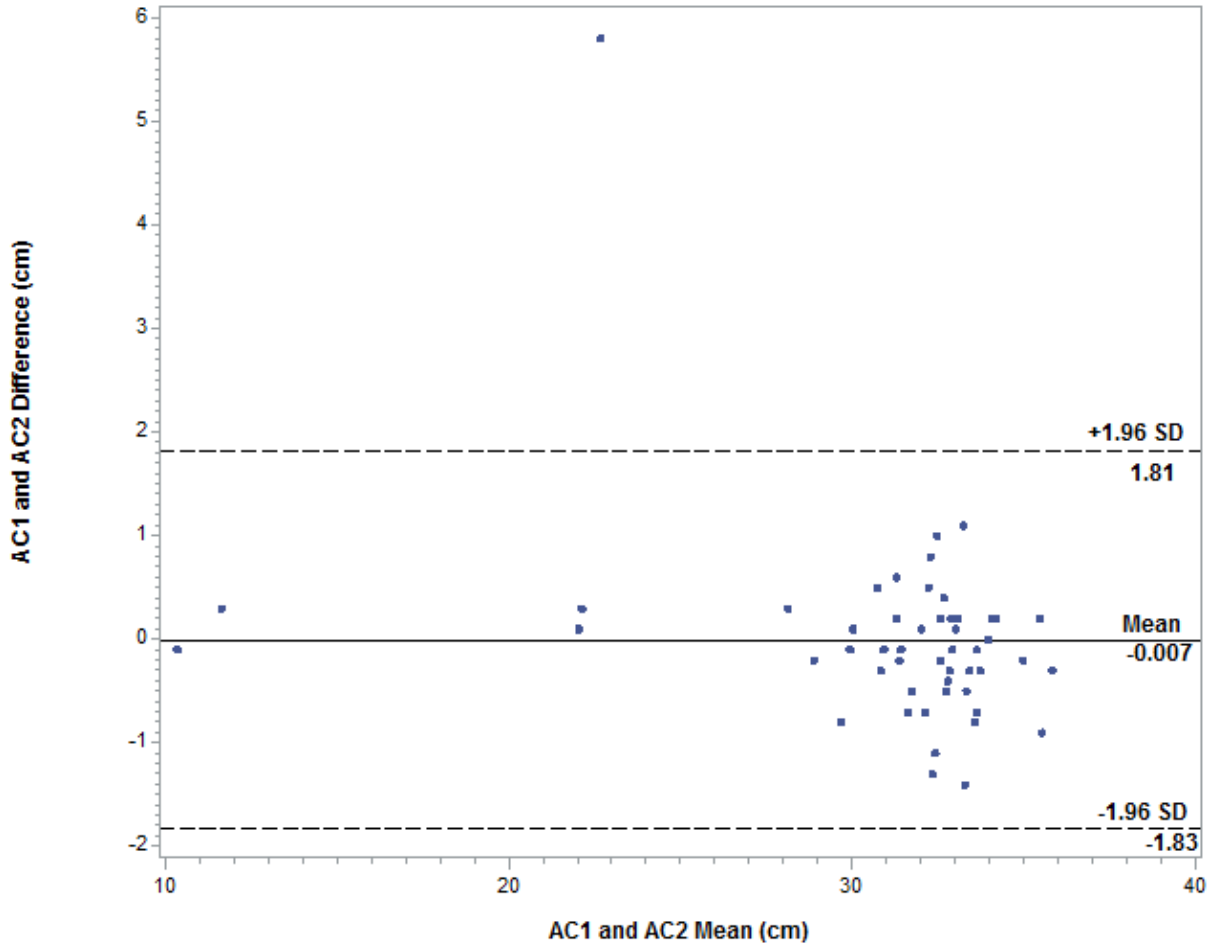


Figure III-6b: Bland Altman plots of abdominal circumference measurements for clinicians 1 and 3

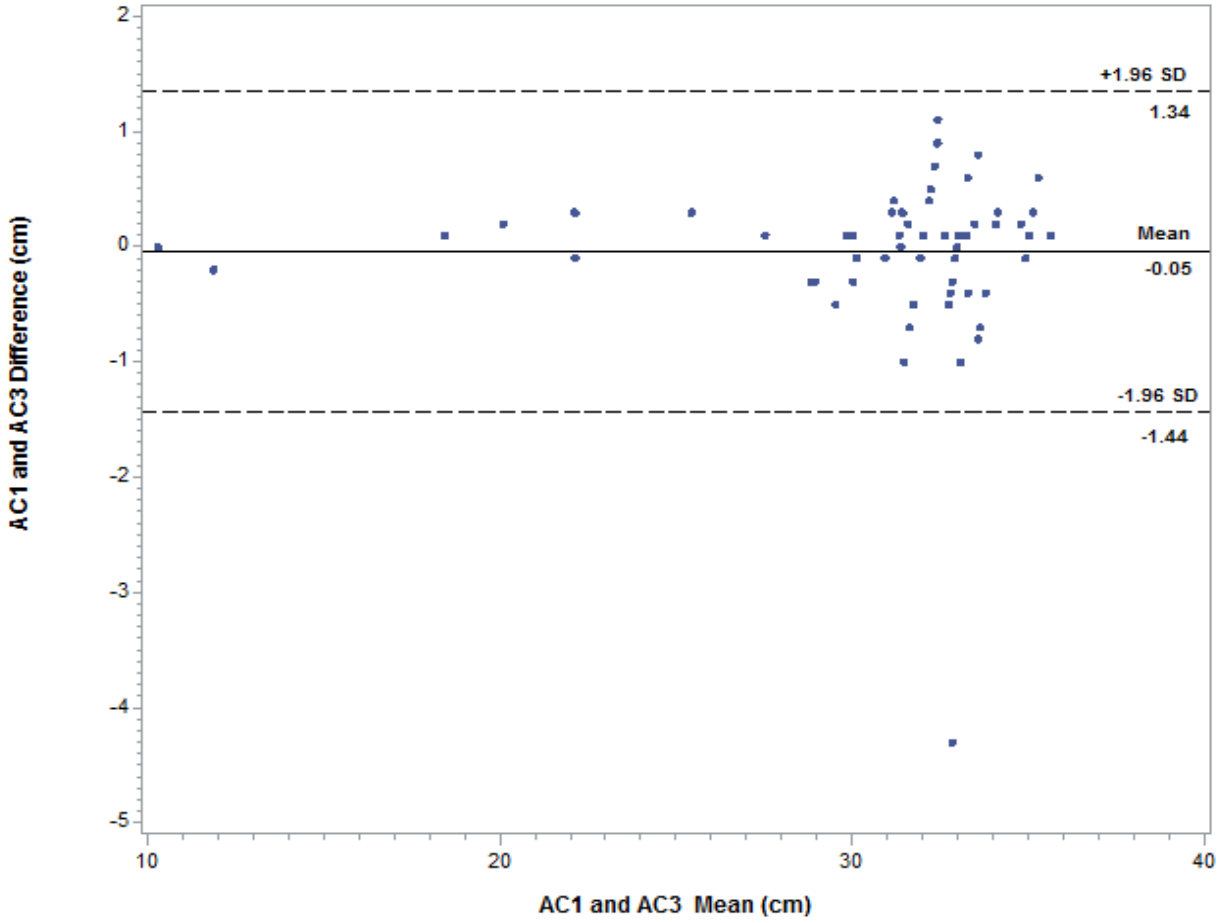


Figure III-6c: Bland Altman plots of abdominal circumference measurements for clinicians 2 and 3

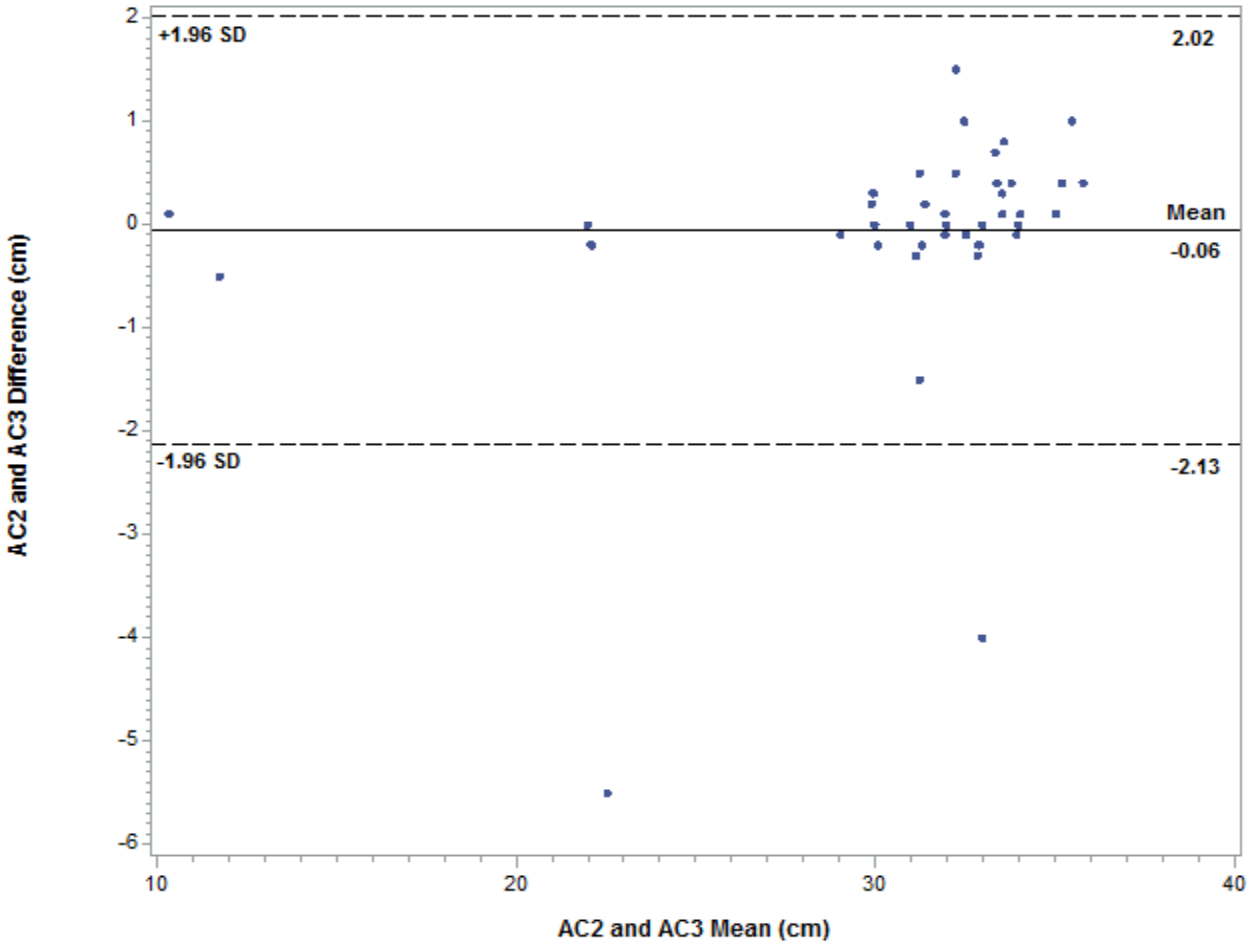


Figure III-7a: Bland Altman plots of biparietal diameter measurements for clinicians 1 and 2

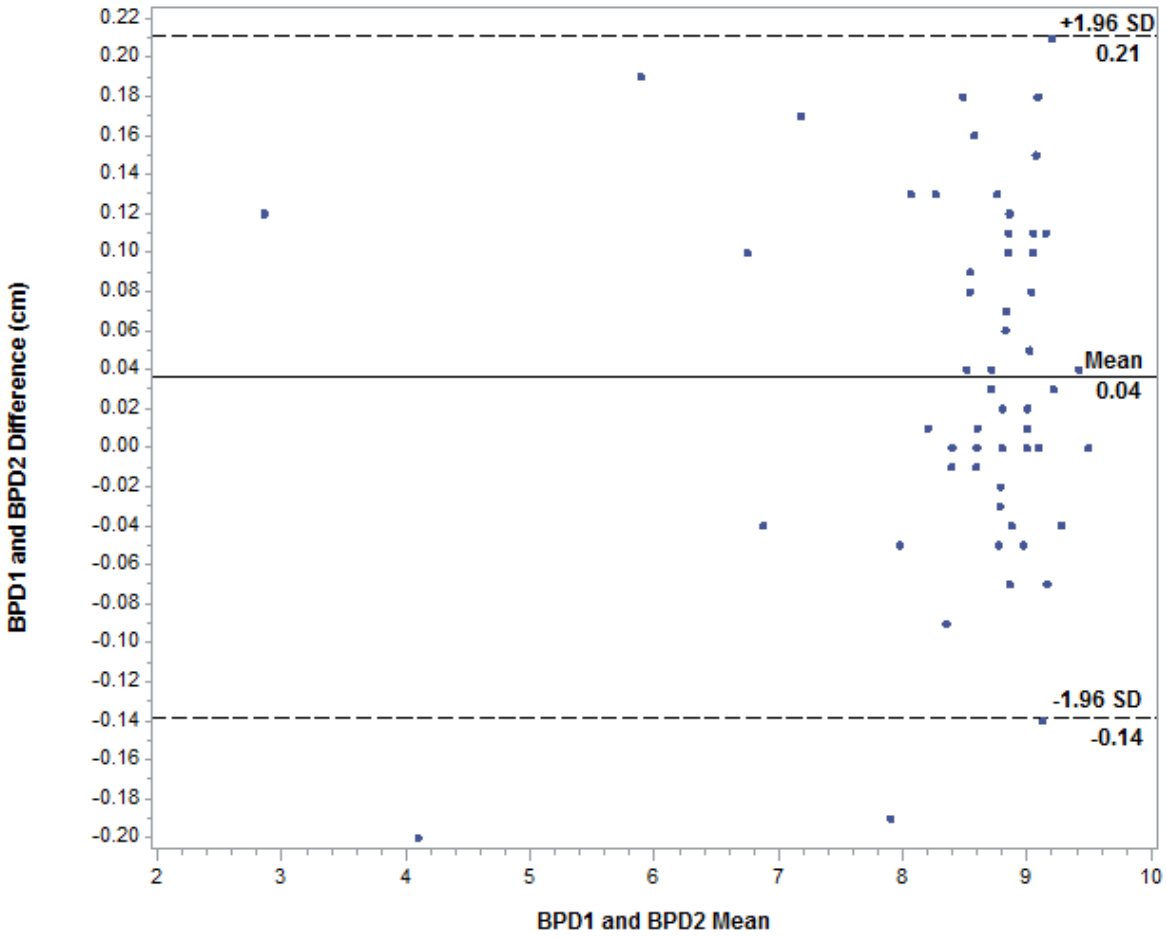


Figure III-7b: Bland Altman plots of biparietal diameter measurements for clinicians 1 and 3

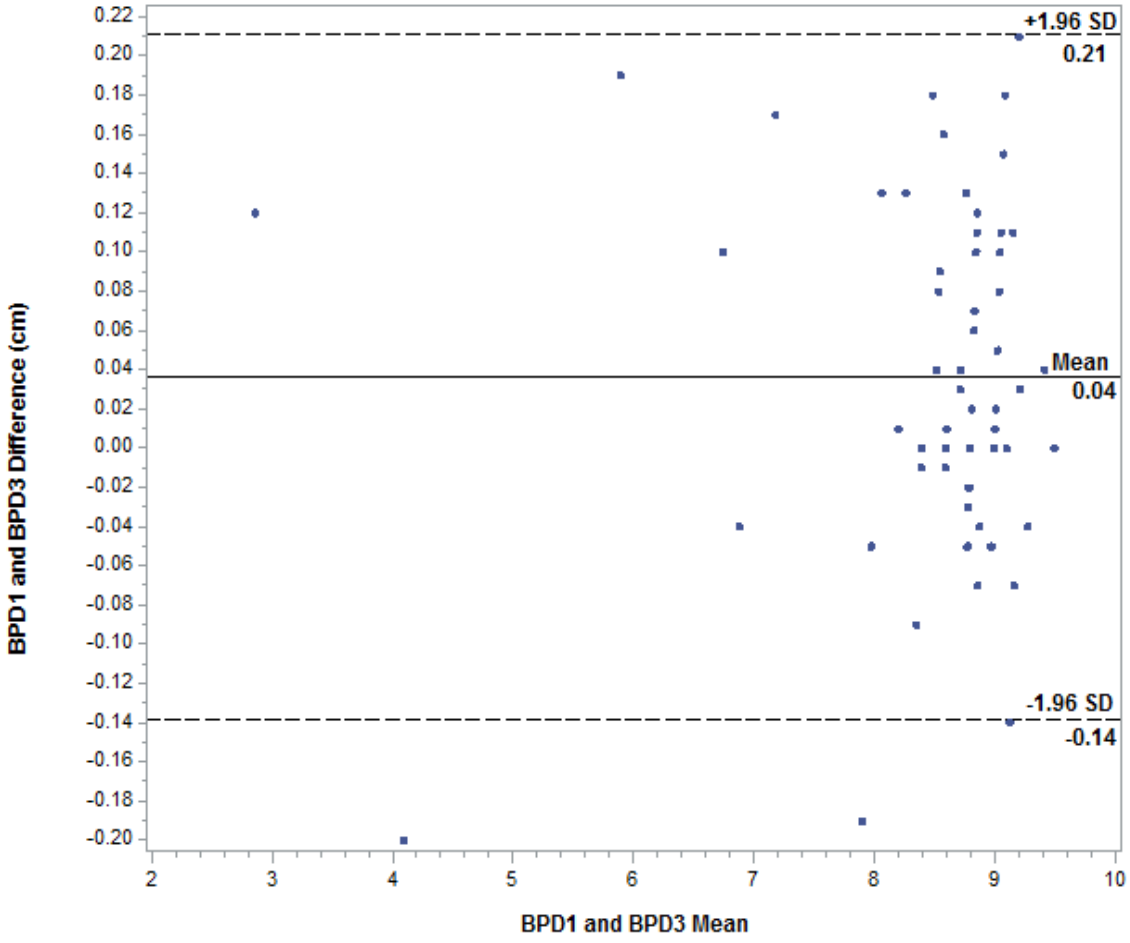


Figure III-7c: Bland Altman plots of biparietal diameter measurements for clinicians 2 and 3

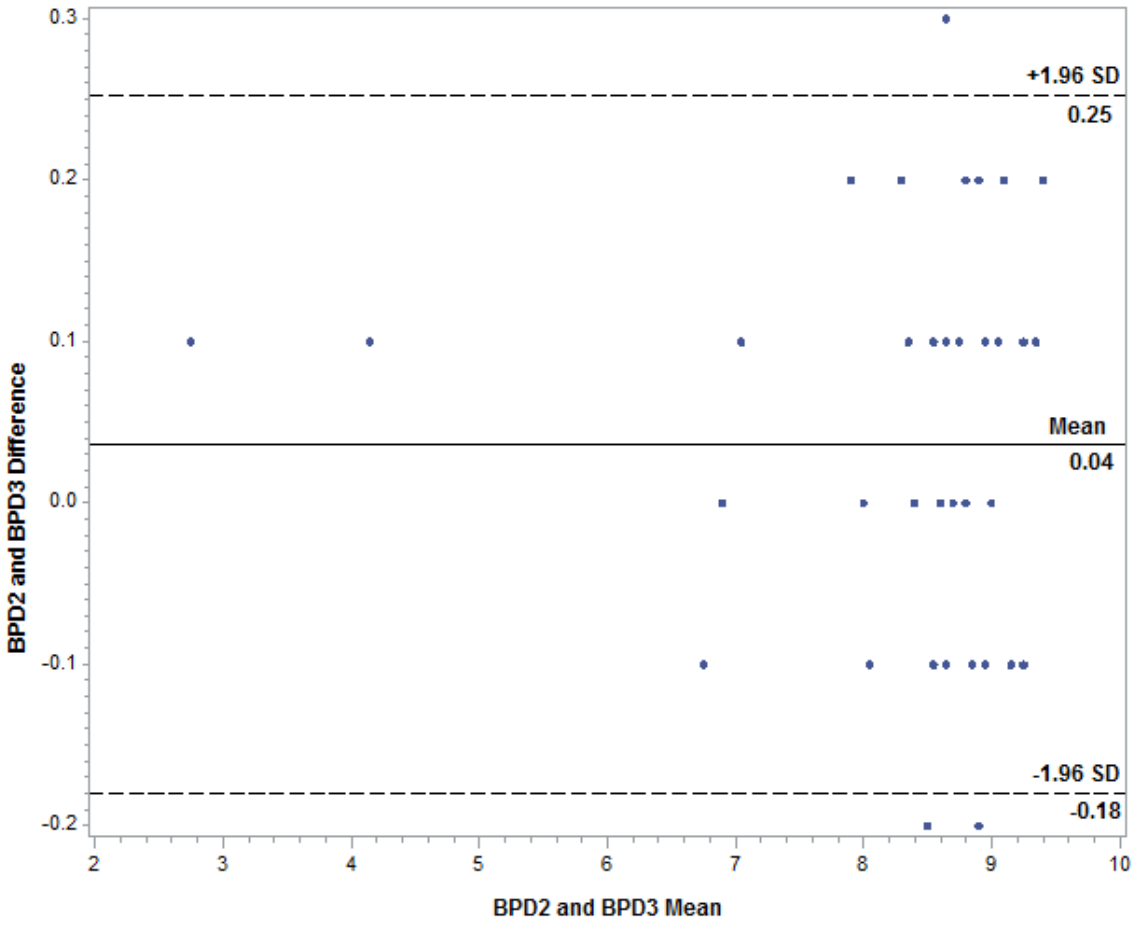


Figure III-8a: Bland Altman plots of femur length measurements for clinicians 1 and 2

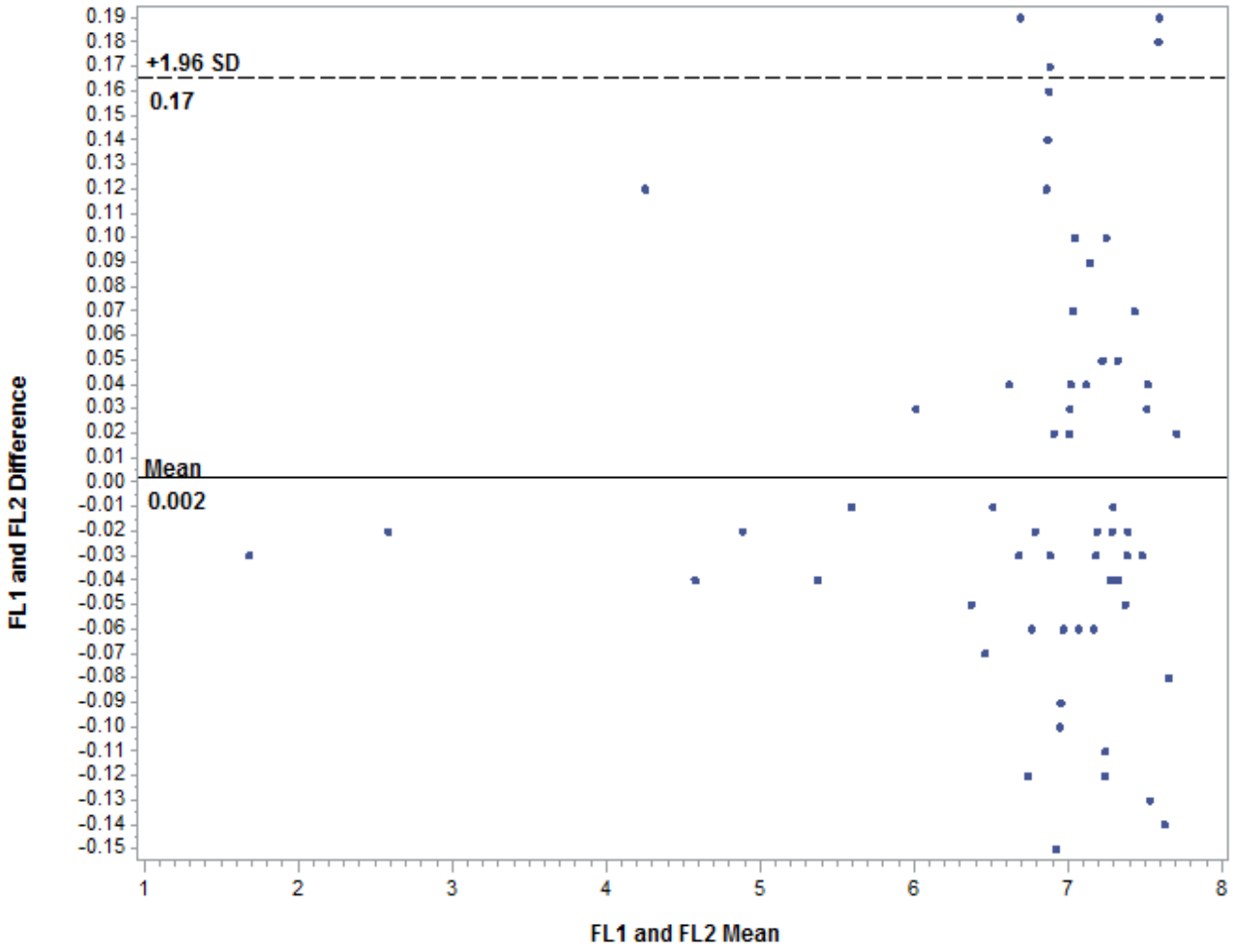


Figure III-8b: Bland Altman plots of femur length measurements for clinicians 1 and 3

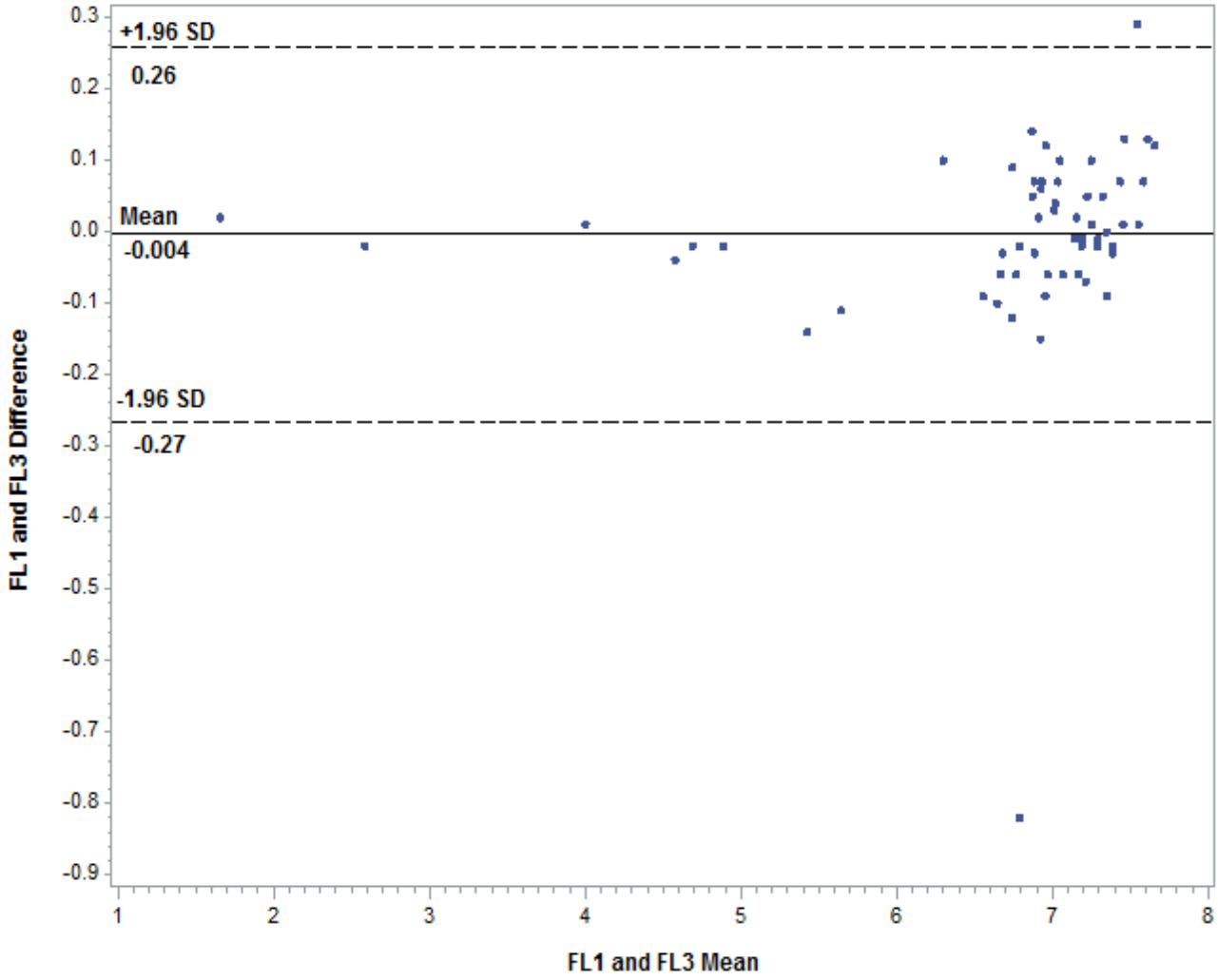


Figure III-8c: Bland Altman plots of femur length measurements for clinicians 2 and 3

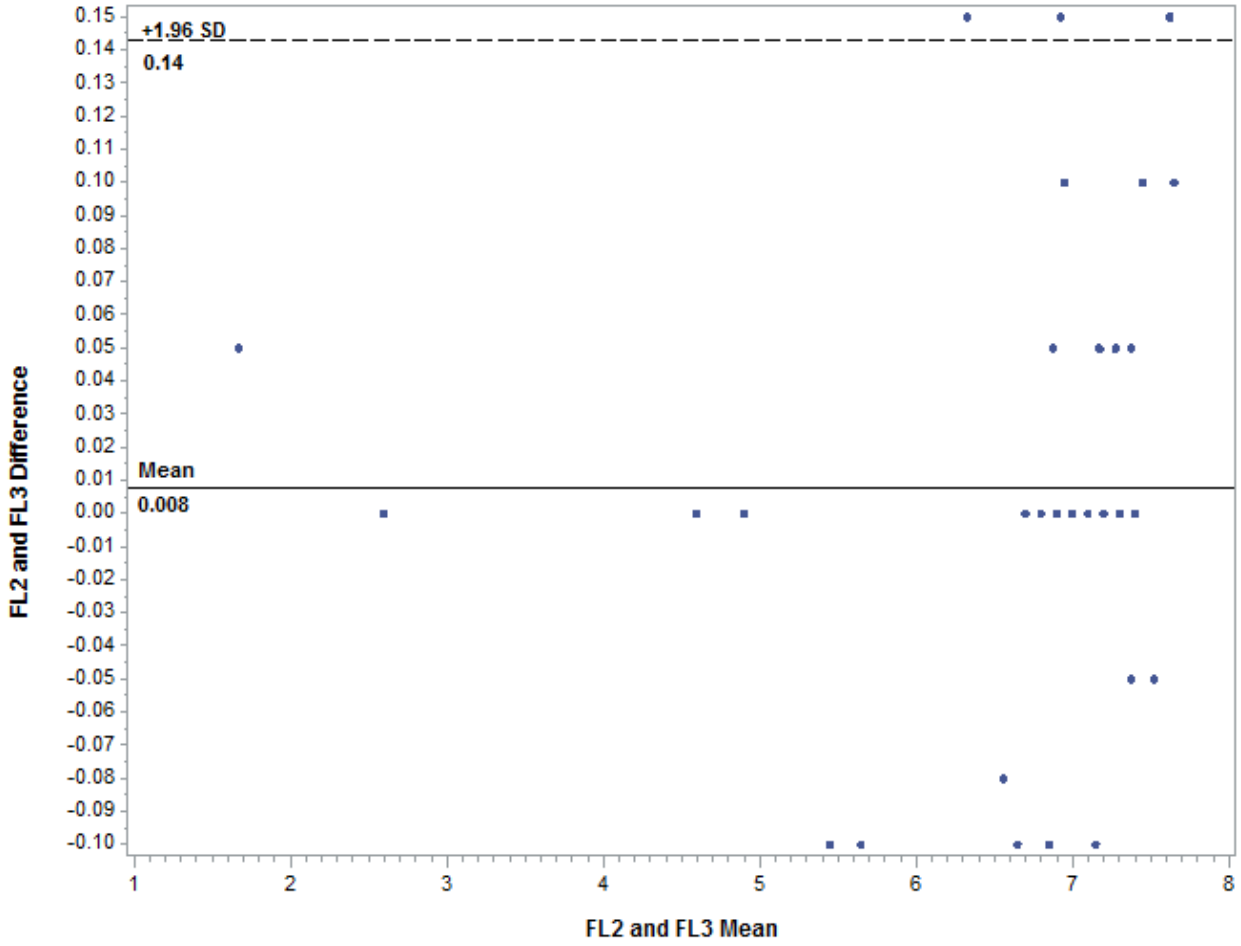


Figure III-9a: Head circumference measurements against gestational age, by clinician

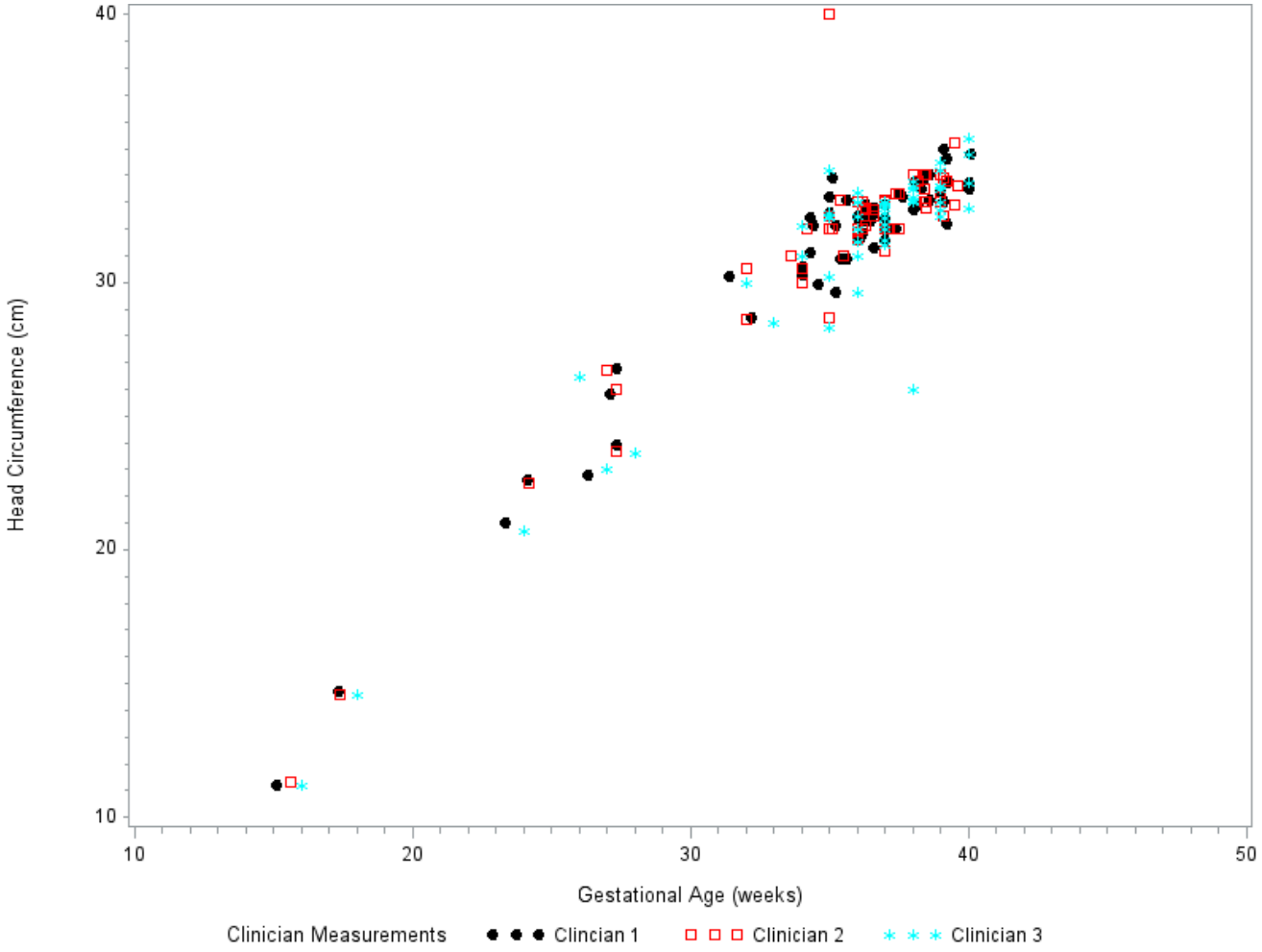


Figure III-9b: Abdominal circumference measurements against gestational age, by clinician

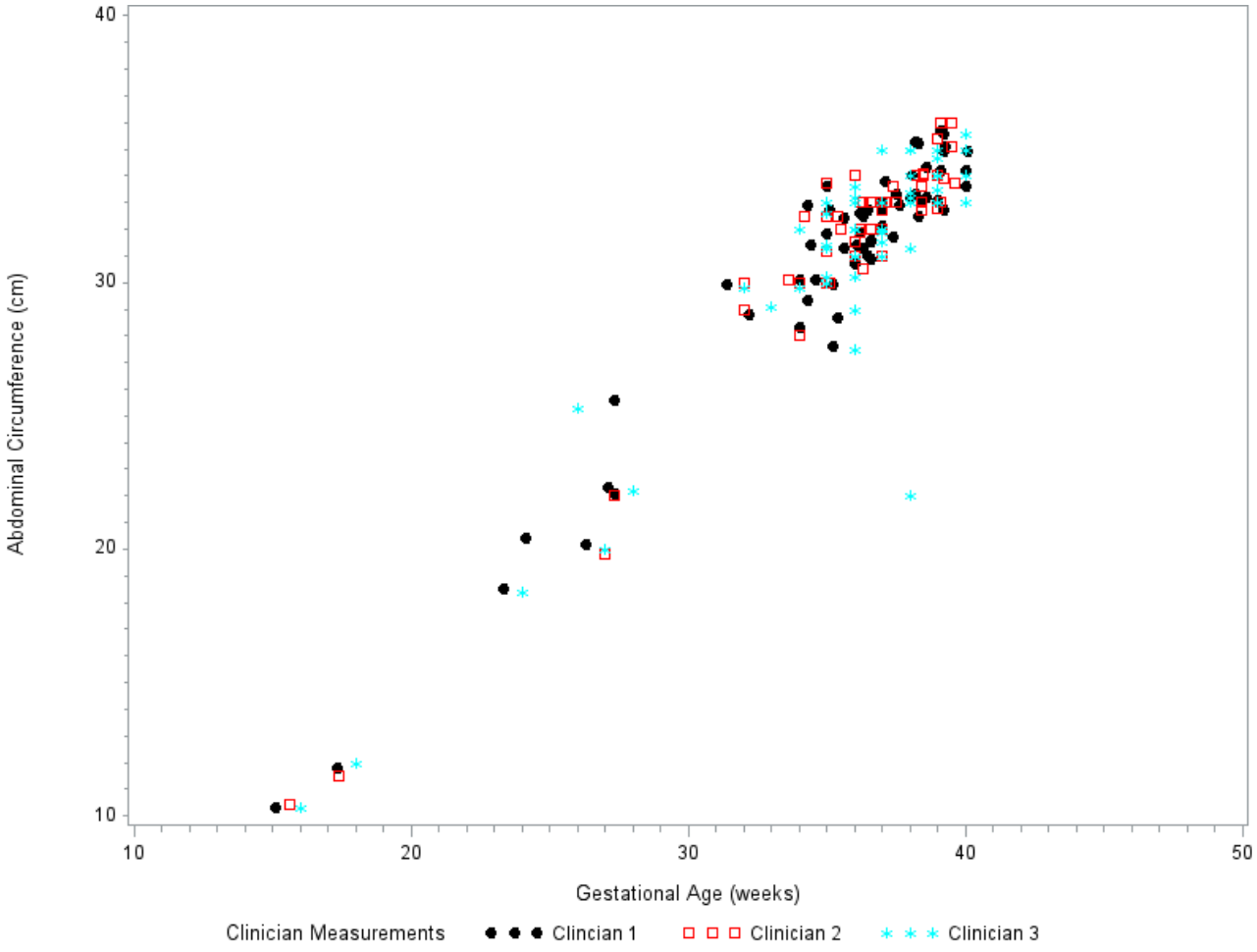


Figure III-9c: Femur length measurements against gestational age, by clinician

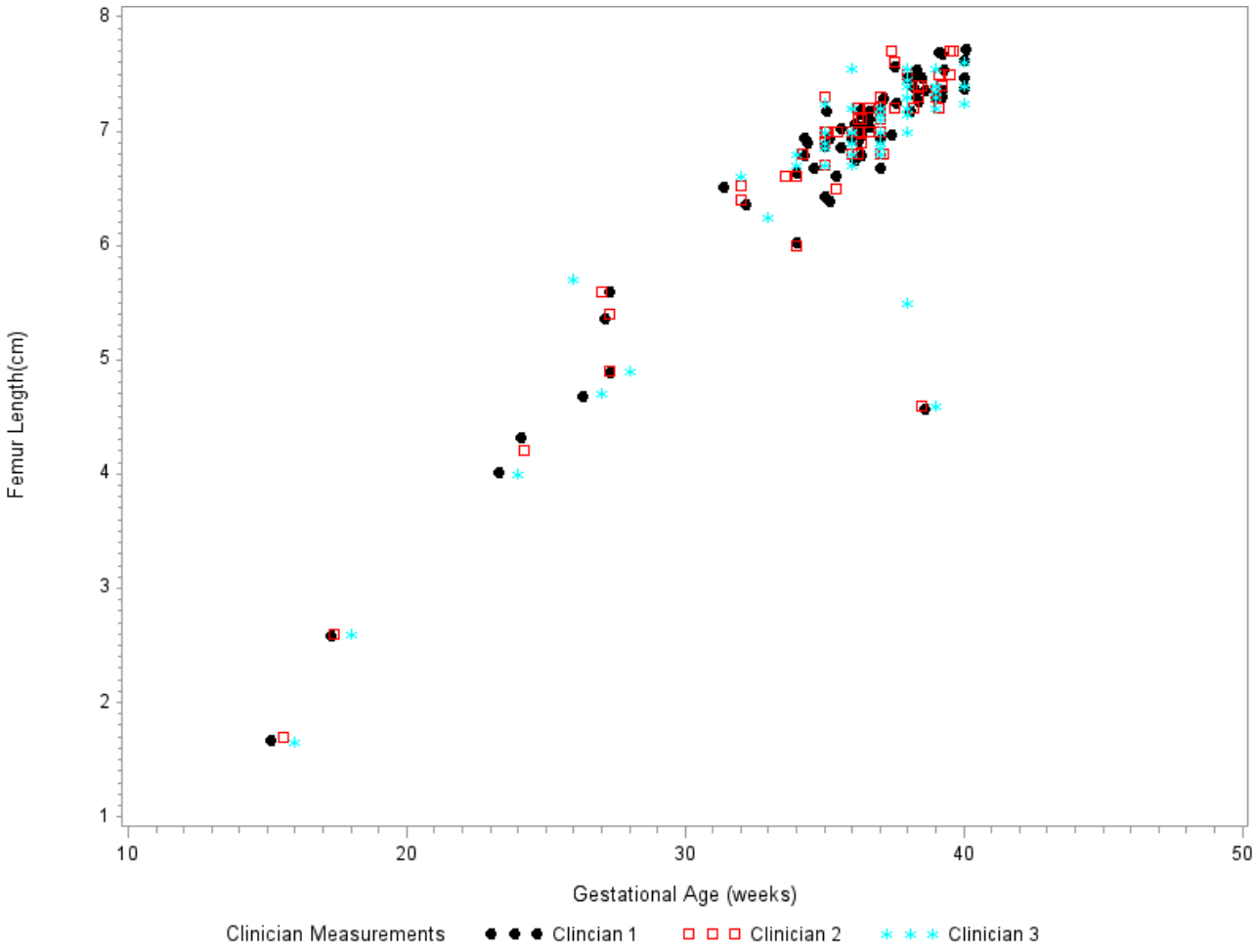


Figure III-9d: Biparietal diameter measurements against gestational age, by clinician

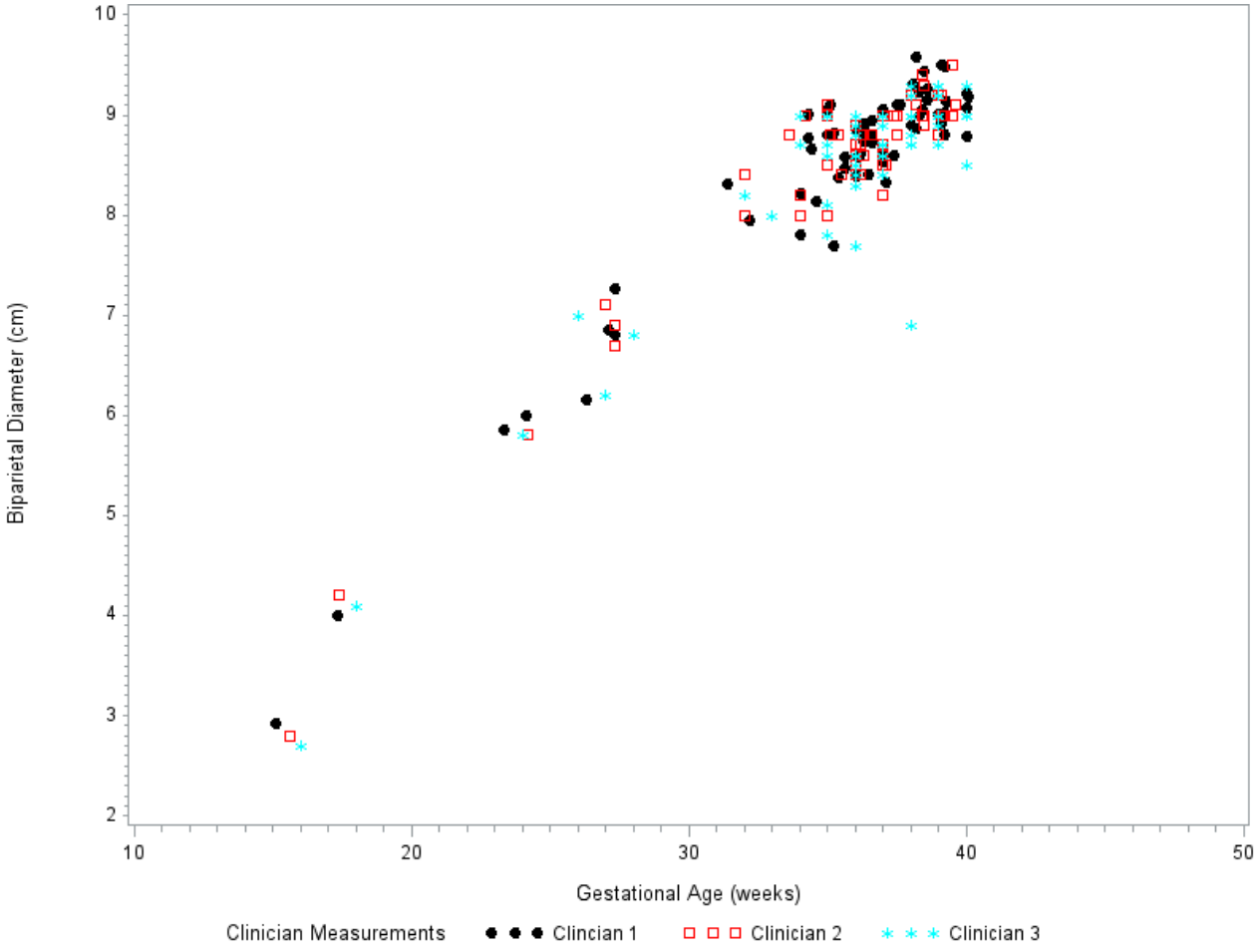


Figure III-10a: Differences in standardized head circumference measurements

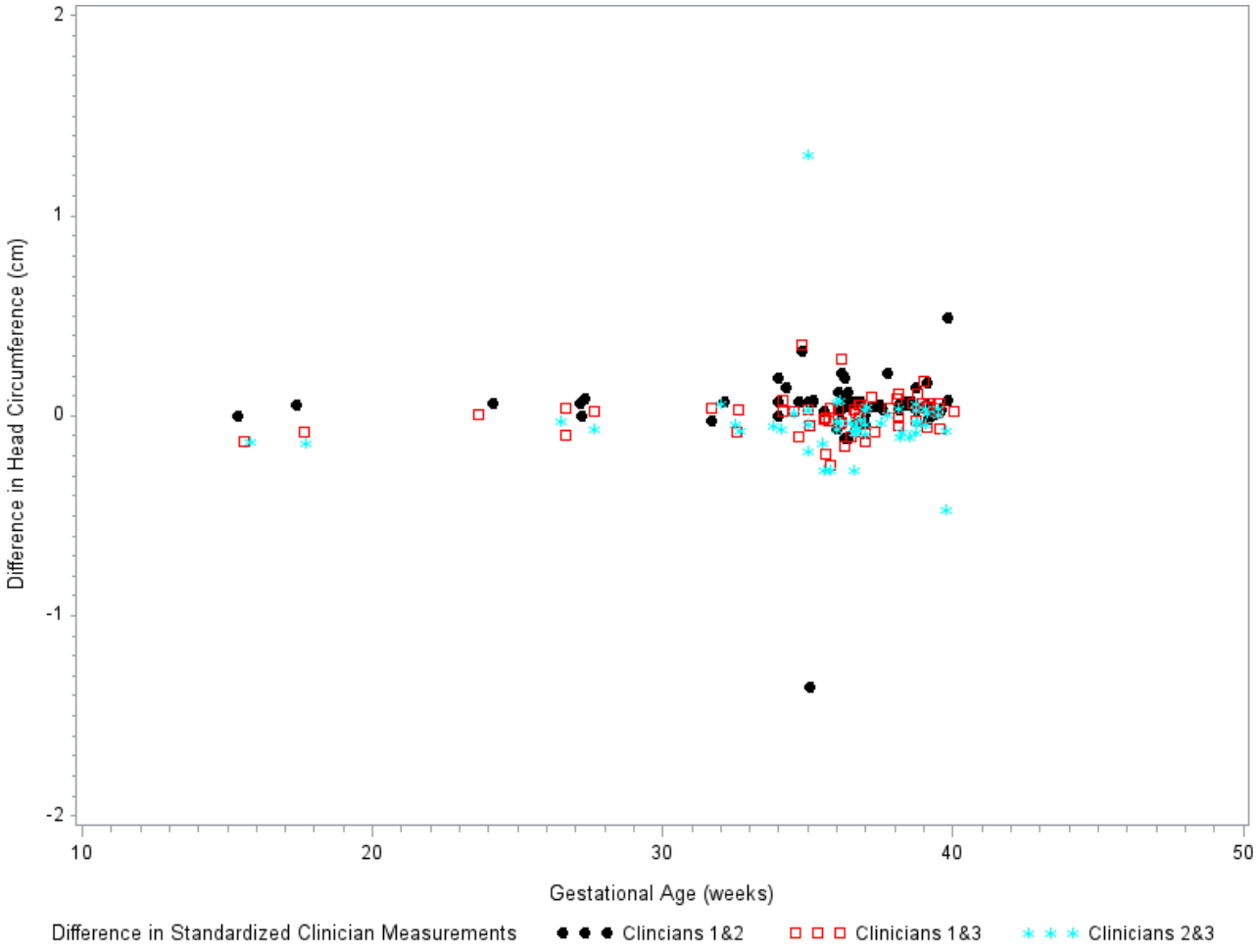


Figure III-10b: Differences in standardized abdominal circumference measurements

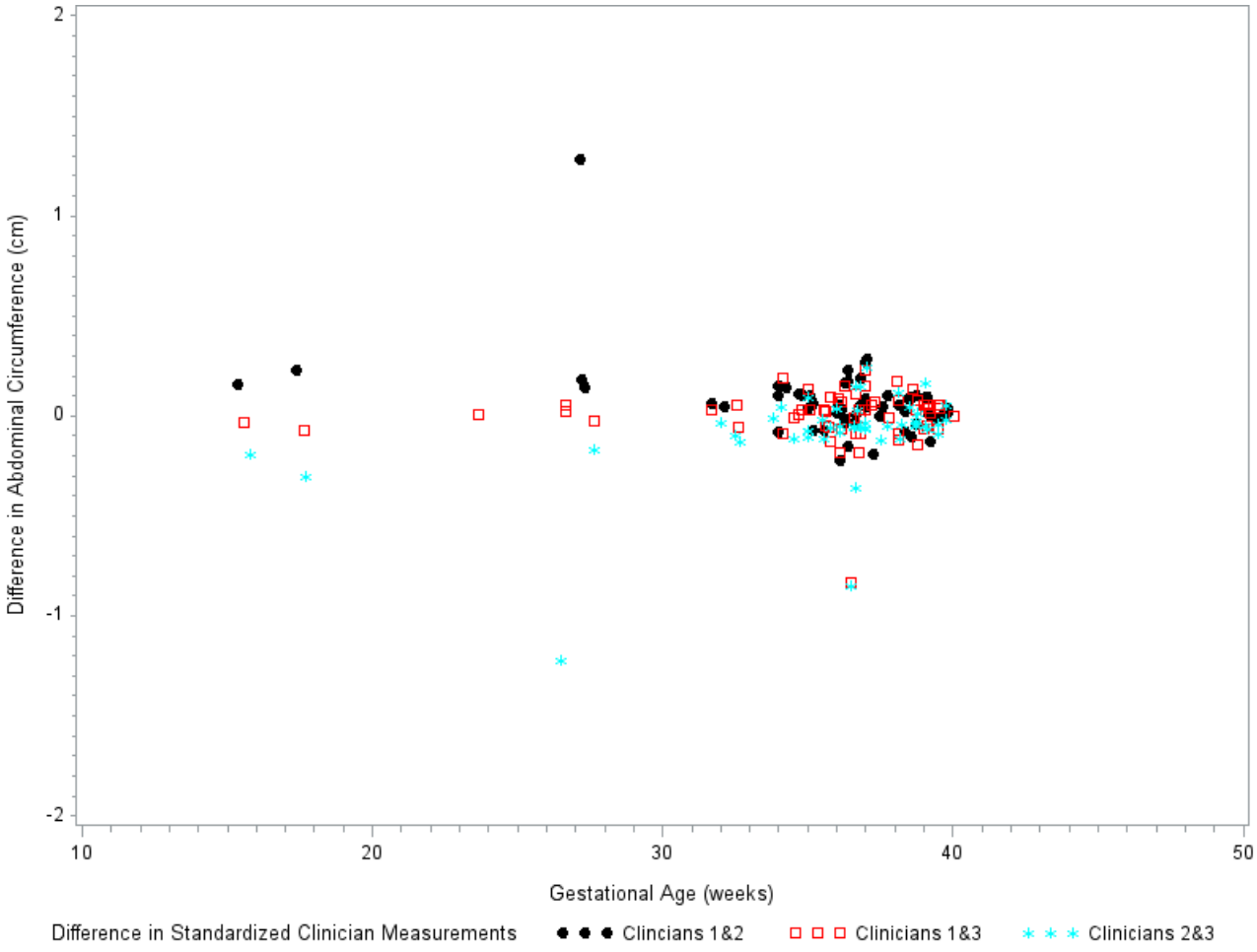


Figure III-10c: Differences in standardized biparietal diameter measurements

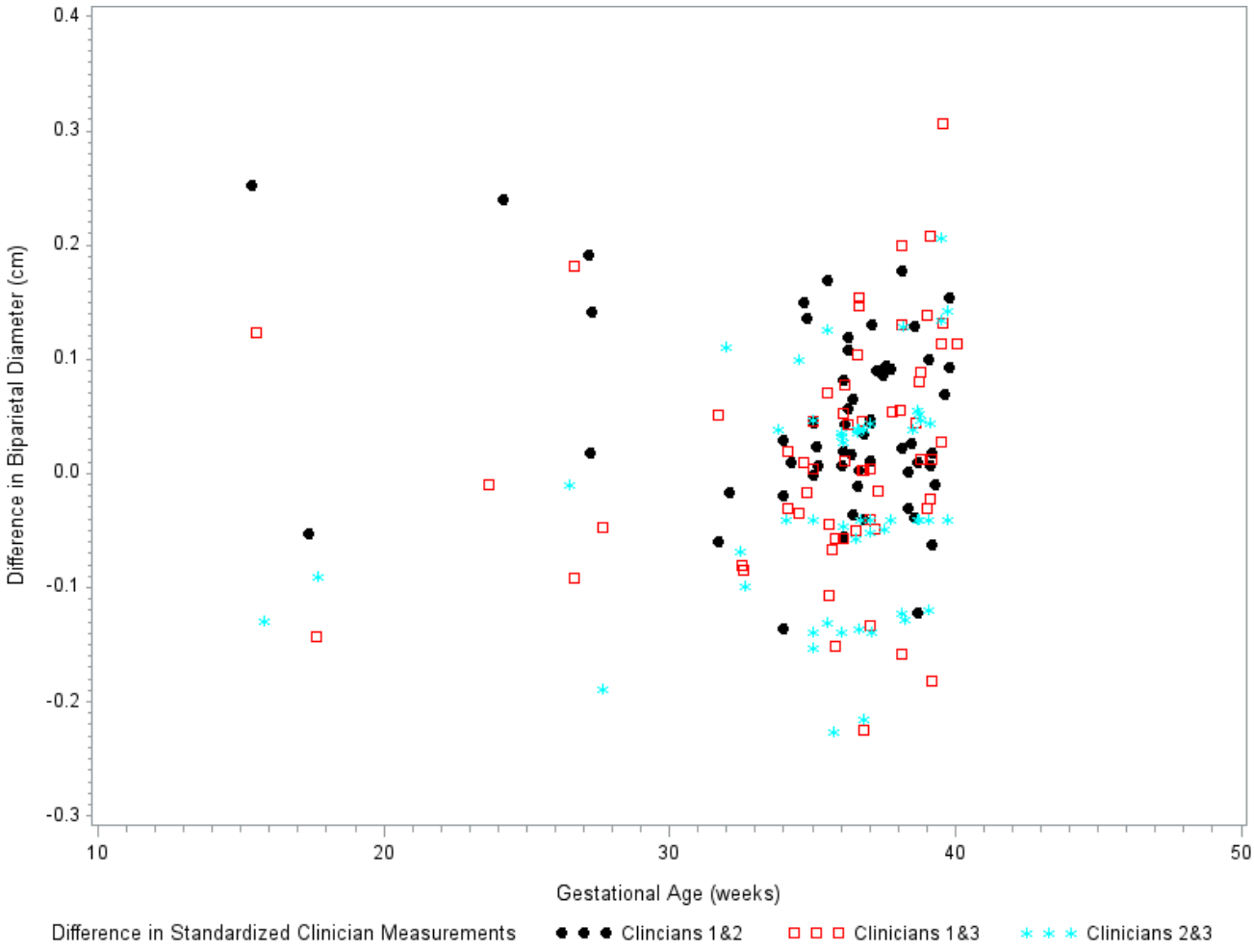
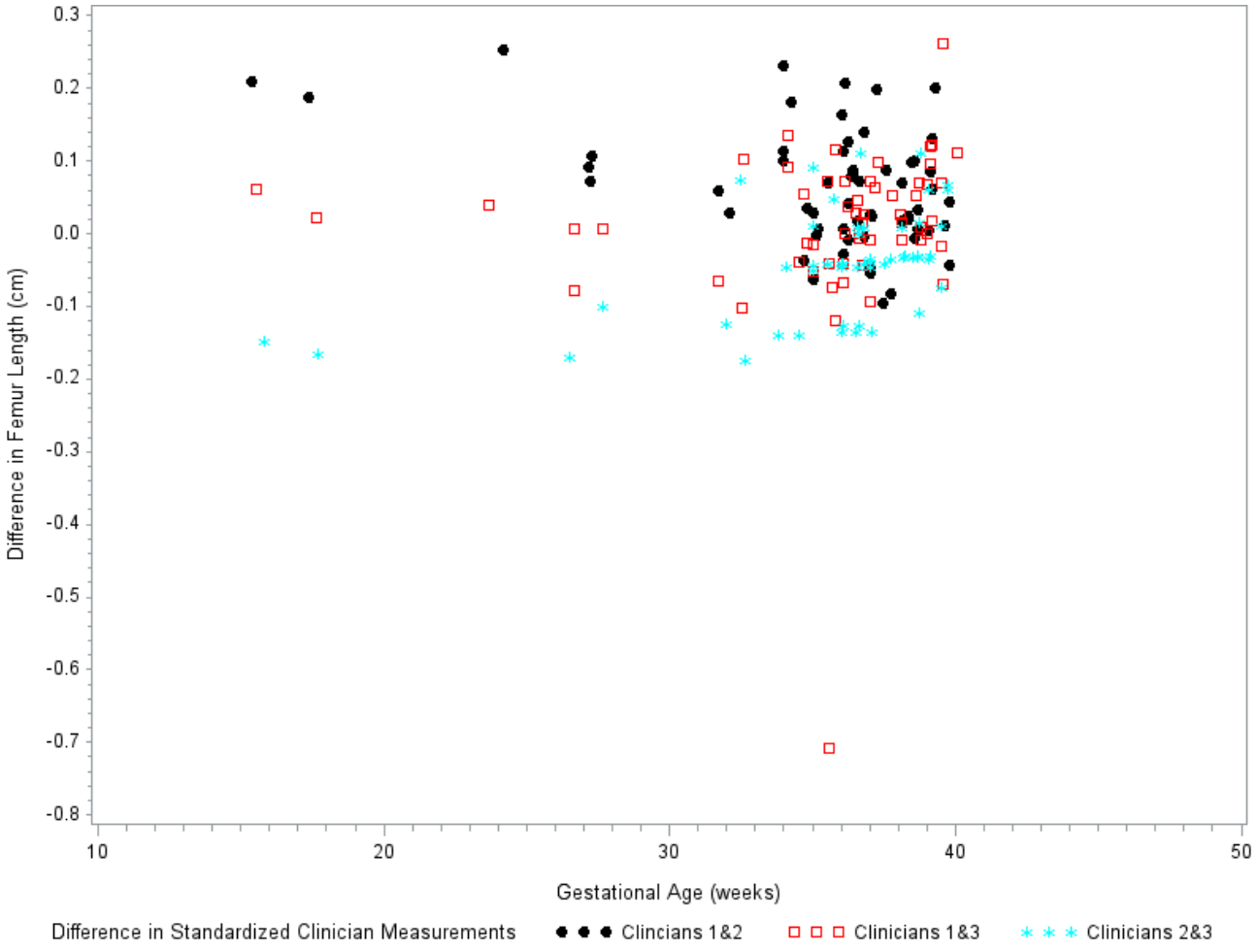


Figure III-10d: Differences in standardized femur length measurements



3.6 REFERENCES

Perni S, Chervenak F, Kalish R, Magherini-Rothe S, Predanic M, Streltzoff J, et al. 2004. Intraobserver and interobserver reproducibility of fetal biometry. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 24(6): 654-658.

Yang F, Leung KY, Lee YP, Chan HY, Tang MH. 2010. Fetal biometry by an inexperienced operator using two- and three-dimensional ultrasound. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 35(5): 566-571.

Sarris I, Ioannou C, Chamberlain P, Ohuma E, Roseman F, Hoch L, et al. 2012. Intra- and interobserver variability in fetal ultrasound measurements. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 39(3): 266-273.

Ioannou C, Talbot K, Ohuma E, Sarris I, Villar J, Conde-Agudelo A, et al. 2012. Systematic review of methodology used in ultrasound studies aimed at creating charts of fetal size. BJOG : an international journal of obstetrics and gynaecology 119(12): 1425-1439.

Smarr MM, Vadillo-Ortega F, Castillo-Castrejon M, O'Neill MS. 2013. The use of ultrasound measurements in environmental epidemiological studies of air pollution and fetal growth. Current Opinion in Pediatrics 25(2): 10.1097/MOP.1090b1013e32835e32831e32874.

O'Neill M, Osornio-Vargas A, Buxton MA, Sanchez BN, Rojas-Bracho L, Castillo-Castrejon M, et al. 2012. Air pollution, inflammation and preterm birth in Mexico City: Study design and methods. The Science of the total environment.

Carrera J. 2001. Ultrasound and Fetal Growth. New York ; London: Parthenon.

Medicine TAIoUi. 2007. AIUM Practice Guidelines.

Lyons JS. 1997. Defining and Measuring Clinical and Utilization Outcomes. In: The Measurement & Management of Clinical Outcomes in Mental Health (Lyons JS, ed): Wiley & Sons, 25-46.

Hughey M. 2005. 2nd and 3rd Trimester Ultrasound Scanning. 2014].

Verburg BO MP, Hofman A, Jaddoe VW, Witteman JC, Steegers EA. 2008. Intra- and interobserver reproducibility study of early fetal growth parameters. Prenatal diagnosis 28(4): 323-331.

CHAPTER IV
AIR POLLUTION AND REPEATED ULTRASOUND MEASURES OF INTRAUTERINE GROWTH IN MEXICO CITY, MEXICO

4.1 Abstract

Maternal exposure to air pollutants during pregnancy has been linked with low birth weight, but few studies have examined this exposure and fetal growth *in utero*. We evaluated exposure to outdoor air pollutants in the first trimester and fetal growth over gestation using repeated fetal ultrasound measures from 625 mother-fetal pairs from a cohort in Mexico City. Fetal head circumference (HC), abdominal circumference (AC), biparietal diameter (BPD) and femur length (FL) were measured by ultrasound. First trimester exposures to ozone (O_3) and particulate matter less than 2.5 micrometers in aerodynamic diameter ($PM_{2.5}$) were estimated using ordinary kriging. Fractional polynomial, mixed-effects models were fit to calculate predicted trajectories of the four fetal parameters and then estimate differences in attained size during pregnancy by interquartile range (IQR) difference in first trimester ozone and $PM_{2.5}$ exposure, controlling for maternal age and fetal sex. For one parameter, BPD, a consistent association was observed with increased maternal exposures to both $PM_{2.5}$ (β's = -0.067 to -0.045 cm, $p<0.05$) during weeks 16-28 and O_3 exposures (IQR 13.29ppb) (β's = -0.064 to -0.045 cm, $p<0.05$) during weeks 22-32 of gestation. Overall, with the exception of fetal AC, increased maternal pollutant exposure during first trimester was uniquely and significantly associated with decreased size of fetal anthropometric parameters during

gestation; parameter results varied by air pollutant. These findings support the need for further research examining air pollution-associated changes in fetal parameters, assessed *in utero*.

4.2 Introduction

Fetal growth is an important component of the survival and health of newborns, both at birth and later in life. In low-middle income countries, including Mexico, most cases of low birth weight (LBW) are growth restricted infants rather than preterm (Balcazar and Haas 1991; Kramer 1987; Semba and Bloem 2008; Carrera 2001). In some studies, intrauterine growth restriction (IUGR), excluding congenital anomalies, has been implicated as a causal factor in 50% of still births (Figueras and Gardosi 2011). In addition to contributing to neonatal death, longer periods of slowed fetal growth *in utero* have been associated with increased perinatal morbidity (Illa et al. 2009). The importance of timing of the growth restriction is illustrated by the findings of a case control study that examined IUGR and cerebral palsy. Risk of cerebral palsy increased with growth restriction occurring closer to term, but not with IUGR during early pregnancy (Figueras and Gardosi 2011).

Several studies have reported on the relationship between air pollution and various pregnancy outcomes (Lee B.E. 2003; Shah and Balkhair 2011; Bell et al. 2010; Parker et al. 2011; Ritz et al. 2007; Maisonet et al. 2004; Rich et al. 2009; Ballester et al. 2010; Choi et al. 2006; Estarlich et al. 2011; J-H Leem et al. 2006; Malmqvist et al. 2011). Although the biological mechanisms that might explain observed associations between air pollution and birth outcomes are not completely understood, Slama and colleagues efficiently summarized hypothesized mechanisms linked to IUGR and preterm

birth (Slama et al. 2008). Many of the potential mechanisms, namely alterations in maternal-placental exchanges of nutrients and oxygen, endocrine disruption and oxidative stress, may be directly linked to IUGR. Others, like pollutant-induced inflammation, are linked to spontaneous preterm birth; these neonates are often growth restricted fetuses. In Mexico City, ozone (O_3) and particulate matter less than 2.5 microns in aerodynamic diameter ($PM_{2.5}$) are considered to be major components of air pollution (Molina et al. 2009; Vallejo et al. 2004) . $PM_{2.5}$ contains a mixture of metals and harmful constituents that can be introduced into the blood stream and result in systemic inflammation (Hackley et al. 2007). Exposure to polycyclic aromatic hydrocarbons (PAHs), a class of compounds that may also be constituents of particulate matter, can lead to the formation of DNA adducts, causing cell mutation or cellular death; this could result in an increase in blood viscosity which reduces the amount of blood flow to the placenta and uterus (Veras et al. 2008; Perera et al. 2003; Detmar et al. 2008) . Additionally, O_3 has been directly linked to increased inflammatory responses as a result of lipid peroxidation, which could lead to alterations in placental function, resulting in fetal growth restriction (Salam et al. 2005). Whatever the mechanism, associations of air pollution exposure with fetal growth can be assessed if the attained size of the fetus at the time of birth or shortly before is known, or repeated ultrasound measures allow estimation of the trajectories of fetal growth over gestation.

Some previous epidemiologic studies on air pollution and birth outcomes have used birth certificates and hospital discharge summaries to obtain fetal and maternal data. These data sources often have limited information on potential confounders of the air pollution and fetal growth association, e.g., nutritional status, since that information is not

readily available in administrative records (Woodruff et al. 2009; Ritz and Wilhelm 2008). IUGR as an outcome is particularly difficult to assess with birth registries; although small for gestational age (SGA) has been used as an IUGR proxy, clinical literature specifies that being growth-restricted and small for gestational age (SGA) are not synonymous (Carrera 2001). The use of birth weight as a proxy for fetal growth may not be the best endpoint since recent literature has shown that birth weight poorly reflects IUGR during the first two trimesters (Kingdom and Baker 2000).

A limited number of studies have used fetal biometry to assess fetal growth in relation to air pollution exposure (Slama 2006; Ritz et al. 2014; Aguilera et al. 2010; Hansen et al. 2008; Slama et al. 2009; van den Hooven et al. 2011; Iniguez et al. 2012a).The purpose of the present analysis was to evaluate the hypothesis that maternal exposure to air pollutants is independently associated with IUGR, as indicated by reduction in attained size and growth trajectories of fetal parameters, using repeated fetal biometry data and other covariates from pregnant women residing in Mexico City.

4.3 Methods

4.3.1 Study Location and Population

Mexico City, the capitol of Mexico, is a mega-city densely settled with a population of 21.2 million in 2013, representing seventy-five percent of Mexico's total population (World Population Review2013). Infants born in Mexico City were estimated to experience a frequency of IUGR of 12% in 2009 (Hernandez-Valencia et al. 2001). Within this population, IUGR is associated with 20% of fetal deaths (Fernández-Carrocera et al. 2003). In addition to adverse birth statistics, Mexico City also has several

sources of environmental pollution, vehicle traffic, petroleum refineries, and dry cleaners, contributing to annual pollution that exceeds health and environmental standards, with much focus being placed on PAHs, O_3 and $PM_{2.5}$ (Marr et al. 2004).

The current analysis was conducted with data from participants in a study of air pollution and adverse birth outcomes in Mexico City, described in detail elsewhere (O'Neill et al. 2012). Briefly, pregnant women residing in various regions across Mexico City were enrolled starting in 2009 and follow up continues; the total study population will number approximately 1,000 women. Inclusion criteria were: women 18 years of age or older, self-reported nonsmokers, having a singleton pregnancy, without the presence of medical complications, and recruited at 18 weeks of gestation or less according to last menstrual period (LMP) recall and confirmed by ultrasound (O'Neill et al. 2012). Once enrolled, women were invited to visit the clinic at the Hospital Materno Infantil Inguaran (Inguaran Maternal Infant Hospital), a public hospital funded by Mexico City's government, on an approximately monthly basis during their pregnancies to have biological samples collected; to receive a clinical exam, including ultrasound; and to provide information on their health status, dietary intake, and activities relevant to air pollution exposure. A global positioning system device was used to record the latitude and longitude of participants' home addresses for use in air pollution exposure assessment.

4.3.2 Ultrasound Measurements

Fetal parameters were measured by the study's clinicians at the Inguaran Hospital. All clinicians were trained using widely accepted methodology according to the obstetric literature (The American Institute of Ultrasound in Medicine Medicine 2007).

Institutional Review Board (IRB) approval was received from all participating institutions: the University of Michigan, the Inguaran Hospital and the Universidad Nacional Autonoma de Mexico (National Autonomous University of Mexico).

Ultrasound measurements of head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur length (FL) were collected during each monthly clinical visit using the Aloka SSD-1000, Hitachi Aloka Medical, Ltd ultrasound equipment. Quality assurance, i.e., estimation of interobserver reliability, was performed to assess possible measurement and observer error, using previously published methodology (Perni et al 2004). Intraclass correlation coefficients of a subset of the fetal measurements made by study clinicians ranged between 0.985-0.996: HC= 0.985, BPD= 0.995, AC= 0.985, FL= 0.996. Cronbach's Alpha coefficients of agreement were consistent with the intraclass correlation coefficient values (HC= 0.995, BPD= 0.999, AC= 0.995, FL=0.999). Mean differences of clinician measurements for the four fetal parameters were close to zero (-0.056 to 0.069 cm), suggesting measurements were highly reliable (unpublished data).

4.3.3 Assessment of Air Pollution Exposure

Outdoor air pollution concentrations were obtained from the Mexico City air quality monitoring system, Sistema de Monitoreo Atmosférico (SIMAT, for its Spanish initials). The monitoring system consisted of 37 stations, according to a 2011 publication (Instituto Nacional de Ecologia (2011)), reporting hourly concentrations for the air pollutants O_3 and particulate matter of two size fractions, PM_{10} (diameter $\leq$ 10 micrometers in aerodynamic diameter) and $PM_{2.5}$. The data collection and United States Environmental Protection Agency audits of the monitoring system have been described

previously. (Edgerton et al. 1999; Shanis Mark 2005) Pollutant data were downloaded as hourly concentrations, then summarized to create the daily (24 hour) average for $PM_{2.5}$ and the 8 hour daily maximum for O_3, to correspond to the health-based standards

Exposures to air pollutants during pregnancy were assessed in two ways. 1) A daily citywide average from all reporting monitors was calculated, reflecting only temporal and not spatial contrasts in exposure for all the women. 2) A daily, woman-specific exposure (encompassing pollutant concentration variation over time and place) was calculated at maternal place of residence, using ordinary kriging, a geo-spatial interpolation method. Kriging is technique that estimates values at un-sampled locations, in this case, maternal place of residence, by interpolating between pollutant concentrations sampled spatially (in this case, the network of monitors sited around Mexico City), to create a spatial surface and assign a unique concentration for the mother's address (Rivera-González 2012). Weekly and first trimester (estimated conception date until 12 completed weeks of gestation) individual-specific kriged estimates of exposure for each mother's home address were derived in SAS version 9.3 (SAS Institute Inc., Cary NC, USA). We assessed the correlations between first trimester air pollutant metrics of exposure (kriged and citywide average) for each pollutant.

4.3.4 Covariates

Information on maternal behaviors and medical history was collected for participants by questionnaires at the initial and sequential follow-up visits. We considered several covariates from these repeated visits based on previously reported risk factors for IUGR (Carrera 2001) as well as covariates suggested for examination as potential confounders and effect modifiers of the relationship between air pollution and

reproductive outcomes (T.J. Woodruff et al. 2009). These included: maternal age, passive smoking status, parity, and pre-pregnancy body mass index (BMI) calculated from pre-pregnancy weight and height, alcohol use and fetal sex. In addition, variables reflecting cooking style (wood or coal stove) were also selected as covariates as proxies for indoor air pollution (Estarlich et al. 2011) . Finally, air pollution concentrations can vary seasonally and season of birth and conception may also be associated with birth outcomes (Strand et al. 2011). Thus, based on the estimated period of conception, an indicator variable for season of conception was created. Primarily, Mexico City has three seasons; cool dry season (November to January), warm dry season February to April), and the wet season (May to October) (Molina et al. 2009; Marr et al. 2004).

From this list, covariates were examined to identify potential confounders, effect modifiers, and mediators. Our goal was to exclude potential mediators, so as not to over-adjust our models and potentially bias our results (Schisterman et al. 2009). Potential confounding factors, identified through the use of directed acyclic graphs (DAGs), were automatically included in adjusted models of air pollution and fetal anthropometric parameters. The first potential confounder was maternal age, a known predictor of IUGR, which could also potentially be associated with systematically different outdoor pollution exposures. The amount of pollutant uptake varies by the physical activity being performed and a person's energy expenditure and basal metabolic rate; all of these factors differ by age according to exposure assessment models like the Environmental Protection Agency's Consolidated Human Activity Database (CHAD) (McCurdy T. 2000). Also, we know that fetal growth assessed at birth differs by the baby's sex (Ghosh et al. 2007) and that sex-specific differences have been found when examining fetal parameters

assessed *in utero* by ultrasound (Schwarzler et al. 2004). However, there is a lack of strong support in the literature of the interaction between air pollution exposure and pregnancy outcomes, especially restricted growth, so we did not address effect modification but instead all models were also adjusted for fetal sex. Maternal education (a socioeconomic status (SES) indicator) was also considered as a potential effect modifier , given that women with lower SES may live in more polluted neighborhoods and may have more physiological and psychological factors that could adversely affect maternal health (O'Neill et al. 2003). SES could explain differential misclassification of ambient exposures among Mexico City women. Women with lower SES may spend more time outdoors as a result of occupation or primary modes of transportation being walking or public transit (Calderon-Garciduenas and Torres-Jardon 2012). Also, it is important to consider that women with a lower SES may have to work more during pregnancy and may spend more time away from home. Finally, known risk factors of IUGR (parity, BMI, and alcohol consumption) were statistically examined to determine if they should be added to the models as precision variables, that is, variables that would also explain variance in model outcomes and improve statistical power, as described in detail later, after regression models are introduced.

4.3.5 Fetal Growth and Growth Trajectories

Since our goal was to evaluate the possible influence of air pollutant exposure on attained size of fetal parameters, we considered the shape of the four fetal parameters' growth trajectories across gestation when choosing our data analysis strategy. The growth trajectory of head circumferences (for example) measured in 4,234 fetuses appeared, on average, linear between 15 and 25 weeks of gestation and then somewhat curvilinear and

beginning to flatten out after 25 weeks (Schwarzler et al. 2004). We thus did not want to assume that our population's fetal parameters would exhibit a simple linear growth rate that is adequately characterized by a single slope throughout gestation, and potentially calculate spurious associations with air pollution by missing these variations in growth rate at different stages of gestation. Because we had repeated measures, an additional issue in our data was that the ultrasound measures were taken during clinic visits at various points in gestation, not always at the same point for each woman. This is referred to as 'unbalanced' data and we wanted to apply methods that would accommodate this feature.

4.3.6 Statistical Analysis

To start, univariate analyses of pollutant exposure variables and covariates of interest were performed to examine variable distributions. To assess potential multicollinearity, the correlations (Pearson or Spearman's, depending on variable distribution) between the covariates were examined. Careful evaluation of the data to identify outliers (data entry errors and/or biologically implausible values) and extreme values (measurements that would not be an outlier at the population level, but would be implausible at the individual level) was performed prior to all analyses. For each of the four fetal parameters, individual panel plots were created for all the women to ensure that parameter values were increasing with gestational age, taking into account potential measurement error using the intraclass correlation coefficients calculated in the previously described validation study. Implausible values were checked for data entry

errors using the originally recorded clinic values, and assigned as missing if they could not be corrected.

4.3.7 Model Selection: Fetal growth trajectories

Univariate analysis was performed to check the normality of each fetal parameter distribution (HC, AC, FL, and BPD). To characterize the growth trajectories of these parameters, we sought a modeling technique that would be flexible enough to accommodate the potential non-linearities in fetal growth mentioned previously. Based on the work of Wen et al (Wen et al. 2012), we applied a fractional polynomial approach which can capture potential nonlinearity at different points in the growth curve to model fetal parameters as a function of gestational age in weeks. We modified the SAS macro they provided (Wen et al. 2012) to allow us to fit 127 candidate models of varying polynomial degrees for each of the four fetal parameters. The expected value of the fetal parameter, e.g., head circumference (HC) was modeled as

$$\textbf{Model A} \qquad E(HC) = b_0 + \sum_{j=1}^{m} b_j Gestational\_Age^{pj},$$

where m is the degree of the model, j is the number of covariates that we are selecting powers for and powers p_j are selected from a fixed set of seven candidate values, ranging from -1 to 3. Although the previously referenced paper selected 8 candidate values, we chose gestational age powers that best fit our data. First, the model covariance structure for the mean model was determined by using the most complex model (the one including

all seven candidate powers) to test several spatial covariance structures by comparing the model fit statistics (smallest Bayes Information Criterion (BIC)). This was done because the data were from an unbalanced study design. The best covariance structure for our data was to assess the random effects only, which was left unstructured for all fetal outcome models. Then, using the selected covariance parameters, we again used the model fit statistic BIC to identify the best fitting degree of polynomial to capture the nonlinearities of fetal outcomes over time. As seen in (Schwarzler et al. 2004), the fetal parameters (HC, AC, BPD, and FL) have growth patterns that are unique from each other, and therefore polynomial models that best fit the data may differ by fetal parameter. To illustrate, a potential fetal parameter (HC) model in which a third degree polynomial was the best fit for our analysis:

Model B $HC_{ij}=\beta_{0i}+ \beta_1$ Gestational $Age_{ij} + \beta_2$ Gestational $Age^2_{ij} + \beta_3$ Log(Gestational $Age^{)}_{ij} +\varepsilon_{ij}$

Here, i represents the i^{th} fetus and j the j^{th} measurement. Because the ultrasound measures are repeated within a fetus, these models are mixed effect models to accommodate that structure.

Once the best fitting mixed effects fractional polynomial model was identified for each of the four fetal parameters, fetal parameter growth trajectories were estimated at both the population and individual level, using the predicted values from the previously described model. We could then plot the average fetal parameter growth curve over gestation in this population sample. The parameters of Model B can be used to estimate attained HC size at any given age (time in gestation).

4.3.8 Assessing Associations between Covariates and Fetal Growth

Once the four trajectory models were chosen, the next step was to add covariates. As previously mentioned, all models were adjusted for maternal age and fetal sex. Additionally, select covariates (parity, BMI, and alcohol consumption) were singularly added to the unadjusted fractional polynomial models of each of the four fetal parameters (Model C), to examine whether they improved statistical power and should be included as precision variables in fully adjusted models.

Model C $HC_{ij}=\beta_{0i}+ \beta_1$ Gestational $Age_{ij} + \beta_2$ Gestational $Age^2_{ij} + \beta_3$ Log(Gestational $Age)_{ij}$
$+ \beta_4$ $Covariate_{ij} + \beta_5$ Gestational Age*$covariate_{ij}+ \beta_6$ Gestational Age^2*covariate $_{ij}$
$+ \beta_7$ Log(Gestational Age) *covariate $_{ij} +\varepsilon_{ij}$

We used the likelihood ratio test to determine if each of the covariates was a significant predictor of fetal parameters. The null hypothesis was that the interaction between the covariate and the gestational age terms (which examine the relationship between the covariates and the fetal parameter outcomes as a function of time) is equal to zero (Ho: $\beta4$ through $\beta7 = 0$). Therefore, if any of these terms did not equal to zero ($p\leq 0.05$), then we rejected the null hypothesis and included all the interaction terms. If all terms were equal to zero ($p\geq 0.05$), we then failed to reject the null and concluded that those predictors were not significantly adding information to the models.

Our main exposures of interest were maternal first-trimester exposures to O_3 and $PM_{2.5}$. First, we added the air pollutant exposure variables (first trimester concentrations) as predictors to the fractional polynomial mixed-effect models (with random effects for the intercept and the linear gestational age term to account for the within person variance

of each fetal measurement) chosen as the best fitting model for each fetal anthropometric parameter. An interaction term between gestational age and the pollutant was used to assess the association between pollutant exposure and changes in fetal parameters over time. Models were fashioned like the example in Model C, with the substitution of pollution variables for covariate variables in the unadjusted pollutant models. Additionally, maternal age was added as a covariate for all these models.

As noted, parameters of the mixed effect fractional polynomial model can be used to estimate attained size at any time in gestation. We chose to look at estimates at 12, 26, and 37 weeks of gestation. These time periods correspond with attained sizes at the end of first trimester and second trimester and at term, respectively. With the pollutant covariates, trajectories of each fetal parameter can be plotted for different levels of exposure, e.g., an inter quartile range (IQR) difference in first trimester ozone concentration. Differences between estimated attained fetal parameter sizes at any given age can be calculated for mothers with first trimester exposures falling in the 75^{th} percentile versus 25^{th} percentile of pollutant concentrations.

Finally, pollutant models adjusting for maternal age and fetal sex were further adjusted for selected precision covariates to enable estimation of fully adjusted air pollution associations with the four fetal parameters. The pollutant predictors were modeled separately, in single pollutant models. All analyses were performed in SAS 9.3 (SAS Institute Inc., Cary, NC, USA.)

4.4 Results

Data was available for 705 mother-fetal pairs. Through the DAG analysis, we identified maternal age, education and fetal sex as potential confounders and/or effect modifiers. However, data for maternal educational attainment was missing for more than eighty percent of the participants, so this variable was not included in the main analysis. We excluded women missing information on potential confounders and effect modifiers; the final analysis dataset consisted of information from 625 mother-fetal pairs. The mean ±standard deviation (SD) age of mothers was 24.84 ± 5.89 years. Table 1 shows demographic and clinical characteristics for the mother-fetal pairs.

Mean first trimester kriged and city-wide average estimates of $PM_{2.5}$ and O_3 for all pregnancies were highly correlated ($r=0.997$ and $r=0.979$, $p<0.001$), respectively (Table 2). Correlations between $PM_{2.5}$ and O_3 first trimester averages were moderately correlated, for both exposure metrics, with all correlation coefficients being greater than 0.47. However, changes in correlations as well as mean first trimester concentrations were evident when exposure was assessed based on season of conception. Rainy season had lowest mean concentrations of $PM_{2.5}$ for both kriged and city-wide average estimates (20.95µg/m^3 and 21.21 µg/m^3), respectively. Highest concentrations of O3 were observed in the warm dry season (66.40 ppb and 69.34 ppb). For all seasons, pollutant concentrations for both exposure metrics remained strong, but between pollutant correlations decreased between the rainy and both dry seasons (warm and cool). For all seasons, citywide average estimates of pollutant exposures were larger than kriged estimates. Therefore, kriged estimates of exposure were used in all of the trajectory models.

Fetal parameters were best fitted using 3rd (HC, BPD and FL) and 4th (AC) degree fractional polynomial, mixed-effects models of gestational age. Model details of fetal parameters as a function of time include: log (gestational age), linear gestational age and gestational age squared for HC and BPD models. Models of FL were fit using the square root of gestational age, linear gestational age and gestational age squared. Finally, AC was modeled using 4 terms of gestational age: log (gestational age), linear gestational age, gestational age squared and gestational age cubed. Pollutant models were all adjusted for maternal age and fetal sex. Likelihood ratio tests did not support further adjustment of prediction models for potential precision variables.

Fetal parameter measurements were collected at each visit; the median number of ultrasound scans per mother-fetal pair was five. Table 3 presents the detailed descriptives of ultrasound measurements by trimester. First trimester scans were limited, with the majority of scans occurring during second trimester exams, and third trimester scans were only slightly fewer than those occurring in the second trimester. In total, over 3,100 ultrasound measurements were collected for 625 fetuses, for each anthropometric parameter.

Comparisons of fetal curves of estimated attained size of fetal HC associated with kriged estimates of first trimester maternal exposure to $PM_{2.5}$ and O_3, adjusting for maternal age, are presented in Figure 1. Attained size of HC was estimated for mean exposures +/- one IQR at each week of gestation (data not shown in table form, but estimates are graphically presented in Figure 1). Figure 1 also displays the plots of the

estimated differences in HC size between two exposure quartiles. Having a one IQR (75^{th} percentile minus the 25^{th} percentile, that is, $8.97\mu g/m^3$) higher first trimester exposure to $PM_{2.5}$ was associated with decrements in attained HC size at 12 to 14 weeks (β's = -0.404 to -0.204 cm, $p<0.05$) but was positively associated with HC growth from weeks 35-42 (β's = 0.113 to 0.360 cm, $p<0.05$). Estimates of association between attained size for the all four fetal parameters, at the end of first and second trimester and at term, and maternal first trimester exposure to $PM_{2.5}$ are presented in Table 4. No evidence of an association was observed between increased exposure to $PM_{2.5}$ and HC prior to model adjustment for maternal age and fetal sex, (β = -0.363 cm, 95% CI: -0.749, 0.024). However, adjusting for potential confounders, an association was seen between maternal first trimester $PM_{2.5}$ exposure and attained fetal HC at gestational week 12(β = -0.404 cm, 95% CI: -0.789, -0.020). Increased first trimester maternal exposure to $PM_{2.5}$ was consistently associated with increased HC attainment at 37 weeks of gestation. Except for the small window of increased HC growth during weeks 35-42 (β's = 0.113 to 0.360 cm, $p<0.05$), which can be seen in Figure 1, no association was observed between increased maternal O_3 exposure in early pregnancy and HC growth.

Growth trajectories at the 75^{th} and 25th quartile exposure categories were more consistent between pollutants for fetal BPD growth curves (Figure 2). Increased maternal $PM_{2.5}$ exposure, adjusting for maternal age and fetal sex, was inversely associated with estimated growth of BPD at 16 –28 weeks of gestation (β's = -0.067 to -0.045 cm, $p<0.05$). Adjusting BPD prediction models for maternal age and fetal sex did not change the strength of association, as seen in Table 4. The crude estimated difference in HC at 26 weeks for a one IQR difference in first trimester $PM_{2.5}$ exposure (β = -0.055 cm, 95%CI:

-0.096, -0.014) was similar to the difference adjusting for maternal age and fetal sex (β = -0.051 cm, 95% CI: -0.091, -0.012). Similarly, increased estimated maternal O_3 exposure in first trimester was associated with reduced BPD growth from 22-32 weeks of gestation (β's = -0.064 to -0.045 cm, $p<0.05$), adjusting for maternal age and fetal sex. The magnitude of effect estimates between pollutant types was within the same range. However, the time period of reduced growth during pregnancy associated with O_3 exposures occurred later in pregnancy compared to the observed association between increased estimated $PM_{2.5}$ exposure and BPD growth. Estimates of difference in BPD size by IQR difference in maternal exposure to O_3 at 12, 26, and 37 weeks of gestation (Table 5) are similar to those reported for $PM_{2.5}$ exposure (Table 4).

Estimated differences in attained FL were not associated with increased first trimester exposure to $PM_{2.5}$ (Figure 3). Although no evidence of association was seen between fetal FL growth and a one IQR difference in maternal exposure at the three selected time periods reported in Table 4, point estimates of effect from the unadjusted model (β = -0.001 cm, 95%CI: -0.035, 0.032) and adjusted model (β = -0.002 cm, 95%CI: -0.035, 0.031) were slightly negative in magnitude for the 26 week point. However, reduced growth of FL during 16-29 weeks of gestation was associated with increased estimated maternal first trimester to O_3 (β's =-0.046 to -0.033 cm, $p<0.05$), Figure 3. Similar to the other anthropometric parameters, effect estimates of the unadjusted model at week 26 (β = -0.041 cm, 95%CI: -0.074, -0.008) did not differ after adjusting for maternal age and fetal sex (β = -0.042 cm, 95%CI: -0.075, -0.010), Table 5.

Finally, fetal AC was the only parameter that showed no significant association between IQR of increased maternal exposure to both O_3 and $PM_{2.5}$ during the first trimester and difference in estimated attained size (Figure 4). Although not significant, point estimates of the difference in attained size of HC by IQR increase of maternal $PM_{2.5}$ exposure were negative during weeks 16 to 20, as seen in Figure 4 . However, models of higher estimated maternal exposure to O_3 during the first trimester had negative point estimates of difference in attained size from 12-34 weeks of gestation. The point estimates from 12, 26 and 37 weeks of gestation for both exposure pollutants (Tables 4 and 5) show similar findings.

4.5 Discussion

Results from this prospective cohort study, beginning early in pregnancy, suggest that increased maternal exposure to air pollution in the first trimester is negatively associated with fetal growth at various periods of gestation for some pollutants and parameters. Heterogeneity in the results may depend on the air pollutant as well as fetal sex for some parameters.

4.5.1 Repeated Measures of Fetal Outcomes and Predictors of Fetal Growth Restriction

To date, few studies have examined the relationship between air pollution and fetal growth restriction with the use of ultrasound measurements to assess fetal growth. The methodology and results of these relevant studies have been discussed in detail in a recent review (M. M. Smarr et al. 2013). Briefly, previous studies varied in sample sizes ranging from 271 to 7,777 mother-fetal pairs, and only one study had repeated ultrasound scans, and only for ≤3 % of their population (Hansen et al. 2008). A more recent study

that was not included in the review (Ritz et al 2014) used data collected from 566 mother-fetal pairs in Los Angeles, California. Ultrasounds were collected during a 2 week window at three time points 19, 28 and 37 weeks of gestation (plus or minus one week)-- with 84% of their study population completing three ultrasound examinations.

The data collected from 625 mother-fetal pairs in the present analysis falls within the sample size range of previously published work and had more repeated ultrasound measures for all four fetal parameters, ranging from 1 to 9 scans, with the median of 5 ultrasound scans per participant. The access to data collected at multiple time periods during pregnancy was a primary strength of this analysis. Repeated measures allowed for a longitudinal analysis of the association between fetal growth and maternal exposure to air pollution.

The ability to assess changes in fetal parameters over time has several advantages. It may help to distinguish between the constitutionally small fetus and the growth restricted fetus (Woodruff et al. 2009), since variations in fetal size are a natural occurrence by the third trimester, and SGA estimates combines fetuses who are both pathologically and constitutionally small (Ananth and Vintzileos 2009). Also, the assessment of IUGR at birth ignores the possibility that injury to growth could occur during one time period, but the fetus could continue to grow and achieve population growth standards by birth (Hemachandra et al. 2006) . Further, a clinical model that examined fetal growth occurring in early and late stages of pregnancy attributed early fetal growth to hyperplasia (cell proliferation), and later growth to hypertrophy (cell growth) Winick 1974). The author proposed that agents that damage the fetus during the first trimester may reduce the cell population, thereby causing a permanent hindrance to

the growth potential, but that the damage caused later in gestation would reduce the cells' size, but the infant could potentially later catch-up to predicted growth (Winick 1974). Having serial ultrasound measurements of fetal growth allows examination of changes occurring *in utero* and may help to distinguish between growth restriction resulting from extrinsic factors (pollution, alcohol, etc.) from intrinsic factors (genetics, malformations, etc.).

Repeated clinical visits also provided data on maternal covariates (smoking, alcohol consumption, nutrition, etc.) during pregnancy. However, one limitation for the present analysis was the amount of missing information for some variables of interest, particularly maternal education, an indicator of SES for our study population. Thus, we were unable to adjust our main prediction models for maternal SES which is a risk factor for IUGR and could potentially explain differential misclassification of exposures. In addition to education, we only had information on ETS exposure for fifty-five percent of the study population. Perhaps this could explain the lack of significance when testing this variable for inclusion the prediction models as a precision variable.

4.5.2 Exposure Assessment of Air Pollutants

Exposure to air pollutants was assessed using two methods 1) citywide averages and 2) ordinary kriging. While citywide average provides a spatially averaged estimate of exposure, kriging uses the geocoded residential address of the women to provide an interpolated estimate. We believed that the kriged metric would provide a more precise estimate of maternal exposure. However, we found the two methods to be highly correlated. This primary reason for the similarity in exposure estimates may be the lack of spatial variability in residence location of our study participants; many of the women

resided in the central region of Mexico City. It has been suggested that for accurate assessment of air pollutant variability on smaller scales (i.e. neighborhood level) the use of land use regression models (LUR) are more effective than basic interpolation methods (i.e. ordinary kriging) (Gilliland et al. 2005). Also, a simulated study of 1,000 women of air pollution and preterm birth in Mexico City (Rivera-González 2012) found pollutant estimates of citywide averages, ordinary kriging and inverse distance weighting (IDW) produced highly correlated results. These three methods all involve averaging of pollutant concentrations from stationary monitors to a certain degree. This lends support to the idea that any method that uses some degree of averaging of pollutant concentrations may not be as distinct from each other, compared to a metric that only uses data from a single monitor (i.e. nearest monitor). Additional planned analyses comparing effect estimates for birth outcomes, including fetal growth, can tell us whether the differences in the exposure metrics result in significantly different associations. We selected first trimester averages of pollutant exposures for the present analysis; future work using other gestational time periods will allow examination of potential critical periods of exposure during gestation.

One limitation is the lack of time activity data available for our study participants at the time of this analysis. Having activity data would help with the weighting of air pollution exposures based on the amount of time that the women spend at home, since we are using their residential addresses to estimate exposure. Also, a more comprehensive assessment model would include geocoded addresses of the women's occupation. This way, interpolation methods could be used but monitors assigning the participant's

exposure would be a combination of the primary locations that she spends her time (home and work). Also, concentrations would be weighted based on where she spends the majority of her time between the two primary locations. Another possible consideration for future studies would be the use of time activity data to create more in-depth assessment models that can estimate uptake of pollutants based on energy expenditure during specific activities at the locations that concentrations are being estimated for. Overall, though, our exposure assessment approach is comparable to those employed in other similar studies, some of which lacked even residential location data.

4.5.3 Air Pollution and Fetal Growth

Although time periods of fetal attained size reduction associated with early maternal exposures to air pollutants varied by a few weeks, these occurrences occurred in the second and early third trimester. For example, increased maternal exposure to O_3 in early pregnancy was significantly, inversely associated with BPD and FL size in gestational weeks during second and early third trimester, and covariate adjustments did not significantly change the strength of associations. Reductions in fetal size in the second trimester of pregnancy are most often the focus of many epidemiological studies, as it is safer to associate these changes with alterations in maternal and fetal environment, compared to third trimester assessment of growth. Given that fetal growth in the late third trimester is expected to slow down, as a natural progression of pregnancy, it is important to note that the changes observed in early third trimester are still worth discussing, as they may contribute to the overall understanding of the effect of air pollution on normal fetal growth.

Hansen and colleagues restricted their analysis to scans collected in mid-gestation (13-26 weeks), which makes it hard to compare the totality of our results, given that they were the only study to previously examine the effects of O_3 on growth (Hansen et al. 2008) While estimates of mean change in fetal parameters were negative for an IQR (8ppb) increase in average monthly O_3 exposure was observed for many of the four sampling periods, a significant reduction was only observed for fetal AC (1.42 mm) in the second trimester with increased during days 31-60. Although it is difficult to compare results between the two studies, AC was the only fetal parameter that we were unable to observe a significant association between reductions in attained size and a one IQR (13.28 ppb) increase in first trimester O_3 exposure. It is also noted that estimated reductions in attained AC size at week 26 in our analysis was similar in magnitude (0.142 cm).

Mexico City does not have established fetal reference curves, so fetuses in our population serve as their own controls from visit to visit. This is different from the Hansen study, which examined fetal parameter measurements collected for the creation of population curves. The large difference in sample size between our analyses could explain similar effect estimates but lack of significance. We also performed a longitudinal analysis, whereas the previous study was cross-sectional; heterogeneity in methodology could explain the difference in magnitude of the results. None of the previously published studies examined $PM_{2.5,}$ so we are unable to compare our findings in that regard.

We used single pollutant models in this analysis. Since air pollution is ubiquitous and is a mixture of various chemicals, a multi-pollutant model is more representative of

'real life' exposures. However, we aimed to obtain results which may help to better identify areas for further study in terms of understanding mechanisms for the relationship between air pollution and birth outcomes. Furthermore, the literature in this area reports single pollutant effects, so our approach enhances comparability. We found consistent reduction of BPD growth in models of increased maternal exposure to air pollution, using IQR differences in kriged estimates of $PM_{2.5}$ and O_3. BPD was not associated with O_3 exposures in the Australian study, the only one to look at this pollutant (Hansen et al. 2008) . In other studies (Ritz et al. 2014; Aguilera et al. 2010; Slama et al. 2009), BPD in second and third trimesters was negatively associated with increased exposures to nitrogen dioxide, markers of vehicle emission (BTEX compounds) and benzene, respectively. Although comparison of our findings with these studies is challenging, as several differences in exist in the methodologies and the pollutant types, the majority of the results seem to suggest that BPD growth is affected by maternal exposure to a pollutant mixture.

We chose to model fetal parameters as a function of time with the use of fractional polynomial, mixed-effects models to capture the non-linearity of our four anthropometric parameters, instead of using splines. While splines are known to have more flexibility in modeling non-parametric relationships, fractional polynomial models are more straightforward in application. Also, a simulation study comparing the use of fractional polynomial, penalized and restricted cubic spline models (Binder et al. 2013) found fractional polynomial models to be better prediction models for simple functions. All of the gestational age terms for the trajectories we fit were 'simple' in nature, meaning that they are not extremely 'wiggly' with regards to the shape of the curves.

4.5.4 Clinical and Epidemiological Relevance

Biological mechanisms explaining associations between maternal exposure to air pollution and pregnancy outcomes are not well defined. However, a potential strength of our analysis is the ability to examine fetal growth and potential growth restriction longitudinally. An important clinical indicator of growth restriction over time is the HC/AC ratio. "Normal" HC at birth but reduced AC results in an inflated HC/AC ratio, which indicates asymmetric growth restriction. While the nature of growth restriction as it relates to the timing of asymmetric growth restriction is debated, it is clearly understood that asymmetric growth restriction is associated with higher risks of neonatal morbidity and mortality(Dashe et al. 2000). An elevated HC/AC ratio in second trimester is believed to be the result of placental insufficiency resulting from external factors, while asymmetric growth restriction in the third trimester is attributed to aneuploidy (Riyami et al. 2011).

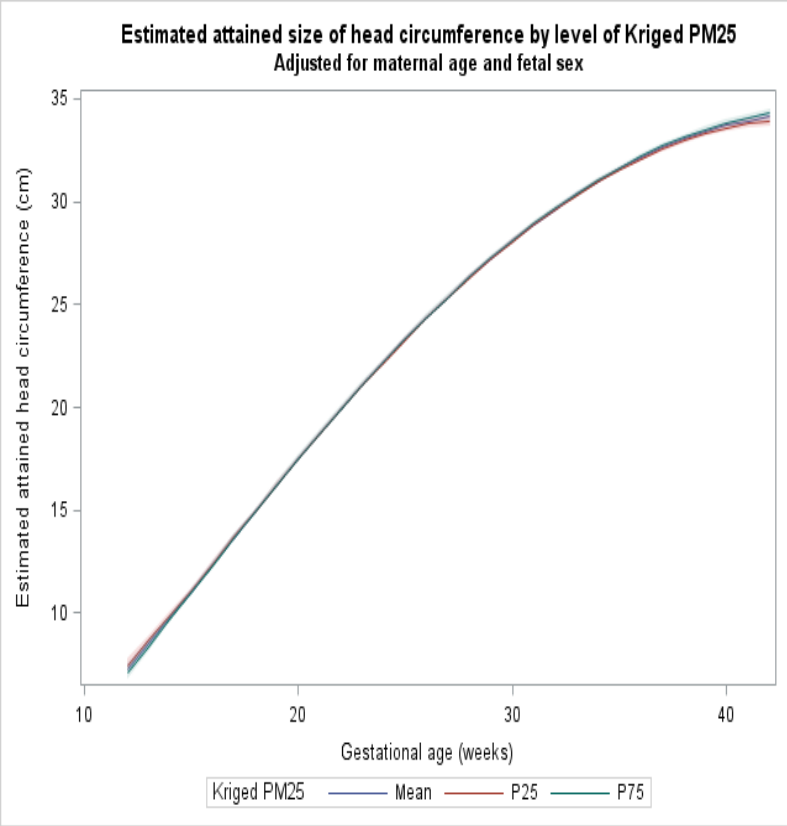
The AC point estimates in the second trimester were negative in models of increased maternal exposure to O_3 in the first trimester. If higher O_3 exposure is in fact involved in reducing attained AC during the second trimester, placental insufficiency could be a mechanism explaining how this pollutant could be relevant to restricted fetal growth, perhaps by affecting vascular function and resulting in insufficient transport of key nutrients across the placental membrane.

4.6 Conclusion

Our results support the growing literature showing that maternal exposure to air pollutants in early pregnancy may alter fetal growth as reflected by attained size in the second trimester of gestation. We applied a modeling approach that was able to capture

differing growth trajectory shapes according to fetal parameter and enabled quantification of differences in attained size by pollutant at multiple weeks across gestation, adjusting for relevant covariates. Future research focusing on changes in fetal growth rates of key anthropometric parameters and clinical indicators of fetal growth would help to improve our understanding of biological mechanisms that may underlie the observed links between environmental exposures and fetal and infant health.

Figure IV-1a**.** Predicted fetal HC trajectories and estimated differences in attained size per IQR increase in first trimester $PM_{2.5}$ exposure



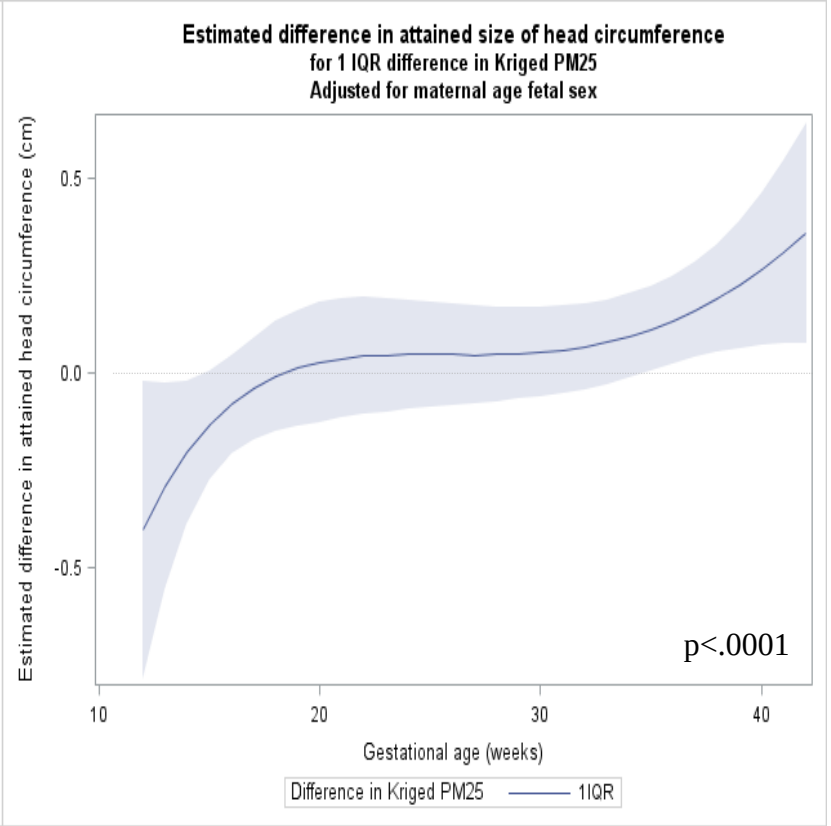


Figure IV- 1a: Predicted fetal HC trajectories, adjusted for maternal age and fetal sex , per maternal first trimester exposure (mean, 25th and 75th percentile) to $PM_{2.5}$ (left). Estimated differences in attained size of fetal HC per 1 IQR difference in exposure (right). Reported p-value from likelihood ratio test of overall association between $PM_{2.5}$ exposure and estimated attained parameter size.

Figure IV-1b**.** Predicted fetal HC trajectories and estimated differences in attained size per IQR increase in first trimester O_3 exposure

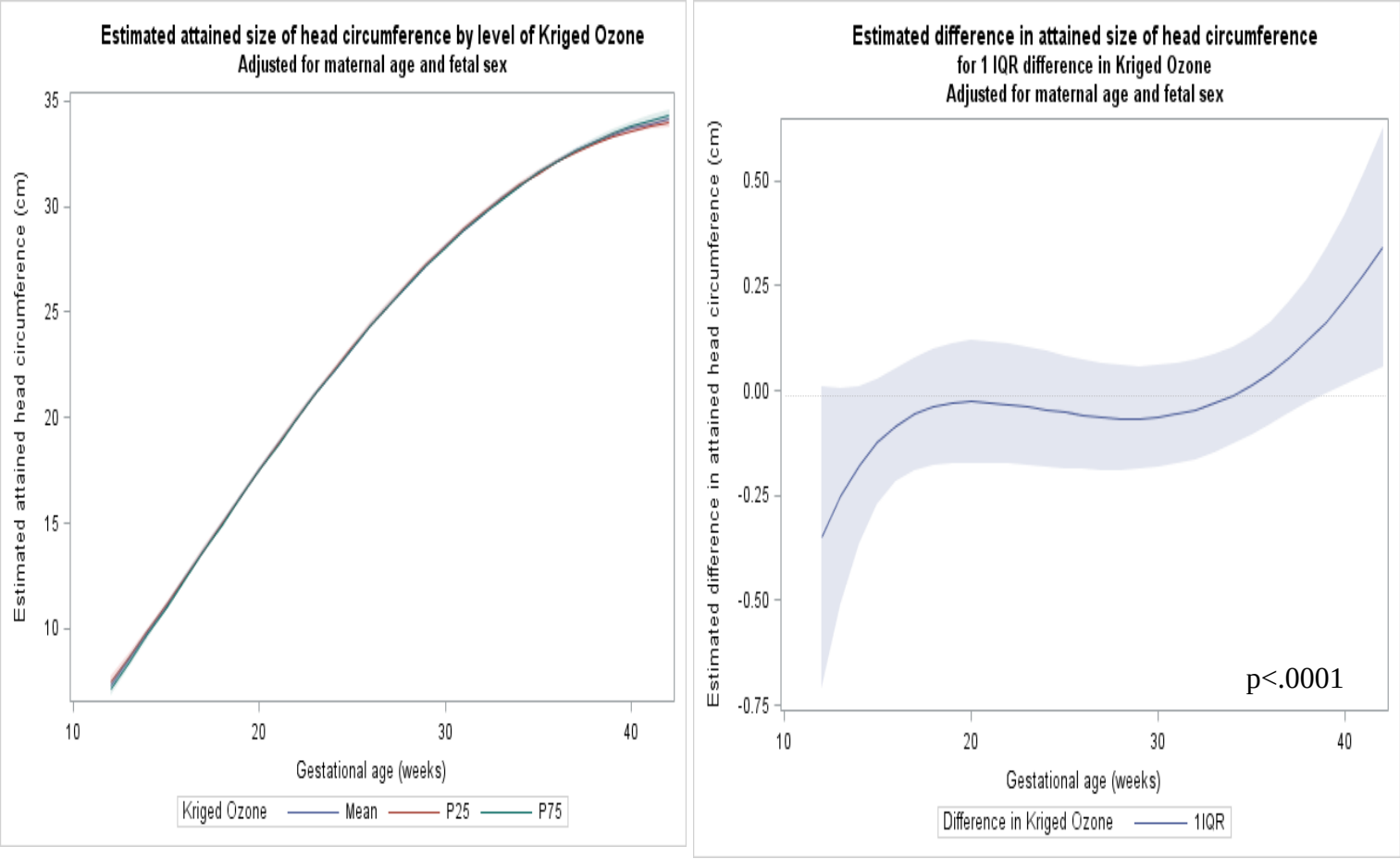


Figure IV-1b: Predicted fetal HC trajectories, adjusted for maternal age and fetal sex , per maternal first trimester exposure (mean, 25th and 75th percentile) to O_3 (left). Estimated differences in attained size of fetal HC per 1 IQR difference in exposure (right). Reported p-value from likelihood ratio test of overall association between O_3 exposure and estimated attained parameter size.

Figure IV-2a. Predicted fetal BPD trajectories and estimated differences in attained size per IQR increase in first trimester $PM_{2.5}$ exposure

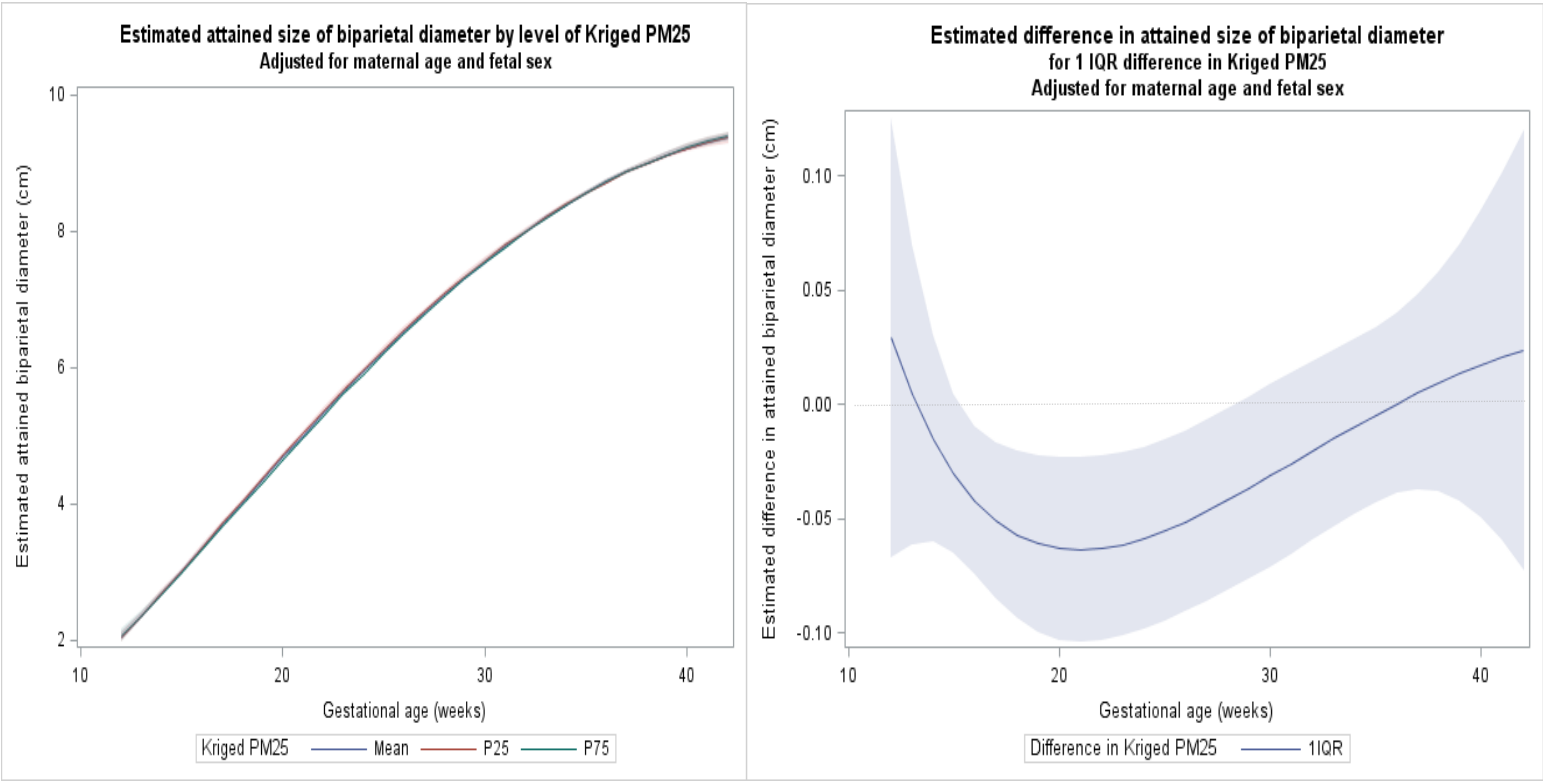
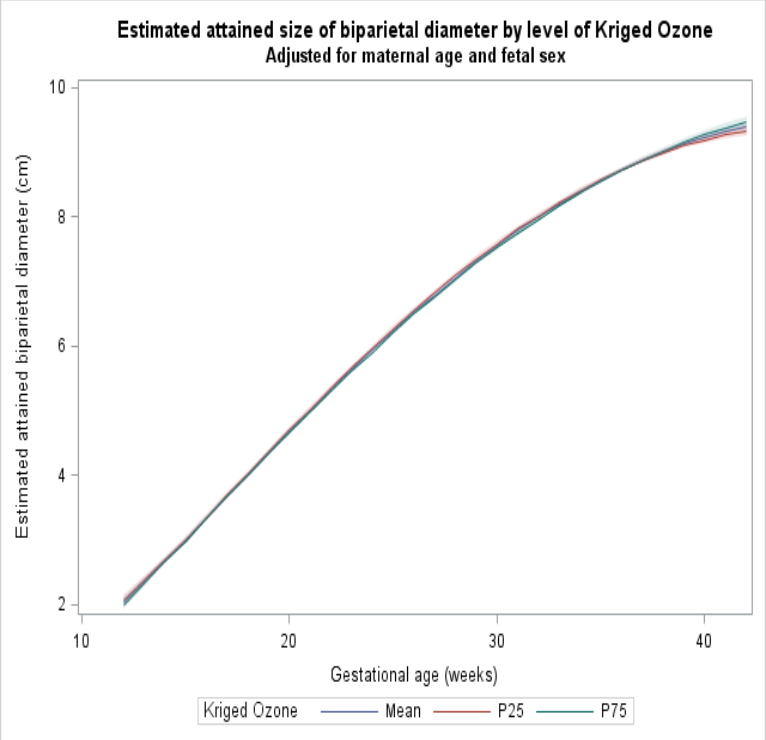


Figure IV-2a: Predicted fetal BPD trajectories, adjusted for maternal age and fetal sex , per maternal first trimester exposure (mean, 25^{th} and 75^{th} percentile) to $PM_{2.5}$ (left). Estimated differences in attained size of fetal BPD per 1 IQR difference in exposure (right). Reported p-value from likelihood ratio test of overall association between $PM_{2.5}$ exposure and estimated attained parameter size.

Figure IV-2b. Predicted fetal BPD trajectories and estimated differences in attained size per IQR increase in first trimester O_3 exposure



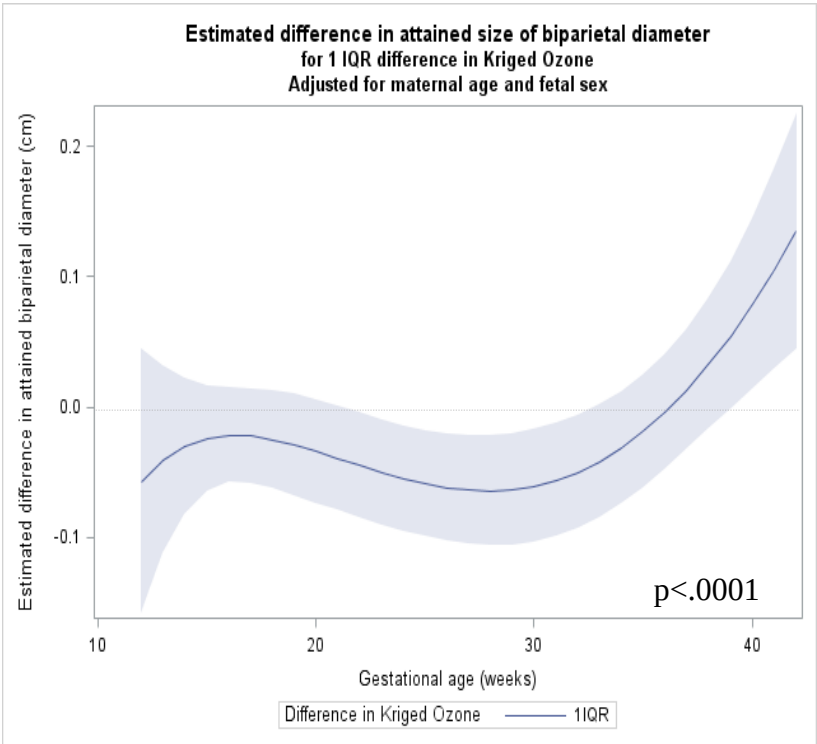


Figure IV-2b: Predicted fetal BPD trajectories, adjusted for maternal age and fetal sex , per maternal first trimester exposure (mean, 25th and 75th percentile) to O_3 (left). Estimated differences in attained size of fetal BPD per 1 IQR difference in exposure (right). Reported p-value from likelihood ratio test of overall association between O_3 exposure and estimated attained parameter size.

Figure IV-3a.Predicted fetal FL trajectories and estimated differences in attained size per IQR increase in first trimester $PM_{2.5}$ exposure

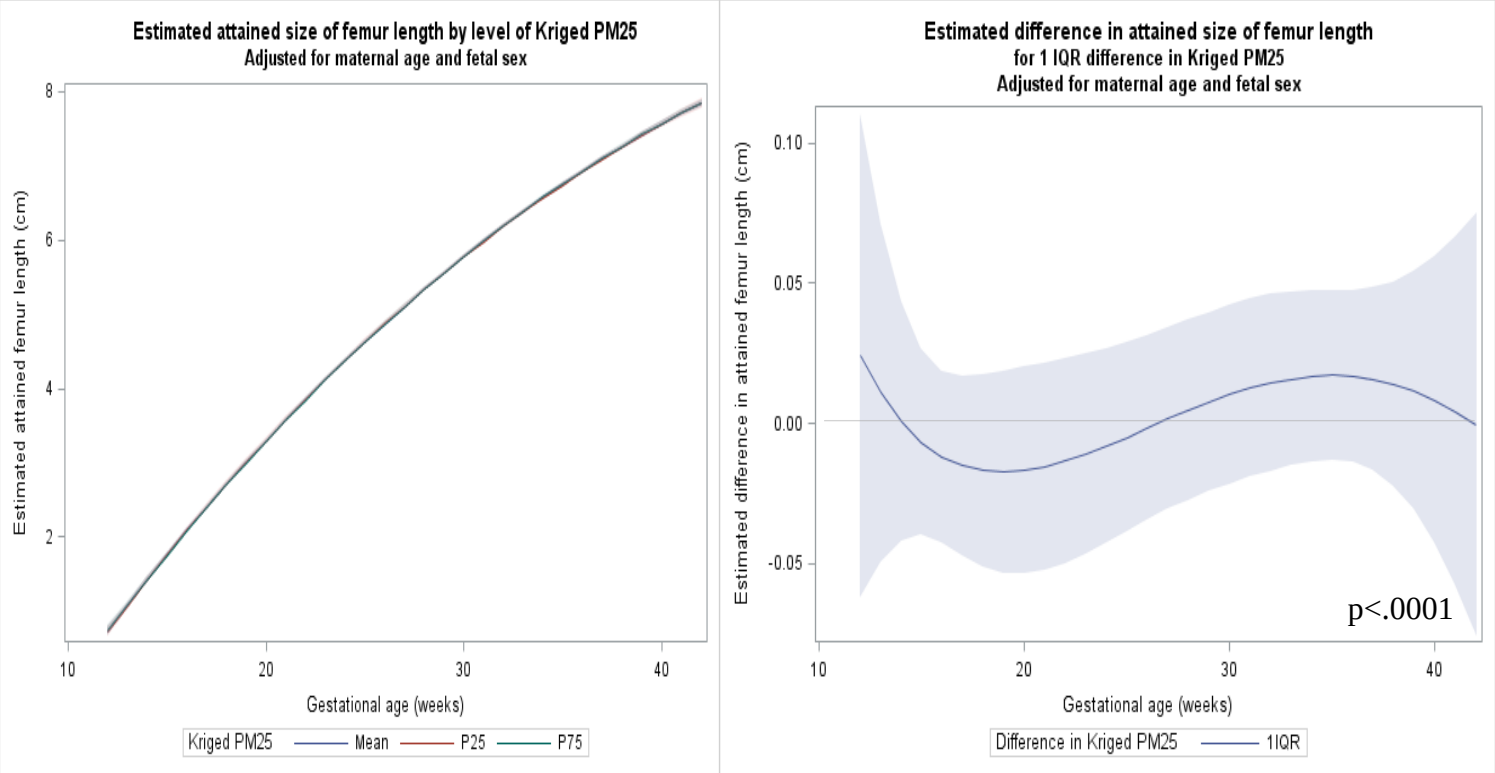


Figure IV-3a: Predicted fetal FL trajectories, adjusted for maternal age and fetal sex , per maternal first trimester exposure (mean, 25^{th} and 75^{th} percentile) to $PM_{2.5}$ (left). Estimated differences in attained size of fetal FL per 1 IQR difference in exposure (right). Reported p-value from likelihood ratio test of overall association between $PM_{2.5}$ exposure and estimated attained parameter size.

Figure IV-3b.Predicted fetal FL trajectories and estimated differences in attained size per IQR increase in first trimester O_3exposure

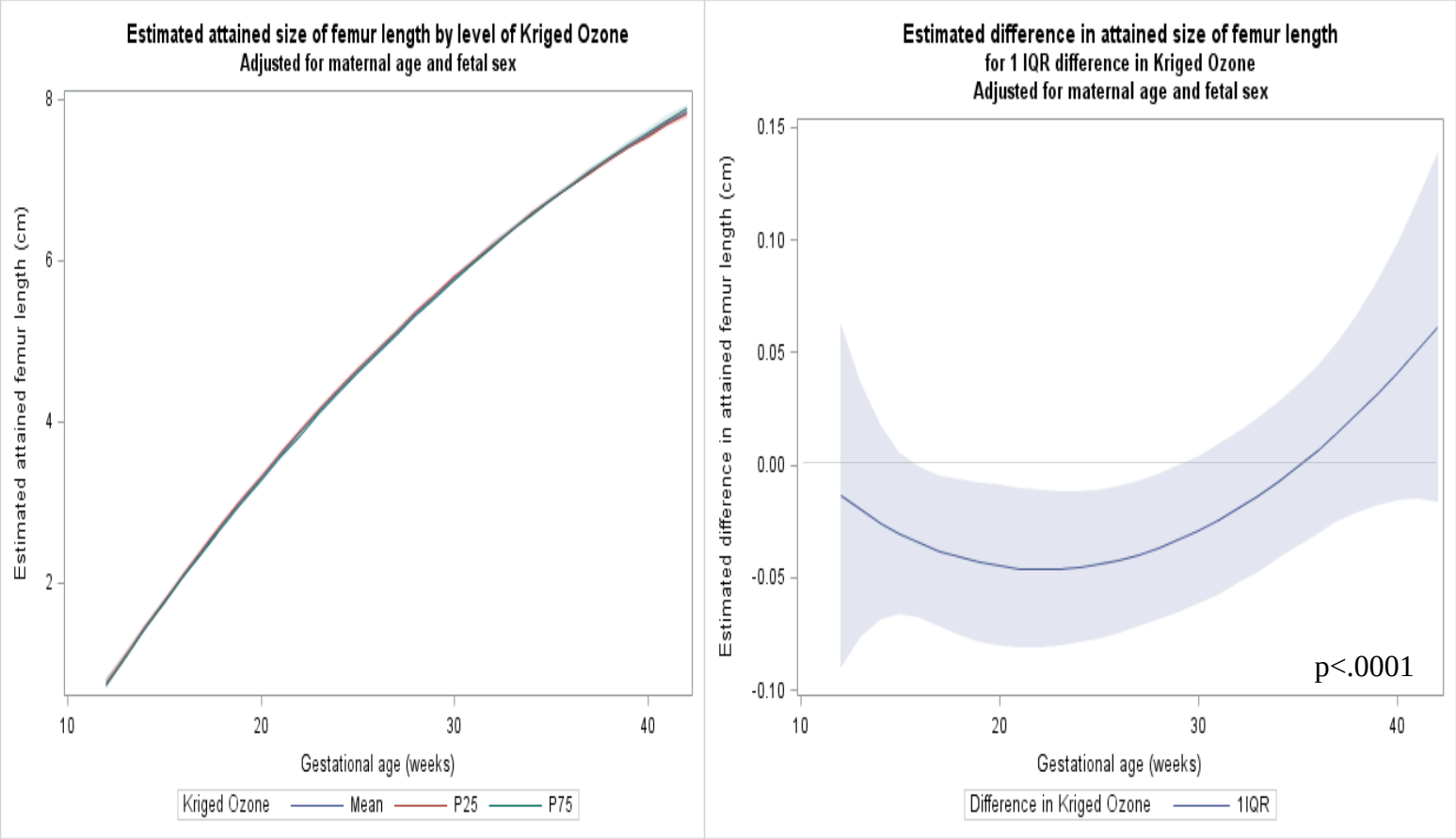


Figure IV-3b: Predicted fetal BPD trajectories, adjusted for maternal age and fetal sex , per maternal first trimester exposure (mean, 25th and 75th percentile) to O_3 (left). Estimated differences in attained size of fetal BPD per 1 IQR difference in exposure (right). Reported p-value from likelihood ratio test of overall association between O_3 exposure and estimated attained parameter size.

Figure IV-4a. Predicted fetal AC trajectories and estimated differences in attained size per IQR increase in first trimester exposure to $PM_{2.5}$

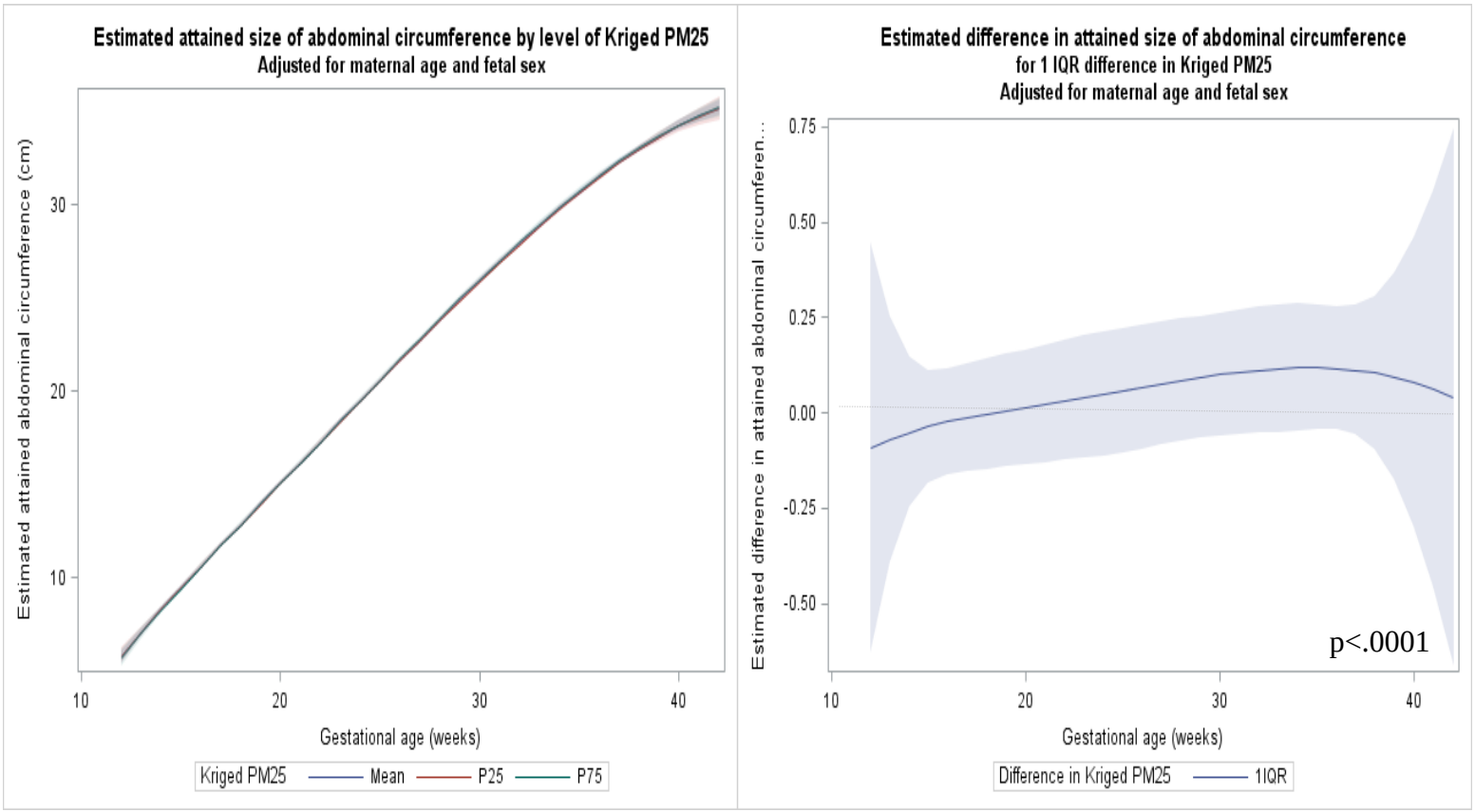


Figure IV-4a: Predicted fetal AC trajectories, adjusted for maternal age and fetal sex , per maternal first trimester exposure (mean, 25th and 75th percentile) to $PM_{2.5}$ (left). Estimated differences in attained size of fetal AC per 1 IQR difference in exposure (right). Reported p-value from likelihood ratio test of overall association between $PM_{2.5}$ exposure and estimated attained parameter size.

Figure IV-4b. Predicted fetal AC trajectories and estimated differences in attained size per IQR increase in first trimester exposure to O_3

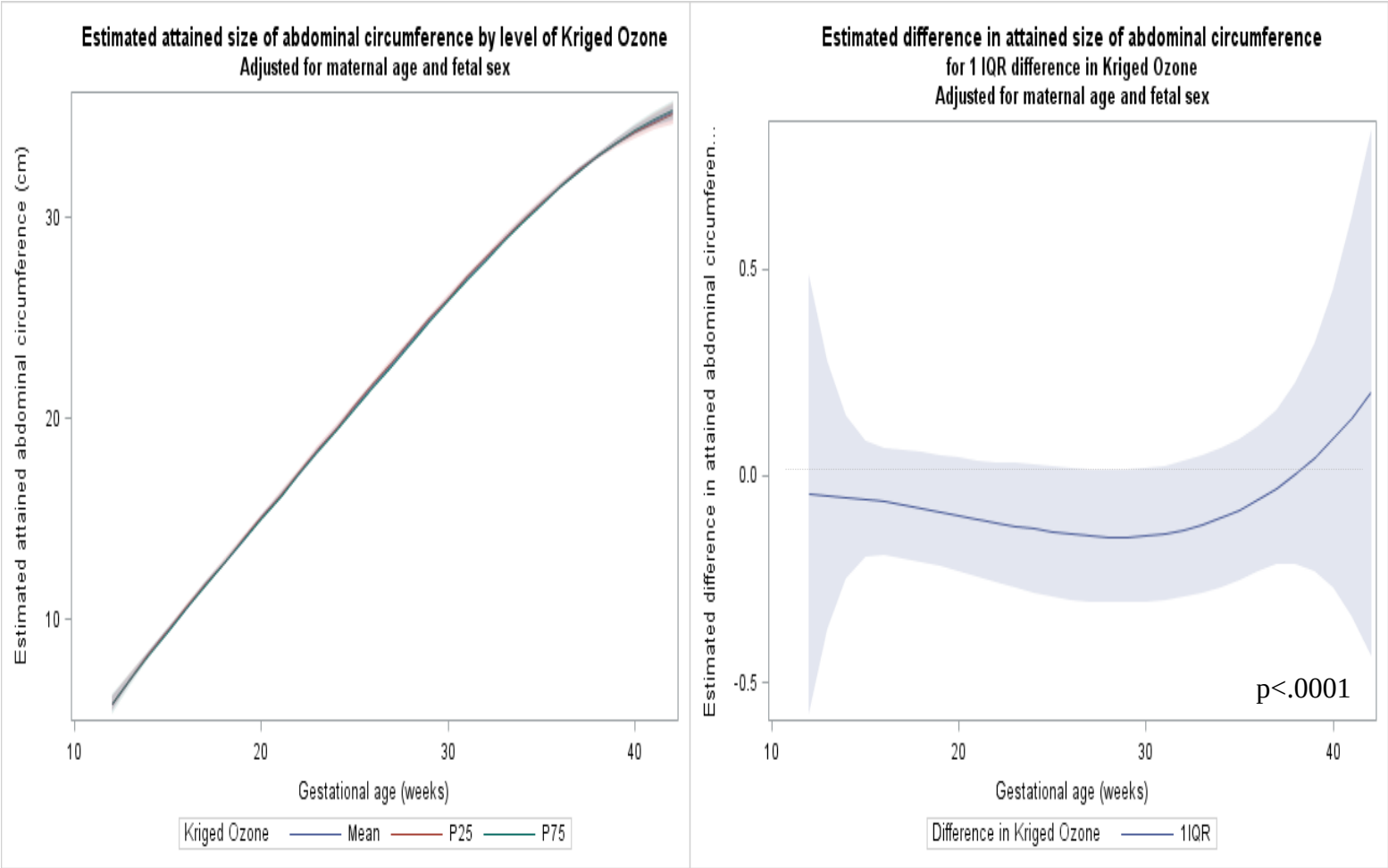


Figure IV-4b: Predicted fetal AC trajectories, adjusted for maternal age and fetal sex , per maternal first trimester exposure (mean, 25th and 75th percentile) to O_3 (left). Estimated differences in attained size of fetal AC per 1 IQR difference in exposure (right). Reported p-value from likelihood ratio test of overall association between O_3 exposure and estimated attained parameter size.

Table IV- 1. Demographics of the 625 mother-fetal pairs

Parameters	**n**	**Mean±SD**	**Min-Max**
Age (years)	625	24.84± 5.89	18.00-46.00
Body Mass Index (kg/m^2)	553	26.03± 5.64	15.18-54.42
Years of education	99	3.23±1.99	0.00 -9.00
First Trimester Kriged $PM_{2.5}$ ($\mu g/m^3$)	625	24.68±5.44	13.28 - 36.02
First Trimester Kriged O_3 (ppb)	625	55.92 ±8.58	38.82 - 77.17
		Frequencies; No. (%)	
Parity:	479		
0		201	41.96
1		155	32.36
2 or more		123	25.68
Time spent with smoker inside the same room:	349		
0 minutes		46	13.18
< 15 minutes		183	52.44
15-30 minutes		43	12.32
31 minutes - 1 hour		23	6.59
1 -1.5 hours		17	4.87
> 1.5 hours		37	10.60
Alcohol consumption:	560		
yes		71	12.68
no		489	87.32
Use of coal stove:	553		
yes		28	5.06
no		525	94.94
Use of wood stove:	550		
yes		16	2.91
no		534	97.09
Fetal sex:	625		
males		295	47.2
females		330	52.8

Table IV- 2. Overall means and Pearson correlation coefficients for first trimester air pollutant metrics

Exposure Metric	Mean ±SD	Range	Kriged $PM_{2.5}$	CWA $PM_{2.5}$	$CWAO_3$	Kriged O_3
All Pregnancies (N=625)						
First Trimester Kriged $PM_{2.5}$ ($\mu g/m^3$)	24.85±5.52	13.28 - 36.02	1.000			
First Trimester Citywide Average $PM_{2.5}$ ($\mu g/m^3$)	24.96± 5.49	13.59 - 34.41	0.997	1.000		
First Trimester Citywide Avergage O_3 (ppb)	55.86 ±8.66	38.15 - 79.06	0.501	0.531	1.000	
First Trimester Kriged O_3 (ppb)	57.74 ±8.86	41. 54 - 80.56	0.474	0.481	0.979	1.000
Rainy Season Conception (N=276)						
First Trimester Kriged $PM_{2.5}$ ($\mu g/m^3$)	20.95±5.87	13.28 - 36.02	1.000			
First Trimester Citywide Average $PM_{2.5}$ ($\mu g/m^3$)	21.21±5.47	13.59 - 34.17	0.998	1.000		
First Trimester Citywide Avergage O_3 (ppb)	51.20±5.62	38.15 - 61.72	0.744	0.734	1.000	
First Trimester Kriged O_3 (ppb)	52.65±5.32	41.54 - 64.78	0.755	0.763	0.981	1.000
Warm Dry Season Conception (N=173)						
First Trimester Kriged $PM_{2.5}$ ($\mu g/m^3$)	26.65±3.57	18.45 - 32.90	1.000			
First Trimester Citywide Average $PM_{2.5}$ ($\mu g/m^3$)	27.13±3.39	19.97 - 33.15	0.990	1.000		
First Trimester Citywide Avergage O_3 (ppb)	69.34±5.25	56.46 - 80.56	0.753	0.791	1.000	
First Trimester Kriged O_3 (ppb)	66.40±4.96	52.73 - 79.06	0.643	0.677	0.920	1.000
Cool Dry Season Conception (N=176)						
First Trimester Kriged $PM_{2.5}$ ($\mu g/m^3$)	27.54±3.95	18.70 - 33.69	1.000			
First Trimester Citywide Average $PM_{2.5}$ ($\mu g/m^3$)	27.99±3.98	19.35 - 34.41	0.997	1.000		
First Trimester Citywide Avergage O_3 (ppb)	53.75±4.12	44.88 - 67.13	0.332	0.325	1.000	
First Trimester Kriged O_3 (ppb)	50.83±3.80	39.84 - 62.08	0.345	0.346	0.870	1.000

$p<0.0001$ for all correlations

Table IV- 3. Descriptives of ultrasound-measured anthropometric parameters for 625 fetuses

	Total		1st Trimester		2nd Trimester		3rd Trimester	
Fetal Characteristics (N=625)	**No. Scans**	**Range**	**No. Scans**	**Mean ± SD**	**No. Scans**	**Mean± SD**	**No. Scans**	**Mean± SD**
Head Circumference (cm)	3119	5.60-35.40	8	7.59 ± 1.43	1646	17.90 ± 4.73	1465	29.99 ± 2.52
Abdominal Circumference (cm)	3112	5.40-38.40	6	7.05 ± 1.31	1638	15.60 ± 4.35	1468	28.60 ± 3.46
Biparietal Diameter (cm)	3114	1.50-9.70	8	2.11 ± 0.38	1638	4.80 ± 1.25	1468	8.10 ± 0.74
Femur Length (cm)	3109	0.80-8.03	7	1.01 ± .18	1646	3.37 ± 1.11	1456	6.33 ± 0.72

Table IV- 4. Estimated attained size of fetal parameters per mean maternal first trimester exposure and difference in predicted size per 1 IQR (8.97 µg/m3) difference in maternal $PM_{2.5}$ exposure

	Unadjusted						Adjusted					
Fetal Parameters	**12 weeks**		**26 weeks**		**37 weeks**		**12 weeks**		**26 weeks**		**37 weeks**	
Head Circumference	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
Attained size (cm)	7.278 (7.071, 7.492)		24.368 (24.457, 24.457)		32.612 (32.059, 32.692)		7.282 (7.071, 7.492)		24.373 (24.457, 24.457)		32.615 (32.693, 32.693)	
Difference in attained size (cm)	-0.363 (-0.749, 0.024)		0.037 (-0.095, 0.169)		**0.145 (0.021, 0.268)**		**-0.404 (-0.789, -0.020)** *		0.047 (-0.082, 0.177)		**0.161 (0.039, 0.283)** *	
Abdominal Circumference												
Attained size (cm)	5.745 (5.402, 6.087)		21.668 (21.562, 21.774)		32.313 (32.195, 32.431)		5.764 (5.423, 6.104)		21.674 (21.570, 21.779)		32.319 (32.202, 32.435)	
Difference in attained size (cm)	-0.108 (-0.646, 0.430)		0.063 (-0.104, 0.229)		0.109 (-0.065, 0.283)		-0.092 (-0.629, 0.445)		0.068 (-0.095, 0.231)		0.112 (-0.056, 0.281)	
Biparietal Diameter												
Attained size (cm)	2.049 (1.984, 2.113)		6.523 (6.496, 6.549)		8.869 (8.842, 8.896)		2.053 (1.989, 2.116)		6.523 (6.497, 6.549)		8.870 (8.843, 8.897)	
Difference in attained size (cm)	0.028 (-0.069, 0.125)		**-0.055 (-0.096, -0.014)***		0.002 (-0.041, 0.045)		0.029 (-0.067, 0.125)		**-0.051 (-0.091, -0.012)***		0.005 (-0.037, 0.048)	
Femur Length												
Attained size (cm)	0.732 (0.682, 0.782)		4.872 (4.851, 4.893)		7.097 (7.075, 7.119)		0.734 (0.684, 0.784)		4.873 (4.852, 4.894)		7.098 (7.076, 7.120)	
Difference in attained size (cm)	0.024 (-0.063, 0.112)		-0.001 (-0.035, 0.032)		0.017 (-0.016, 0.050)		0.024 (-0.062, 0.110)		-0.002 (-0.035, 0.031)		0.016 (-0.017, 0.048)	

Models were adjusted for maternal age and fetal sex

* $p<0.05$

Table IV- 5. Estimated attained size of fetal parameters per mean maternal first trimester exposure and difference in predicted size per 1 IQR (13.28 ppb) difference in maternal O_3 exposure

	Unadjusted						Ajdusted					
Fetal Parameters	**12 weeks**		**26 weeks**		**37 weeks**		**12 weeks**		**26 weeks**		**37 weeks**	
Head Circumference	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**	**Est.**	**95% CI**
Attained size (cm)	7.290	(7.070, 7.509)	24.366	(24.281, 24.452)	32.616	(32.536, 32.696)	7.296	(7.083, 7.510)	24.371	(24.287, 24.455)	32.619	(32.541, 32.698)
Difference in attained size (cm)	-0.330	(-0.692, 0.032)	-0.057	(-0.189, 0.076)	0.070	(-0.065, 0.205)	-0.351	(-0.709, 0.008)	-0.058	(-0.188, 0.072)	0.077	(-0.055, 0.209)
Abdominal Circumference												
Attained size (cm)	5.785	(5.434, 6.137)	21.669	(21.564, 21.774)	32.314	(32.196, 32.431)	5.807	(5.458, 6.156)	21.676	(21.572, 21.780)	32.319	(32.204, 32.435)
Difference in attained size (cm)	-0.059	(-0.591, 0.472)	-0.140	(-0.302, 0.022)	-0.026	(-0.217, 0.165)	-0.046	(-0.579, 0.488)	-0.142	(-0.301, 0.018)	-0.030	(-0.217, 0.158)
Biparietal Diameter												
Attained size (cm)	2.037	(1.974, 2.099)	6.523	(6.497, 6.550)	8.873	(8.846, 8.900)	2.041	(1.979, 2.104)	6.524	(6.498, 6.550)	8.873	(8.846, 8.900)
Difference in attained size (cm)	-0.059	(-0.162, 0.044)	**-0.062**	**(-0.104, -0.020)***	0.013	(-0.034, 0.059)	-0.057	(-0.159, 0.045)	**-0.062**	**(-0.103, -0.021)***	0.013	(-0.033, 0.059)
Femur Length												
Attained size (cm)	0.731	(0.682, 0.781)	4.872	(4.850, 4.893)	7.098	(7.075, 7.120)	0.734	(0.684, 0.784)	4.873	(4.852, 4.894)	7.099	(7.077, 7.121)
Difference in attained size (cm)	-0.014	(-0.091, 0.063)	**-0.041**	**(-0.074, -0.008)***	0.015	(-0.025, 0.056)	-0.014	(-0.090, 0.063)	**-0.042**	**(-0.075, -0.010)***	0.015	(-0.025, 0.055)

Models were adjusted for maternal age and fetal sex

* $p<0.05$

3.6 REFERENCES

Kramer MS. 1987. Determinants of low birth weight: methodological assessment and meta-analysis. Bulletin of World Health Organization 65(5): 663-737.

Balcazar H, Haas JD. 1991. Retarded Fetal Growth and Early Neonatal Mortality in a Mexico City Population. Bulletin of PAHO.

Semba RD, Bloem MW. 2008. Nutrition and health in developing countries. NJ , USA: Humana Press, 63-86.

Carrera J. 2001. Ultrasound and Fetal Growth. New York ; London: Parthenon.

Figueras F, Gardosi J. 2011. Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. American journal of obstetrics and gynecology 204(4): 288-300.

Illa M, Coloma JL, Eixarch E, Meler E, Iraola A, Gardosi J, et al. 2009. Growth deficit in term small-for-gestational fetuses with normal umbilical artery Doppler is associated with adverse outcome. Journal of perinatal medicine 37(1): 48-52.

Ballester F, Estarlich M, Iniguez C, Llop S, Ramon R, Esplugues A, et al. 2010. Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. Environmental health : a global access science source 9: 6.

Choi H, Jedrychowski W, Spengler J, Camann D, Whyatt R, Rauh V, et al. 2006. International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. Environmental health perspectives 114(11): 1744-1750.

Estarlich M, Ballester F, Aguilera I, Fernández-Somoano A, Lertxundi A, Llop S, et al. 2011. Residential Exposure to Outdoor Air Pollution during Pregnancy and Anthropometric Measures at Birth in a Multicenter Cohort in Spain. Environmental health perspectives 119(9).

Shah PS, Balkhair T. 2011. Air pollution and birth outcomes: a systematic review. Environment international 37(2): 498-516.

Maisonet M, Correa A, Misra D, Jaakkola JJ. 2004. A review of the literature on the effects of ambient air pollution on fetal growth. Environmental research 95(1): 106-115.

Lee B.E. HEH, Park H.S., Kim Y.J., Hong Y.C., Kim H, Lee J.T. 2003. Exposure to air pollution during different gestational phases contributes to risks of low birth weight. Human Reproduction 18(3): 638-643.

Leem J-H, Kaplan BM, Shim YK, Pohl HR, Gotway CA, Bullard SM, et al. 2006. Exposures to Air Pollutants during Pregnancy and Preterm Delivery. Environmental health perspectives 114(6): 905-910.

Bell ML, Belanger K, Ebisu K, Gent JF, Lee HJ, Koutrakis P, et al. 2010. Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources. Epidemiology 21(6): 884-891.

Parker JD, Rich DQ, Glinianaia SV, Leem JH, Wartenberg D, Bell ML, et al. 2011. The international collaboration on air pollution and pregnancy outcomes: initial results. Environmental health perspectives 119(7): 1023-1028.

Rich DQ, Demissie K, Lu SE, Kamat L, Wartenberg D, Rhoads GG. 2009. Ambient air pollutant concentrations during pregnancy and the risk of fetal growth restriction. Journal of epidemiology and community health 63(6): 488-496.

Ritz B, Wilhelm M, Hoggatt KJ, Ghosh JK. 2007. Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles. American journal of epidemiology 166(9): 1045-1052.

Malmqvist E, Rignell-Hydbom A, Tinnerberg H, Bjork J, Stroh E, Jakobsson K, et al. 2011. Maternal exposure to air pollution and birth outcomes. Environmental health perspectives 119(4): 553-558.

Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. 2008. Meeting report: atmospheric pollution and human reproduction. Environmental health perspectives 116(6): 791-798.

Molina LT, de Foy B, Martinez OC, Figueroa VHP. 2009. <air quality in mexico city molina.pdf>. WMO Bulletin 58(1): 48-53.

Vallejo M, Lerma C, Infante O, Hermosillo AG, Riojas-Rodriguez H, Cardenas M. 2004. Personal exposure to particulate matter less than 2.5 microm in Mexico City: a pilot study. Journal of exposure analysis and environmental epidemiology 14(4): 323-329.

Hackley B, Feinstein A, Dixon J. 2007. Air pollution: impact on maternal and perinatal health. Journal of midwifery & women's health 52(5): 435-443.

Perera FP, Rauh V, Tsai W-Y, Kinney P, Camann D, Barr D, et al. 2003. Effects of Transplacental Exposure to Environmental Pollutants on Birth Outcomes in a Multiethnic Population. Environmental health perspectives 111(2): 201-205.

Detmar J, Rennie MY, Whiteley KJ, Qu D, Taniuchi Y, Shang X, et al. 2008. Fetal growth restriction triggered by polycyclic aromatic hydrocarbons is associated with altered placental vasculature and AhR-dependent changes in cell death. American journal of physiology Endocrinology and metabolism 295(2): E519-530.

Veras M, Damaceno-Rodrigues N, Caldini E, Maciel R, Mayhew T, Saldiva P, et al. 2008. Particulate urban air pollution affects the functional morphology of mouse placenta. Biology of reproduction 79(3): 578-584.

Salam MT, Millstein J, Li YF, Lurmann FW, Margolis HG, Gilliland FD. 2005. Birth outcomes and prenatal exposure to ozone, carbon monoxide, and particulate matter: results from the Children's Health Study. Environmental health perspectives 113(11): 1638-1644.

Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. 2009. Methodological issues in studies of air pollution and reproductive health. Environmental research 109(3): 311-320.

Ritz B, Wilhelm M. 2008. Ambient Air Pollution and Adverse Birth Outcomes: Methodologic Issues in an Emerging Field. Journal compilation: Nordic Pharmacological Society Basic & Clinical Pharmacology & Toxicology102: 182-190.

Kingdom J, Baker P. 2000. Definitions of Intrauterine Growth Restriction In: Intrauterine Growth Restriction : Aetiology and Management, (Philip B, ed). London:Springer, 1-4.

Aguilera I, Garcia-Esteban R, Iniguez C, Nieuwenhuijsen MJ, Rodriguez A, Paez M, et al. 2010. Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA Sabadell cohort. Environmental health perspectives 118(5): 705-711.

Hansen CA, Barnett AG, Pritchard G. 2008. The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. Environmental health perspectives 116(3): 362-369.

Slama R, Thiebaugeorges O, Goua V, Aussel L, Sacco P, Bohet A, et al. 2009. Maternal personal exposure to airborne benzene and intrauterine growth. Environmental health perspectives 117(8): 1313-1321.

van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW, et al. 2011. Air Pollution Exposure During Pregnancy, Ultrasound Measures of Fetal Growth, and Adverse Birth Outcomes: A Prospective Cohort Study. Environmental health perspectives.

Iniguez C, Ballester F, Estarlich M, Esplugues A, Murcia M, Llop S, et al. 2012. Prenatal exposure to traffic-related air pollution and fetal growth in a cohort of pregnant women. Occupational and environmental medicine 69(10): 736-744.

Slama RS-T, S ; Thiebaugeorges, O ; Goua, V ; Forhan, A ; Ducot, B ; Annesi-Maesano, I ; Heinrich, J ; Schweitzer, M ; Magnin, G ; Bouyer, J ; Kaminski, M ; Charles, M A. . 2006. Relation Between Atmospheric Pollutants and Head Circumference in Utero and at Birth: a Cohort Study Relying on Ultrasound Imaging During Pregnancy.[Abstract]. Epidemiology 17(6 (Suppl)): S129-S130.

Ritz B, Qiu J, Lee PC, Lurmann F, Penfold B, Erin Weiss R, et al. 2014. Prenatal air pollution exposure and ultrasound measures of fetal growth in Los Angeles, California. Environmental research 130: 7-13.

[Anonymous]. 2013. Mexico City Population 2013 - World Population Review. Available: http://worldpopulationreview.com/world-cities/mexico-city-population/ [accessed January 17, 2014 2014].

Hernandez-Valencia M, Zarate A, Ochoa R, Fonseca ME, Amato D, De Jesus Ortiz M. 2001. Insulin-like growth factor I, epidermal growth factor and transforming growth factor beta expression and their association with intrauterine fetal growth retardation, such as development during human pregnancy. Diabetes, obesity & metabolism 3(6): 457-462.

Fernández-Carrocera LA, Chávez-Torres R, Casanueva E, Barrera-Reyes RH, del Pilar Ibarra-Reyes M, Martínez-Cruz C. 2003. Intrauterine growth retardation and neurodevelopment at one year of age in Mexican children. Nutrition Research 23(1): 1-8.

Marr LC, Grogan LA, Wohrnschimmel H, Molina LT, Molina MJ, Smith TJ, et al. 2004. Vehicle traffic as a source of particulate polycyclic aromatic hydrocarbon exposure in the Mexico City metropolitan area. Environmental science & technology 38(9): 2584-2592.

O'Neill M, Osornio-Vargas A, Buxton MA, Sanchez BN, Rojas-Bracho L, Castillo-Castrejon M, et al. 2012. Air pollution, inflammation and preterm birth in Mexico City: Study design and methods. The Science of the total environment.

American Institute of Ultrasound Medicine. 2007. AIUM Practice Guidelines.

Perni S, Chervenak F, Kalish R, Magherini-Rothe S, Predanic M, Streltzoff J, et al. 2004. Intraobserver and interobserver reproducibility of fetal biometry. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 24(6): 654-658.

Ecologia INd. 2011. Zona Metropolitana del Valle de México. In: Cuarto almanaque de datos y tendencias de la calidad del aire en 20 ciudades mexicanas (2000-2009). Mexico City, Mexico, 410.

Edgerton SA, Arriaga JL, Archuleta J, Bian X, Bossert JE, Chow JC, et al. 1999. Particulate Air Pollution in Mexico City A Col laborative Research Project. J Air Waste Manage 49: 1221-1229.

Shanis Mark WM. 2005. Report from the Performance Audit of the Mexico City Ambient Air Monitoring Network. USEPA.

Rivera-González LO. 2012. Environmental Toxicants and Preterm Birth in Mexico City:A Geo-Spatial Approach for Use in Epidemiology. Ann Arbor, Michigan: University of Michigan.

Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. 2009. Methodological issues in studies of air pollution and reproductive health. Environmental research 109(3): 311-320.

Strand LB, Barnett AG, Tong S. 2011. Methodological challenges when estimating the effects of season and seasonal exposures on birth outcomes. BMC medical research methodology 11: 49.

Schisterman EF, Cole SR, Platt RW. 2009. Overadjustment Bias and Unnecessary Adjustment in Epidemiologic Studies. Epidemiology 20(4): 7.

McCurdy T. GG, Smith L., Lakkadi Y. 2000. The National Exposure Research Laboratory's Consolidated Human Activity Database. J Exp Anal Envrion Epidem(10): 566-578.

Ghosh R, Rankin J, Pless-Mulloli T, Glinianaia S. 2007. Does the effect of air pollution on pregnancy outcomes differ by gender? A systematic review. Environmental research 105(3): 400-408.

Schwarzler P, Bland JM, Holden D, Campbell S, Ville Y. 2004. Sex-specific antenatal reference growth charts for uncomplicated singleton pregnancies at 15-40 weeks of gestation. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 23(1): 23-29.

O'Neill MS, Jerrett M, Kawachi I, Levy JI, Cohen AJ, Gouveia N, et al. 2003. Health, Wealth, and Air Pollution: Advancing Theory and Methods. Environmental health perspectives 111(16): 1861-1870.

Calderon-Garciduenas L, Torres-Jardon R. 2012. Air Pollution, Socioeconomic Status, and Children's Cognition in Megacities: The Mexico City Scenario. Frontiers in psychology 3: 217.

Wen X, Kleinman K, Gillman MW, Rifas-Shiman SL, Taveras EM. 2012. Childhood body mass index trajectories: modeling, characterizing, pairwise correlations and socio-demographic predictors of trajectory characteristics. BMC medical research methodology 12: 38.

Smarr MM, Vadillo-Ortega F, Castillo-Castrejon M, O'Neill MS. 2013. The use of ultrasound measurements in environmental epidemiological studies of air pollution and fetal growth. Curr Opin Pediatr 25(2): 240-246.

Hemachandra AH, Klebanoff MA, Duggan AK, Hardy JB, Furth SL. 2006. The association between intrauterine growth restriction in the full-term infant and high blood pressure at age 7 years: results from the Collaborative Perinatal Project. International journal of epidemiology 35(4): 871-877.

Winick M. 1974. Nutrition and fetal development. . New York: : Wiley.

Ananth CV, Vintzileos AM. 2009. Distinguishing pathological from constitutional small for gestational age births in population-based studies. Early Hum Dev 85(10): 653-658.

Gilliland F, Avol E, Kinney P, Jerrett M, Dvonch T, Lurmann F, et al. 2005. Air pollution exposure assessment for epidemiologic studies of pregnant women and children: lessons learned from the Centers for Children's Environmental Health and Disease Prevention Research. Environmental health perspectives 113(10): 1447-1454.

Jedrychowski W, Perera F, Mrozek-Budzyn D, Mroz E, Flak E, Spengler JD, et al. 2009. Gender differences in fetal growth of newborns exposed prenatally to airborne fine particulate matter. Environmental research 109(4): 447-456.

Binder H, Sauerbrei W, Royston P. 2013. Comparison between splines and fractional polynomials for multivariable model building with continuous covariates: a simulation study with continuous response. Statistics in medicine 32(13): 2262-2277.

Dashe JS, McIntire DD, Lucas MJ, Leveno KJ. 2000. Effects of Symmetric and Asymmetric Fetal OGrowth on Pregnancy Outcomes. Obstetrics and gynecology 96(3): 321-327.

Riyami Na, Walker MG, Proctor LK, Yinon Y, Windrim RC, Kingdom JCP. 2011. Utility of Head/Abdomen Circumference Ratio in the Evaluation of Severe Early-Onset Intrauterine Growth Restriction. J Obstet Gynaecol Can 33(7): 718-719.

CHAPTER V
CONCLUSION

5.1. Summary of Research Findings

The objectives of this dissertation research were to 1) identify and establish the use of ultrasound measurements of fetal anthropometric parameters as a novel method to assess fetal growth in epidemiological studies; 2) perform a quality control and assessment (QA/QC) of the ultrasound measurements of four fetal parameters [head circumference (HC), abdominal circumference (AC), biparietal diameter (BPD) and femur length (FL)] collected by multiple clinicians; and 3) assess the relationship between maternal exposure to air pollution during the first trimester of pregnancy and fetal growth assessed by repeated ultrasound measurements. This chapter briefly summarizes the findings of each specific research aim including results, strengths and limitations, with emphasis being placed on public health relevance and implications and consideration of future research directions.

In chapter two of this dissertation, a systematic review of the literature identified a small but increasing number of epidemiological studies that have used ultrasound measurements to assess potential relationships between fetal growth *in utero* and maternal exposures to air pollution. These findings are a preliminary response to a major gap in the literature; the need for methodology that allows for the distinction between the

overlapping etiologies that contribute to low birth weight (e.g., preterm birth, intrauterine growth restriction). While these studies offer insights that will help to elucidate the biological mechanisms explaining adverse pregnancy outcomes related to gestational exposures to air pollution, collectively they lack homogeneity in various areas. A published review of methodological issues in studies of air pollution and reproductive health found that variability in study design and analysis was a key issue limiting comparisons and syntheses of these studies (T.J. Woodruff et al. 2009). To date, only seven studies address maternal exposure to air pollution and fetal growth with the use of ultrasound data collected during pregnancy. Even with this small number of studies, it was difficult to compare results between studies as a result of heterogeneity in the air pollutants examined, the time period of exposure and fetal growth assessment, and analytic methods. However, two similarities in the findings were worth noting. First most of the studies identified early pregnancy exposures to be a potential window of special relevance, with several air pollutants in early pregnancy being negatively associated with the growth of fetal anthropometric parameters. The second similarity across the studies, with one exception (the Generation R study in the Netherlands), was the lack of quantification of measurement error associated with fetal parameter data.

The results of chapter three addressed the latter concern, specific to our study population. The amount of measurement error associated with fetal anthropometric parameters collected by various clinicians, using the same ultrasound equipment on the same participants on the same day, was found to be minimal between clinicians. Statistical analyses accounted for differences in gestational age since measurement error increases as these parameters increase in size. This analysis also allowed for the

calculation of standard measurement error, which aided in the decision to use data collected on BPD by the various clinicians although the mean difference in measurements between each clinician pair was statistically significant. Overall, the results of the interobserver reliability assessment reduced concerns of measurement uncertainty that may have biased the results of effect estimates in the epidemiological analysis of air pollution and fetal growth.

Finally, the results of the analysis in chapter four suggest that longitudinal assessment of the growth of fetal anthropometric parameters may help to further the understanding of the relationship between early gestational exposures to air pollutants and adverse birth outcomes. The use of single pollutant models showed that associations between intrauterine growth and air pollutant exposures not only vary by the fetal parameter or the timing period of growth assessment, but by the air pollutant of interest. The use of prediction models to assess alterations in fetal parameters' growth across pregnancy also lent support to the concept that the effects of air pollution on fetal growth may be transient and that catchup growth may explain the lack of reduction in parameter size estimated at later periods in pregnancy. In addition to the studies that have addressed air pollution and fetal growth (Aguilera et al. 2010; Hansen et al. 2008; Slama et al. 2009; Iniguez et al. 2012a; van den Hooven et al. 2012), this has also been seen in previous studies of tobacco smoke and fetal growth parameters (Lampl et al. 2003; Iniguez et al. 2012b). Although active and passive smoking variables were not primary exposures of interest in this analysis, that literature is relevant given similar potential mechanisms and similar adverse health outcomes observed for both smoking and air pollution.

5.2. Public Health Implications

From a public health perspective, the accurate identification of a fetus who is growth restricted could help to reduce the risk of infant mortality and childhood morbidity associated with IUGR. Therefore, the use of serial ultrasound measurements may help to distinguish between the pathologically growth restricted and the constitutionally smaller fetuses. Clinically, early identification of IUGR sets the course of management and treatment options for the at-risk fetus, and could potentially reduce the number of IUGR related stillbirths. From an epidemiological perspective, using a methodology to assess fetal growth prior to birth would help to reduce misclassification of smaller neonates as at-risk growth restricted neonates. This would ultimately help to improve estimations of both incidence and prevalence of IUGR.

While the clinical relevance of changes in some of the fetal parameters and ratios of the fetal parameters may differ depending on the timing of restriction, the magnitude of the reduction, and if changes are on the individual or population level, parameter assessment *in utero* is still beneficial. Assessing changes in the size and growth rate of fetal anthropometric parameters that are associated with air pollution exposure could inform health educators who make maternal health recommendations, such as altering maternal behaviors in order to reduce risk of having a growth restricted fetus. For example, if the inhalation of pollutants containing reactive oxygen species is associated reduction of fetal AC, oxidative stress may be a hypothesized mechanism, and mothers who live in heavily polluted areas may be advised to increase their consumption of more foods that are rich in antioxidants as a preventative measure. Results of these studies could also provide a foundation for future toxicology studies with the use of fetal animal

models to further test hypotheses of biological mechanisms, since the physiology explaining the normal growth and development of specific anthropometric parameters may be previously understood and the effect of exposure to air pollutants can be explored in a more controlled setting.

Finally, one of the principal motivations behind most research on air pollution and human health is the hope of effecting change at a policy level. Pollution abatement is a constant concern and the supporting policy is ever changing. Results of this research and previous literature of air pollution and fetal growth suggest that maternal exposure to air pollution may adversely affect fetal growth and development. Therefore, continued research of this nature may encourage future discussions of air quality as it pertains to environmental and human health standards.

5.3. Future Directions

The focus of this dissertation was placed on $PM_{2.5}$ and O_3, as these pollutants exceed health standards set by the Instituto Nacional de Ecologia in Mexico. One consideration for future explorations relevant to my dissertation research is to examine other ambient air pollutants and fetal growth in Mexico City.

Carbon monoxide's ability to cross the placenta by simple diffusion interferes with oxygen transport to the fetus and makes this a pollutant of interest. The affinity of hemoglobin is substantially greater for CO (210-300 times) than for oxygen, and the half-life of carbon monoxide hemoglobin (COHb) in fetal blood is three times that of maternal blood (Hackley et al. 2007). As a result of NO_2 exposure, inflammatory

reaction in lung may cause the release of cytokines that may trigger preterm birth (Devalia et al. 1993). Nitrogen dioxide exposure suppresses antioxidant defense systems, increased lipid peroxidation in humans and biomarkers of exposure are associated with poor birth outcomes (Tabacova et al. 1998). Others studies report that early exposure to NO_2 is associated with adverse pregnancy outcomes (Maroziene and Grazuleviciene 2002). Much like NO_2, exposure to SO_2 may result in aggravated symptoms of pre-existing respiratory disease and may affect fetal health through similar hypothesized mechanisms. Adverse associations between maternal exposures to SO_2 and various birth outcomes like low birth weight (Dugandzic et al. 2006) and preterm birth (JH Leem et al. 2006) have been reported in previous literature. Another class of environmental hazards that would be interesting to study within the scope of this research is polycyclic aromatic hydrocarbons (PAHs). PAHs are ubiquitous in the environment and are known carcinogenic, mutagenic and teratogenic compounds, often resulting from the incomplete combustion of coal, gas, and other materials (US EPA 2001) . In Mexico City, exposure assessment of PAH concentrations have reported higher concentration of various PAHs during the dry season compared to the rainy season, and for many of these compounds trends were linear (Amador-Muñoz et al. 2013; Valle-Hernandez et al. 2010). PAHs and birth outcomes have been studied employing various study designs (Choi et al. 2006; Jedrychowski W 2004). Biomarkers of exposure, PAH DNA adducts, have been associated with adverse birth outcomes like reduced weight, HC, and length (Perera et al. 2005; Perera Frederica P. 1998; Tang D 2006; Perera FP 2004). To date, PAH-adducts have not been reported for maternal and fetal pairs in Mexico City, however studies have shown that PAH-adducts are higher among those non-smoking adults in Mexico City

during the dry season compared to the rainy season. Therefore assessing maternal exposure to PAHs from various sources of exposure (air pollution, diet and tobacco smoke), by quantifying PAH-DNA adduct formation in maternal blood and umbilical cord blood samples, and fetal growth of anthropometric parameters during pregnancy with repeated ultrasound measurements would be novel in terms of study population and study design.

Lastly, while it is important to have a better understanding of the relationship between the various air pollutants previously described and fetal growth in Mexico City, consideration should also be given to how these pollutants interact with each other. Therefore, the use of multi-pollutant models to assess changes in fetal growth in response to air pollution exposures should also be considered for future analyses. While the approach of this dissertation was to use single-pollutant models as an attempt to better understand the relationship between each pollutant and each fetal parameter, as it may be easier to hypothesize biological explanations in this manner, the potential synergistic effect of air pollution should be furthered explored. In reality, ambient air pollution is a mixture of various gases and particulates, some of which are strongly correlated with each other. It is then suggested that models in future analyses explore maternal exposure to air pollution and pregnancy outcomes with the use of multi-pollutant models. The results of such models may lend support to increased discussion regarding air quality standards that are established to protect the environment and human health, especially among those belonging to susceptible sub-groups like the pregnant woman and her unborn fetus.

5.4. REFERENCES

Peleg D, Kennedy CM, Hunter SK. 1998. Intrauterine Growth Restriction: Identification and Management. Am Fam Physician 58(2): 453-460.

Imdad A, Yakoob MY, Siddiqui S, Bhutta ZA. 2011. Screening and triage of intrauterine growth restriction (IUGR) in general population and high risk pregnancies: a systematic review with a focus on reduction of IUGR related stillbirths. BMC public health 11 Suppl 3: S1.

Figueras F, Gardosi J. 2011. Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. American journal of obstetrics and gynecology 204(4): 288-300.

Mandruzzato G, Antsaklis A, Botet F, Chervenak FA, Figueras F, Grunebaum A, et al. 2008. Intrauterine restriction (IUGR). Journal of perinatal medicine 36(4): 277-281.

Organization WH. 2011. 2.6 million babies stillborn in 2009 : New global and country estimates published in Lancet Series.

Illa M, Coloma JL, Eixarch E, Meler E, Iraola A, Gardosi J, et al. 2009. Growth deficit in term small-for-gestational fetuses with normal umbilical artery Doppler is associated with adverse outcome. Journal of perinatal medicine 37(1): 48-52.

Balcazar H, Haas JD. 1991. Retarded Fetal Growth and Early Neonatal Mortality in a Mexico City Population. Bulletin of PAHO.

Kannan S, Misra DP, Dvonch JT, Krishnakumar A. 2006. Exposures to Airborne Particulate Matter and Adverse Perinatal Outcomes: A BiologicallyPlausible Mechanistic Framework for Exploring Potential Effect Modification by Nutrition. Environmental health perspectives 114(11): 1636-1642.

Lee B.E. HEH, Park H.S., Kim Y.J., Hong Y.C., Kim H, Lee J.T. 2003. Exposure to air pollution during different gestational phases contributes to risks of low birth weight. Human Reproduction 18(3): 638-643.

Kramer MS. 1987. Determinants of low birth weight: methodological assessment and meta-analysis. Bulletin of World Health Organization 65(5): 663-737.

Gardosi J, Figueras F, Clausson B, Francis A. 2011. The customised growth potential: an international research tool to study the epidemiology of fetal growth. Paediatric and perinatal epidemiology 25(1): 2-10.

Haas J, Balcazar H, Caulfield L. 1987. variation in early neonatal mortality for different types of fetal growth retardation. American Journal Of Physical Anthropology 73(467-473).

Semba RD, Bloem MW. 2008. Nutrition and health in developing countries

NJ , USA: Humana Press, 63-86.

Carrera J. 2001. Ultrasound and Fetal Growth. New York ; London: Parthenon.

Saleem T, Sajjad N, Fatima S, Habib N, Ali SR, Qadir M. 2011. Intrauterine growth retardation--small events, big consequences. Italian journal of pediatrics 37: 41.

Kingdom J, Baker P. 2000. Definitions of Intrauterine Growth Restriction In: Intrauterine Growth Restriction : Aetiology and Management, (Philip B, ed). London:Springer, 1-4.

Hemachandra AH, Klebanoff MA, Duggan AK, Hardy JB, Furth SL. 2006. The association between intrauterine growth restriction in the full-term infant and high blood pressure at age 7 years: results from the Collaborative Perinatal Project. International journal of epidemiology 35(4): 871-877.

Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. 2009. Methodological issues in studies of air pollution and reproductive health. Environmental research 109(3): 311-320.

Hemachandra AH, Klebanoff MA. 2006. Use of serial ultrasound to identify periods of fetal growth restriction in relation to neonatal anthropometry. American journal of human biology : the official journal of the Human Biology Council 18(6): 791-797.

Winick M. 1974. Nutrition and fetal development. . New York: : Wiley.

EPA US. 2012. National Ambient Air Quality Standards (NAAQS).

Ecologia INd. 2011. Zona Metropolitana del Valle de México. In: Cuarto almanaque de datos y tendencias de la calidad del aire en 20 ciudades mexicanas (2000-2009). Mexico City, Mexico, 410.

Girard JE. 2010a. The Earth's Atmosphere. In: Principles of Environmental Chemistry, Part Second. Sudbury Massachusetts Jones and Bartlett, 40-65.

Anderson JO, Thundiyil JG, Stolbach A. 2012. Clearing the air: a review of the effects of particulate matter air pollution on human health. Journal of medical toxicology : official journal of the American College of Medical Toxicology 8(2): 166-175.

Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D. 2004. Particulate air pollution and fetal health: a systematic review of the epidemiologic evidence. Epidemiology 15(1): 36-45.

EPA) USEPAU. 2012. Ground-level Ozone. Available: http://www.epa.gov/glo/basic.html [accessed January 14, 2014.

Girard JE. 2010b. Chemistry of the Troposphere. In: Principles of Environmental Chemistry Part Second. Sadbury, Massachusetts:Jones and Bartlett, 92-123.

O'Neill MS, Loomis D, Borja-Aburto VH. 2004. Ozone, area social conditions, and mortality in Mexico City. Environmental research 94(3): 234-242.

Salam MT, Millstein J, Li YF, Lurmann FW, Margolis HG, Gilliland FD. 2005. Birth outcomes and prenatal exposure to ozone, carbon monoxide, and particulate matter: results from the Children's Health Study. Environmental health perspectives 113(11): 1638-1644.

Hackley B, Feinstein A, Dixon J. 2007. Air pollution: impact on maternal and perinatal health. Journal of midwifery & women's health 52(5): 435-443.

Med R. illness information. Available: http://www.rxmed.com/b.main/b1.illness/b1.1.illnesses/anemia_during_pregnancy.html.

Shah PS, Balkhair T. 2011. Air pollution and birth outcomes: a systematic review. Environment international 37(2): 498-516.

Veras M, Damaceno-Rodrigues N, Caldini E, Maciel R, Mayhew T, Saldiva P, et al. 2008. Particulate urban air pollution affects the functional morphology of mouse placenta. Biology of reproduction 79(3): 578-584.

Veras M, Caldini E, Dolhnikoff M, Saldiva P. 2010. Air pollution and effects on reproductive-system functions globally with particular emphasis on the Brazilian population. Journal of toxicology and environmental health Part B, Critical reviews 13(1): 1-15.

Hecht SS. 2008. Human Phenanthrene Metabolites as Probes for the Metabolic Activation and Detoxification of Carcinogenic Polycyclic Aromatic Hydrocarbons. 1-22.

Perera FP, Tang D, Rauh V, Lester K, Tsai WY, Tu YH, et al. 2005. Relationships among Polycyclic Aromatic Hydrocarbon–DNA Adducts, Proximity to the World Trade Center, and Effects on Fetal Growth. Environmental health perspectives 113(8): 1062-1067.

Perera Frederica P. WRM, Jedrychowski Wieslaw, Rauh Virginia, Manchester David, Santella Regina M., Ottman Ruth 1998. A Study of the Effects of Environmental Polycyclic Aromatic Hydrocarbons on Birth Outcomes in Poland. American journal of epidemiology 147(3): 309-314.

Slama R, Darrow L, Parker J, Woodruff TJ, Strickland M, Nieuwenhuijsen M, et al. 2008. Meeting report: atmospheric pollution and human reproduction. Environmental health perspectives 116(6): 791-798.

Bell ML, Belanger K, Ebisu K, Gent JF, Lee HJ, Koutrakis P, et al. 2010. Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources. Epidemiology 21(6): 884-891.

Parker JD, Rich DQ, Glinianaia SV, Leem JH, Wartenberg D, Bell ML, et al. 2011. The international collaboration on air pollution and pregnancy outcomes: initial results. Environmental health perspectives 119(7): 1023-1028.

Ritz B, Wilhelm M, Hoggatt KJ, Ghosh JK. 2007. Ambient air pollution and preterm birth in the environment and pregnancy outcomes study at the University of California, Los Angeles. American journal of epidemiology 166(9): 1045-1052.

Maisonet M, Correa A, Misra D, Jaakkola JJ. 2004. A review of the literature on the effects of ambient air pollution on fetal growth. Environmental research 95(1): 106-115.

Rich DQ, Demissie K, Lu SE, Kamat L, Wartenberg D, Rhoads GG. 2009. Ambient air pollutant concentrations during pregnancy and the risk of fetal growth restriction. Journal of epidemiology and community health 63(6): 488-496.

Dejmek J, Selevan SG, Benes I, Solansky I, Srim RJ. 1999. Fetal Growth and Maternal Exposure to Particulate Matter during Pregnancy. Environmental health perspectives 107(6): 475-480.

Rojas-Bracho L, Suh HH, Koutrakis P. 2000. Relationships among personal, indoor, and outdoor fine and coarse particle concentrations for individuals with COPD. Journal of Exposure Analysis & Environmental Epidemiology 10(3): 294-306.

Sarnat JA, Brown KW, Schwartz J, Coull BA, Koutrakis P. 2005. Ambient gas concentrations and personal particulate matter exposures: implications for studying the health effects of particles. Epidemiology 16(3): 385-395.

Woodruff TJ, Parker JD, Darrow LA, Slama R, Bell ML, Choi H, et al. 2009. Methodological issues in studies of air pollution and reproductive health. Environmental research 109(3): 311-320.

Slama RS-T, S ; Thiebaugeorges, O ; Goua, V ; Forhan, A ; Ducot, B ; Annesi-Maesano, I ; Heinrich, J ; Schweitzer, M ; Magnin, G ; Bouyer, J ; Kaminski, M ; Charles, M A. . 2006. Relation Between Atmospheric Pollutants and Head Circumference in Utero and at Birth: a Cohort Study Relying on Ultrasound Imaging During Pregnancy.[Abstract]. Epidemiology 17(6 (Suppl)): S129-S130.

Perni S, Chervenak F, Kalish R, Magherini-Rothe S, Predanic M, Streltzoff J, et al. 2004. Intraobserver and interobserver reproducibility of fetal biometry. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 24(6): 654-658.

Ritz B, Qiu J, Lee PC, Lurmann F, Penfold B, Erin Weiss R, et al. 2014. Prenatal air pollution exposure and ultrasound measures of fetal growth in Los Angeles, California. Environmental research 130: 7-13.

Ioannou C, Talbot K, Ohuma E, Sarris I, Villar J, Conde-Agudelo A, et al. 2012. Systematic review of methodology used in ultrasound studies aimed at creating charts of

fetal size. BJOG : an international journal of obstetrics and gynaecology 119(12): 1425-1439.

Smarr MM, Vadillo-Ortega F, Castillo-Castrejon M, O'Neill MS. 2013. The use of ultrasound measurements in environmental epidemiological studies of air pollution and fetal growth. Current Opinion in Pediatrics 25(2): 10.1097/MOP.1090b1013e32835e32831e32874.

O'Neill MS, Loomis D, Torres Meza V, Retama A, Gold D. 2002. Estimating particle exposure in the Mexico City metropolitan area. Journal of exposure analysis and environmental epidemiology 12(2): 145-156.

Michael Brauer, Cornel Lencar, Lillian Tamburic, Mieke Koehoorn, Paul Demers, Karr C. 2008. A Cohort Study of Traffic-Related Air Pollution Impacts on Birth Outcomes. Environmental health perspectives 116(5): 680-686.

Rivera-González LO. 2012. ENVIRONMENTAL TOXICANTS AND PRETERM BIRTH IN MEXICO CITY:

A GEO-SPATIAL APPROACH FOR USE IN EPIDEMIOLOGY. Ann Arbor, Michigan: University of Michigan.

Yang F, Leung KY, Lee YP, Chan HY, Tang MH. 2010. Fetal biometry by an inexperienced operator using two- and three-dimensional ultrasound. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 35(5): 566-571.

Sarris I, Ioannou C, Chamberlain P, Ohuma E, Roseman F, Hoch L, et al. 2012. Intra- and interobserver variability in fetal ultrasound measurements. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 39(3): 266-273.

O'Neill M, Osornio-Vargas A, Buxton MA, Sanchez BN, Rojas-Bracho L, Castillo-Castrejon M, et al. 2012. Air pollution, inflammation and preterm birth in Mexico City: Study design and methods. The Science of the total environment.

Medicine TAIoUi. 2007. AIUM Practice Guidelines.

Lyons JS. 1997. Defining and Measuring Clinical and Utilization Outcomes. In: The Measurement & Management of Clinical Outcomes in Mental Health (Lyons JS, ed): Wiley & Sons, 25-46.

Hughey M. 2005. 2nd and 3rd Trimester Ultrasound Scanning. 2014].

Verburg BO MP, Hofman A, Jaddoe VW, Witteman JC, Steegers EA. 2008. Intra- and interobserver reproducibility study of early fetal growth parameters. Prenatal diagnosis 28(4): 323-331.

Ballester F, Estarlich M, Iniguez C, Llop S, Ramon R, Esplugues A, et al. 2010. Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia, Spain. Environmental health : a global access science source 9: 6.

Choi H, Jedrychowski W, Spengler J, Camann D, Whyatt R, Rauh V, et al. 2006. International studies of prenatal exposure to polycyclic aromatic hydrocarbons and fetal growth. Environmental health perspectives 114(11): 1744-1750.

Estarlich M, Ballester F, Aguilera I, Fernández-Somoano A, Lertxundi A, Llop S, et al. 2011. Residential Exposure to Outdoor Air Pollution during Pregnancy and Anthropometric Measures at Birth in a Multicenter Cohort in Spain. Environmental health perspectives 119(9).

Leem J-H, Kaplan BM, Shim YK, Pohl HR, Gotway CA, Bullard SM, et al. 2006. Exposures to Air Pollutants during Pregnancy and Preterm Delivery. Environmental health perspectives 114(6): 905-910.

Malmqvist E, Rignell-Hydbom A, Tinnerberg H, Bjork J, Stroh E, Jakobsson K, et al. 2011. Maternal exposure to air pollution and birth outcomes. Environmental health perspectives 119(4): 553-558.

Molina LT, de Foy B, Martinez OC, Figueroa VHP. 2009. <air quality in mexico city molina.pdf>. WMO Bulletin 58(1): 48-53.

Vallejo M, Lerma C, Infante O, Hermosillo AG, Riojas-Rodriguez H, Cardenas M. 2004. Personal exposure to particulate matter less than 2.5 microm in Mexico City: a pilot study. Journal of exposure analysis and environmental epidemiology 14(4): 323-329.

Perera FP, Rauh V, Tsai W-Y, Kinney P, Camann D, Barr D, et al. 2003. Effects of Transplacental Exposure to Environmental Pollutants on Birth Outcomes in a Multiethnic Population. Environmental health perspectives 111(2): 201-205.

Detmar J, Rennie MY, Whiteley KJ, Qu D, Taniuchi Y, Shang X, et al. 2008. Fetal growth restriction triggered by polycyclic aromatic hydrocarbons is associated with altered placental vasculature and AhR-dependent changes in cell death. American journal of physiology Endocrinology and metabolism 295(2): E519-530.

Ritz B, Wilhelm M. 2008. Ambient Air Pollution and Adverse Birth Outcomes: Methodologic Issues in an Emerging Field. Journal compilation: Nordic Pharmacological Society Basic & Clinical Pharmacology & Toxicology

102: 182-190.

Aguilera I, Garcia-Esteban R, Iniguez C, Nieuwenhuijsen MJ, Rodriguez A, Paez M, et al. 2010. Prenatal exposure to traffic-related air pollution and ultrasound measures of fetal growth in the INMA Sabadell cohort. Environmental health perspectives 118(5): 705-711.

Hansen CA, Barnett AG, Pritchard G. 2008. The effect of ambient air pollution during early pregnancy on fetal ultrasonic measurements during mid-pregnancy. Environmental health perspectives 116(3): 362-369.

Slama R, Thiebaugeorges O, Goua V, Aussel L, Sacco P, Bohet A, et al. 2009. Maternal personal exposure to airborne benzene and intrauterine growth. Environmental health perspectives 117(8): 1313-1321.

van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW, et al. 2011. Air Pollution Exposure During Pregnancy, Ultrasound Measures of Fetal Growth, and Adverse Birth Outcomes: A Prospective Cohort Study. Environmental health perspectives.

Iniguez C, Ballester F, Estarlich M, Esplugues A, Murcia M, Llop S, et al. 2012a. Prenatal exposure to traffic-related air pollution and fetal growth in a cohort of pregnant women. Occupational and environmental medicine 69(10): 736-744.

[Anonymous]. 2013. Mexico City Population 2013 - World Population Review. Available: http://worldpopulationreview.com/world-cities/mexico-city-population/ [accessed January 17, 2014 2014].

Hernandez-Valencia M, Zarate A, Ochoa R, Fonseca ME, Amato D, De Jesus Ortiz M. 2001. Insulin-like growth factor I, epidermal growth factor and transforming growth factor beta expression and their association with intrauterine fetal growth retardation, such as development during human pregnancy. Diabetes, obesity & metabolism 3(6): 457-462.

Fernández-Carrocera LA, Chávez-Torres R, Casanueva E, Barrera-Reyes RH, del Pilar Ibarra-Reyes M, Martínez-Cruz C. 2003. Intrauterine growth retardation and neurodevelopment at one year of age in Mexican children. NUTRITION RESEARCH 23(1): 1-8.

Marr LC, Grogan LA, Wohrnschimmel H, Molina LT, Molina MJ, Smith TJ, et al. 2004. Vehicle traffic as a source of particulate polycyclic aromatic hydrocarbon exposure in the Mexico City metropolitan area. Environmental science & technology 38(9): 2584-2592.

Edgerton SA, Arriaga JL, Archuleta J, Bian X, Bossert JE, Chow JC, et al. 1999. Particulate Air Pollution in Mexico City A Col laborative Research Project. J Air Waste Manage 49: 1221-1229.

Shanis Mark WM. 2005. Report from the Performance Audit of the Mexico City Ambient Air Monitoring Network. USEPA.

Strand LB, Barnett AG, Tong S. 2011. Methodological challenges when estimating the effects of season and seasonal exposures on birth outcomes. BMC medical research methodology 11: 49.

Schisterman EF, Cole SR, Platt RW. 2009. Overadjustment Bias and Unnecessary Adjustment in Epidemiologic Studies. Epidemiology 20(4): 7.

McCurdy T. GG, Smith L., Lakkadi Y. 2000. The National Exposure Research Laboratory's Consolidated Human Activity Database. J Exp Anal Envrion Epidem(10): 566-578.

Ghosh R, Rankin J, Pless-Mulloli T, Glinianaia S. 2007. Does the effect of air pollution on pregnancy outcomes differ by gender? A systematic review. Environmental research 105(3): 400-408.

Schwarzler P, Bland JM, Holden D, Campbell S, Ville Y. 2004. Sex-specific antenatal reference growth charts for uncomplicated singleton pregnancies at 15-40 weeks of gestation. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology 23(1): 23-29.

O'Neill MS, Jerrett M, Kawachi I, Levy JI, Cohen AJ, Gouveia N, et al. 2003. Health, Wealth, and Air Pollution: Advancing Theory and Methods. Environmental health perspectives 111(16): 1861-1870.

Calderon-Garciduenas L, Torres-Jardon R. 2012. Air Pollution, Socioeconomic Status, and Children's Cognition in Megacities: The Mexico City Scenario. Frontiers in psychology 3: 217.

Wen X, Kleinman K, Gillman MW, Rifas-Shiman SL, Taveras EM. 2012. Childhood body mass index trajectories: modeling, characterizing, pairwise correlations and socio-demographic predictors of trajectory characteristics. BMC medical research methodology 12: 38.

Smarr MM, Vadillo-Ortega F, Castillo-Castrejon M, O'Neill MS. 2013. The use of ultrasound measurements in environmental epidemiological studies of air pollution and fetal growth. Curr Opin Pediatr 25(2): 240-246.

Ananth CV, Vintzileos AM. 2009. Distinguishing pathological from constitutional small for gestational age births in population-based studies. Early Hum Dev 85(10): 653-658.

Gilliland F, Avol E, Kinney P, Jerrett M, Dvonch T, Lurmann F, et al. 2005. Air pollution exposure assessment for epidemiologic studies of pregnant women and children: lessons learned from the Centers for Children's Environmental Health and Disease Prevention Research. Environmental health perspectives 113(10): 1447-1454.

Binder H, Sauerbrei W, Royston P. 2013. Comparison between splines and fractional polynomials for multivariable model building with continuous covariates: a simulation study with continuous response. Statistics in medicine 32(13): 2262-2277.

Dashe JS, McIntire DD, Lucas MJ, Leveno KJ. 2000. Effects of Symmetric and Asymmetric Fetal OGrowth on Pregnancy Outcomes. Obstetrics and gynecology 96(3): 321-327.

Riyami Na, Walker MG, Proctor LK, Yinon Y, Windrim RC, Kingdom JCP. 2011. Utility of Head/Abdomen Circumference Ratio in the Evaluation of Severe Early-Onset Intrauterine Growth Restriction. J Obstet Gynaecol Can 33(7): 718-719.

van den Hooven EH, Pierik FH, de Kluizenaar Y, Willemsen SP, Hofman A, van Ratingen SW, et al. 2012. Air pollution exposure during pregnancy, ultrasound measures of fetal growth, and adverse birth outcomes: a prospective cohort study. Environmental health perspectives 120(1): 150-156.

Lampl M, Kuzawa CW, Jeanty P. 2003. Prenatal smoke exposure alters growth in limb proportions and head shape in the midgestation human fetus. American journal of human biology : the official journal of the Human Biology Council 15(4): 533-546.

Iniguez C, Ballester F, Amoros R, Murcia M, Plana A, Rebagliato M. 2012b. Active and passive smoking during pregnancy and ultrasound measures of fetal growth in a cohort of pregnant women. Journal of epidemiology and community health 66(6): 563-570.

Devalia JL, Campbell AM, Sapsford RJ, Rusznak C, Quint D, Godard P, et al. 1993. Effect of Nitrogen Dioxide on Synthesis of Inflammatory Cytokines Expressed by Human Bronchial Epithelial Cells In Vitro. American Journal of Respiratory Cell and Molecular Biology 9(3): 271-278.

Tabacova S, Baird DD, Balabaeva L. 1998. Exposure to oxidized nitrogen: lipid peroxidation and neonatal health risk. Archives of environmental health 53(3): 214-221.

Maroziene L, Grazuleviciene R. 2002. Maternal exposure to low-level air pollution and pregnancy outcomes: a population-based study. Environmental health : a global access science source 1(1): 6.

Dugandzic R, Dodds L, Stieb D, Smith-Doiron M. 2006. The association between low level exposures to ambient air pollution and term low birth weight: a retrospective cohort study. Environmental health : a global access science source 5: 3.

Leem JH, Kaplan BM, Shim YK, Pohl HR, Gotway CA, Bullard SM, et al. 2006. Exposures to air pollutants during pregnancy and preterm delivery. Environmental health perspectives 114(6): 905-910.

EPA) USEPAU. 2001. Emergency Planning and Community Right-to-Know Act – Section 313: Guidance for Reporting Toxic Chemicals: Polycyclic Aromatic Compounds Category, EPA 260-B-01-03. Washington, DC.

Amador-Muñoz O, Bazán-Torija S, Villa-Ferreira SA, Villalobos-Pietrini R, Bravo-Cabrera JL, Munive-Colín Z, et al. 2013. Opposing seasonal trends for polycyclic aromatic hydrocarbons and PM10: Health risk and sources in southwest Mexico City. Atmospheric Research 122: 199-212.

Valle-Hernandez BL, Mugica-Alvarez V, Salinas-Talavera E, Amador-Munoz O, Murillo-Tovar MA, Villalobos-Pietrini R, et al. 2010. Temporal variation of nitro-

polycyclic aromatic hydrocarbons in PM10 and PM2.5 collected in Northern Mexico City. The Science of the total environment 408(22): 5429-5438.

Jedrychowski W BI, Flak E, Penar A, Jacek R, Kaim I, Spengler JD, Camann D, Perera FP. 2004. Estimated Risk for Altered Fetal Growth Resulting from Exposure to Fine Particles during Pregnancy: An Epidemiologic Prospective Cohort Studyin Poland. Environmental health perspectives 112(14): 1398-1402.

Tang D LT-y, Liu JJ,Chen Y, Qu L, Perera F. 2006. PAH–DNA Adducts in Cord Blood and Fetal and Child Development in a Chinese Cohort. Environmental health perspectives 114(8).

Perera FP TD, Tu YH, Cruz LA, Borjas M, Bernert T, Whyatt RM. 2004. Biomarkers in Maternal and Newborn Blood Indicate Heightened Fetal Susceptibility to Procarcinogenic DNA Damage. Environmental health perspectives 112(10).