ABSTRACT

Background: Cardiovascular disease (CVD) and type 2 diabetes mellitus (DM) are major causes of mortality and disease burden among developing countries, yet maternal and child undernutrition are also still of great concern. The objective of this dissertation is to evaluate whether intrauterine growth, prenatal zinc, and the development of the fetal autonomic nervous system influence the risk of long-term health outcomes with emphasis on cardiometabolic disease.

Methods: Data of pregnant women taking part in a maternal zinc supplementation trial in Lima, Peru between 1998 and 2000, and a follow-up of their offspring when they were approximately 4.5 years of age were used. A total of 242 women were randomized to receiving an oral supplement containing 60 mg iron (ferrous sulfate) and 250 μg folic acid, with or without 25 mg of zinc (zinc sulfate) daily throughout pregnancy. The health and nutritional status of the mother were assessed at enrollment (10-16 weeks of gestation), and again at 28 and 36 weeks. Fetal growth and neurobehavioral development (heart rate and movement patterns) were evaluated using ultrasound and electronic fetal monitoring at 20, 24, 28, 32, 36, and 38 weeks, respectively. In 2003, follow-up assessments were conducted with 184 children born during this trial, at the age of 4.5 years, to evaluate the effect of prenatal zinc on health, nutritional and developmental outcomes, including cardiorespiratory monitoring. In 2011, markers of cardiometabolic risk (lipid profile and insulin resistance) were measured in cryopreserved plasma samples collected from the children as part of the follow-up.

Results: At 4.5 years, on average children were shorter (height for age Z-score = -0.85, SD = 0.79) and heavier (BMI for age Z-score = 0.77, SD = 0.95) than children of their same sex and
age, when using the WHO growth standards. After adjusting for prenatal zinc supplement, gestational age at delivery and selected maternal characteristics, estimated fetal weight (EFW) was positively associated ($P<0.05$) with child height, weight, head circumference, fat mass index, sum of 4 skinfolds, and waist circumference in males and females. Relations between EFW and child BMI and fat-free mass index were also positive, but were statistically significant only among females. EFW was inversely associated with the ratio of subscapular/triceps skinfolds, but this association was statistically significant only among males. All associations tend to be strongest at 32 weeks and effect sizes correspond generally to moderate relationships (~0.3 SD).

In general, associations of EFW with measures of child size were stronger for males, whereas associations between EFW and measures of child body composition were stronger for females. Similar associations were observed for weight at birth. The direction of the associations between individual fetal proportions (head circumference – HC, abdominal circumference – AC, or femur diaphysis length – FL) and measures of child size and body composition were similar. Prenatal zinc supplementation was not associated with an increased risk of the metabolic syndrome or any of its individual components at 4.5 years. Fetal heart rate (FHR) was inversely associated with child heart period (HP, the distance in milliseconds between two heart beats), all measures of cardiac variability, and vagal tone ($V$, a measure of parasympathetic control) at 4.5 years. Two measures of cardiac variability in the fetal period, the range of HR, and the number of episodic accelerations of heart rate, were also significantly associated with childhood measures of heart period and variability.

**Conclusions:** Overall, the findings of the present study support the hypothesis that intrauterine growth and development have an association with anthropometric and cardiometabolic parameters in early childhood that are known to be precursors of cardiometabolic disease later in adulthood; however, zinc supplementation received by the pregnant mother did not appear to influence the presence of cardiometabolic conditions in early childhood.
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Thesis readers: Dr. Kerry Schulze, Dr. Janet DiPietro, and Dr. Eliseo Guallar
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started working with him and two years later I started my doctoral studies at Johns Hopkins. Claudio has been a great mentor and friend, and has been instrumental in shaping my professional and personal life in many ways; I look forward to continue working with him to improve the public health of our country.

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Last but not least, I would like to thank the women and children who participated in this study. The time and information they provided made this work possible.

I would like to dedicate this work to my children Piero and Ivana. I hope they find it inspiring in the future.
ABBREVIATIONS

AC = abdominal circumference
ADA = American Diabetes Association
ANS = autonomic nervous system
ATP = adenosine triphosphate
BMI = body mass index
bpm = beats per minute
CHDS = Child Health and Development Study
CI = confidence interval
cm = centimeters
COHORTS = Consortium of Health-Oriented Research in Transitioning Societies
CRED = crecimiento y desarrollo (growth and development)
CRL = crown-rump length
CVD = cardiovascular disease
DALY = disability adjusted year
DBP = diastolic blood pressure
DM = type 2 diabetes mellitus
DNA = deoxyribonucleic acid
DOHaD = developmental origins of health and disease
EAR = estimated average requirement
ECG = electrocardiography
EFM = electronic fetal monitoring
EFW = estimated fetal weight
FFM = fat-free mass
FFMI = fat-free mass index
FHR = fetal heart rate
FL = femur diaphysis length
FM = fat mass
FMI = fat mass index
g = grams
GH = growth hormone
HC = head circumference
HDL = high-density lipoprotein
hGH-V = human growth hormone variant
HOMA-IR = homeostasis model assessment for insulin resistance
HP = heart period
HPA = hypothalamic-pituitary-adrenal (axis)
HP-SD = standard deviation of heart period
hPL = human placental lactogen
HR = heart rate
HR-SD = standard deviation of heart rate
HRV = heart rate variability
ICC = intra-class correlation coefficient
IGF = insulin-like growth factor
IIN = Instituto de Investigacion Nutricional (Nutrition Research Institute)
IUGR = intrauterine growth restriction
JHSPH = Johns Hopkins School of Public Health
Kcal = kilocalories
kg = kilograms
LDL = low-density lipoprotein
LMIC = low- and middle-income countries
MAP = mean arterial blood pressure
mg = milligrams
mm = millimeters
mmol/L = millimoles per liter
MMS = multiple micronutrient supplementation
ms = milliseconds
MSSD = mean square of successive differences
mU/L = milliunits per liter
µg = micrograms
NCD = non-communicable disease
NCPP = National Collaborative Perinatal Project
NHANES = National Health and Nutrition Examination Survey
PNS = parasympathetic nervous system
r = Pearson’s correlation coefficient
RR = relative risk
RSA = respiratory sinus arrhythmia
SBP = systolic blood pressure
SCL = skin conductance level
SD = standard deviation
SF = skinfold
SGA = small for gestational age
SNS = sympathetic nervous system
SS = subscapular (skinfold thickness)
TBW = total body water
Tri = triceps (skinfold thickness)
U.K. = United Kingdom
U.S. = United States
V = vagal tone
y = years
WC = waist circumference
WHO = World Health Organization
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CHAPTER 1: INTRODUCTION

Cardiovascular and metabolic diseases are important causes of morbidity and mortality worldwide; further increases in their burden are expected in the near future. It has been projected that for the year 2030, ischaemic heart disease, cerebrovascular disease, and type 2 diabetes mellitus (DM) will be the 1st, 2nd, and 7th top causes of death worldwide and 3rd, 6th, and 11th top causes of disease burden, respectively, and together will account for 27% of all deaths and 10% of disability adjusted life years (DALYs) globally (1). It is expected that most of the increase in disease burden of cardiometabolic diseases will occur in low- and middle-income countries.

Peru, like many other Latin-American countries, has experienced major economic, demographic, and epidemiological changes, over the past few decades. The increase in urbanization together with the economic growth observed in the region have been associated with a decrease in maternal and child mortality and in the incidence of communicable diseases, and an increase in life expectancy and in the incidence of non-communicable disease (2). At the country-level, 15.4% of all deaths are due to cardiovascular disease, and 1.7% from diabetes mellitus (3). The presence of cardiometabolic risk factors in the adult population in Peru is relatively high. It has been estimated that 14.5% of the adult population in Peru has high blood pressure, 7.4% has raised blood sugar, 20.2% has dyslipidemia, 20.4% is obese, and 41.9% of the population is considered to have a sedentary lifestyle (4, 5).

Traditionally, the effect of concurrent lifestyle and behavioral factors influencing the development of chronic diseases has been the most studied; however, over the past few decades, the role of early-life exposures on the development of later disease has gained increased attention. The importance of stimuli taking place during the prenatal period and infancy affecting adult disease was first emphasized by Professor David Barker and colleagues in the 1980s, when they showed a geographic correlation between neonatal and infant mortality, and ischaemic heart disease mortality rates in the United Kingdom (6). Many studies have been published since then.
that show an inverse association between size at birth and later hypertension, insulin resistance, and hyperlipidemia, among other chronic conditions (7). These findings have helped to put together the so called “Developmental Origins of Health and Disease (DOHaD) hypothesis”. The current working model of DOHaD maintains that, besides genetic and lifestyle factors, certain environmental factors acting in utero and in the early postnatal period alter the development of the organism through developmental plasticity and possibly epigenetic modification, affecting its capacity to cope with environmental influences throughout life (8).

Nutrition across the life course is crucial in the development of cardiometabolic diseases, with either overnutrition or undernutrition increasing an individual’s risk of developing these diseases, depending on the timing at which the nutritional status is measured. Undernutrition in the prenatal period and infancy and excess adiposity in late childhood and/or adulthood have both been associated with an elevated risk of developing cardiometabolic conditions, with the highest risk being observed among individuals in whom both are experienced (9, 10). Although cardiometabolic diseases have traditionally been considered pathologies of the most affluent populations, they are increasing in importance in populations from the developing world in whom maternal and child undernutrition often coexist with adult overnutrition, and, therefore, in whom early life programming may have its greatest effects (11, 12). Yet, most of the evidence in this field has been generated from studies conducted in populations from developed countries. Very few studies have been conducted in developing countries; therefore, the effect of differences in the nutritional environment throughout the life cycle among the most disadvantaged populations is less clear, making it difficult to develop clear recommendations, at the public health level for chronic disease prevention.

Within the context of programming studies, nutrition during the prenatal period is of particular interest. Nutrient supply to the fetus is considered a major regulator of fetal survival, growth and development, and reduced nutrient availability to the fetus leads to permanent structural and metabolic changes that can continue later in life (13). Characterizing the nutritional
fetal environment is challenging. Most studies in this area use size at birth as a proxy indicator of the fetal nutritional environment, although it is widely accepted that it is an imperfect measure. Size at birth is dependent not only on the nutritional environment but also on the innate growth potential of the individual, and fetal development can be affected without affecting fetal size, and insults at different points of time in pregnancy can affect fetal growth and development in different ways. Due to methodological constraints, very few studies include measures of the intrauterine nutritional environment, or measures to characterize fetal growth and development (14, 15).

Both macronutrient and micronutrient deficiencies during pregnancy have been linked to an increased risk for cardiometabolic conditions, among other chronic diseases, later in life (15-17). Although it is widely accepted that an adverse intrauterine environment affects later risk of disease, the mechanisms through which it operates are still not completely understood. There is evidence suggesting that homeostatic systems mediating stress can be affected, predisposing individuals to long-term disease. The hypothalamic-pituitary-adrenal (HPA) axis has been the most widely studied (18). Less studied is the autonomic nervous system (ANS) which coordinates automated organ functions critical during homeostasis and in response to environmental stimuli. The development of the ANS begins in the prenatal period and is affected by the intrauterine nutritional environment.

For several reasons it seems plausible to hypothesize that an adverse prenatal nutritional environment – specifically zinc status – can “program” the structure and function of the developing ANS which, in turn, can result in an increased risk for cardiometabolic conditions later in life. First, it is known that development of the ANS starts during the prenatal period and remains moderately stable into the postnatal period, and that imbalances in the autonomic nervous function in adulthood are associated with negative health outcomes, including an increased mortality from cardiometabolic disease and all major risk factors influencing its development (19-22). Second, studies in animals show that zinc deficiency during pregnancy results in altered
growth and development of nervous system structures and in an adverse cardiometabolic profile in the offspring (decreased number and size of nephrons, glomerular filtration rate, lean body mass and insulin response to glucose, and increased systolic blood pressure and body fat) (23-26). Finally, prenatal zinc supplementation in humans is associated with both improved fetal autonomic control (which continues into the childhood period) and a reduction in the risk of microalbuminuria and peripheral adiposity in childhood; all suggestive of an improved cardiometabolic profile (27-30).

**Goal**

The overall goal of this project was to evaluate whether intrauterine growth, prenatal zinc, and the development of the fetal ANS influence the risk of long-term health outcomes, with emphasis in cardiometabolic disease. Data were used from pregnant mothers participating in a prenatal zinc supplementation trial conducted in Lima, Peru, and from a follow-up conducted when children born during the trial were approximately 4.5 years of age. To characterize intrauterine growth and autonomic control, we used fetal biometric measures obtained by ultrasound and intrauterine measures of fetal heart rate (FHR), respectively. To assess the cardiometabolic profile of children, we used anthropometric measures of body size and composition, blood pressure, lipid profile, insulin sensitivity, and cardiac measures of autonomic control. Although the direct health effects of differences in the cardiometabolic profile in young children are not well understood, it is known that these differences continue to emerge into adulthood, and, therefore, can be considered surrogates of potential differences in cardiometabolic risk later in life (31). **Figure 1.1** shows the conceptual framework used for the development of this study.

The specific aims and hypotheses of the study are:

**Specific Aim 1:** To evaluate whether fetal size and growth in the second half of gestation are associated with body size and composition at 4.5 years of age
**Hypothesis 1:** Greater fetal size and faster growth between 20 and 36 weeks of gestation (measured using head circumference – HC, abdominal circumference – AC, femur diaphysis length – FL, and estimated fetal weight – EFW) will be positively associated with overall body size and lean mass, and inversely associated with adiposity at 4.5 years of age.

**Specific Aim 2:** To examine the effect of supplementation during pregnancy with iron, folic acid, and zinc compared to iron and folic acid as the control on the cardiometabolic profile of children at 4.5 years of age.

**Hypothesis 2:** Children of mothers who receive the supplement containing zinc will have lower risk of cardiometabolic disease (lower adiposity, lower blood pressure, lower insulin resistance, and improved lipid profile) than those who receive the supplement without zinc.

**Specific Aim 3:** To assess the association between cardiac measures of autonomic control during the second half of pregnancy and at 4.5 years of age.

**Hypothesis 3:** Fetuses with higher baseline HR and lower heart rate variability (HRV) between 20 and 38 weeks of gestation will have lower heart period (HP, higher HR) and lower HRV at 4.5 years of age.

The rationale, results, and conclusions of this study are presented in different chapters, as described below.

Chapter 2, is a review of the literature for this study, focusing on describing selected factors influencing fetal growth and development and their long-term effects with emphasis on cardiometabolic risk. Then described are the current working model of DOHaD and the challenges of conducting studies in this area of research. Also described is the development of the fetal ANS, as is its role in the development of cardiometabolic diseases in the context of programming. Finally, previous findings from studies conducted by researchers involved in the
current study are summarized to support the conceptual and methodological approach of this dissertation.

Chapter 3 corresponds to the overview of the study design of the prenatal zinc supplementation trial and the follow-up conducted approximately 5 years later. This chapter also describes the new measurements conducted to address the aims of this study.

In Chapters 4, 5, and 6 results are presented corresponding to each of the aims of this study. Each of these articles includes an abstract, introduction, methods, results, and discussion section. In Chapter 4, the association between fetal size and growth during the second half of pregnancy and body size and composition at 4.5 years are evaluated. In Chapter 5, the effect of prenatal zinc supplementation on the cardiometabolic profile at 4.5 years is investigated. Finally, in Chapter 6, whether the individual differences in cardiac measures of autonomic control in the second half of gestation continue to 4.5 years of age is assessed.

In Chapter 7, key findings of this study are summarized, followed by a discussion of the methodological strengths and limitations, and the public health significance of the results, and the implications for the directions of future research in this area are presented.
Figure 1.1 Hypothesized associations of the long-term influence of fetal growth and the development of the autonomic nervous system on the risk of cardiometabolic disease
REFERENCES


CHAPTER 2: REVIEW OF THE LITERATURE


Non-communicable diseases (NCDs) are leading causes of global disease and deaths and are, therefore, a major public health problem. In 2008, there were 57 million deaths in the world, of which 63% or 36 million were due to NCDs (1). This was initially perceived as a problem exclusive of developed countries, but it is now recognized as a problem affecting developing societies as well. In fact, in 2005, 80% of the estimated 35 million deaths caused by NCDs occurred in low and middle income countries (2). Of particular interest are cardiovascular and metabolic diseases. According to the World Health Organization, in 2012, the two major types of cardiovascular diseases (CVD), ischaemic heart disease and cerebrovascular disease, together accounted for approximately 25% of all deaths and 11% of disability adjusted life years (DALYs), globally (1). Type 2 diabetes mellitus is also an important contributor to disease worldwide. In 2014, it was estimated that 9% of adults in the world had diabetes and in 2012, it was estimated that 1.5 million deaths were directly caused by diabetes (3). Projections for the year 2030 suggest that the global burden of cardiovascular and metabolic disease will continue to increase in importance (1). In Peru, 52% of all deaths are from non-communicable diseases; of those, one third is due to cardiovascular disease or diabetes (4).

The World Health Organization (WHO) has identified six factors associated with NCDs as the leading global risk factors for death: high blood pressure, high glucose levels, excess weight, high cholesterol levels, physical inactivity, and tobacco use (5). All six are well-known factors associated with increased risk of cardiometabolic diseases, and the first four can be programmed in utero (6-9). Experiences during critical periods of growth and development, including those occurring during the fetal period, permanently affect how the organism responds to environmental exposures later in life, influencing its long-term morbidity and mortality risk.
1. Determinants of human fetal growth and development

Achieving optimal fetal growth and development is an important goal to improve perinatal survival and short- and long-term health. Intrauterine growth restriction (IUGR) is associated with adverse fetal and neonatal outcomes, such as fetal distress, fetal death, birth asphyxia, neonatal morbidity and mortality, poor nutrition and immune function (10-12). Further, poor fetal growth has been associated with negative long-term outcomes such as adult stunting (and its associated consequences – poor schooling, decreased economic productivity, adverse maternal outcomes) and increased risk for certain chronic diseases (10, 13). Although alterations in fetal development can take place without necessarily affecting fetal growth, they commonly coexist. Fetal growth and development represent the result of the interplay of genetic inheritance with the fetal environment, and are influenced by the interaction between the mother, the fetus, and the placenta (14). The human pregnancy can be divided into two somewhat defined stages that represent a continuum: 1) early pregnancy, which corresponds to the first trimester, when embryogenesis, placental development, and organogenesis take place, and 2) late pregnancy, which includes the remaining two trimesters, when most fetal growth occurs (15).

An adequate supply of energy and nutrients is the major environmental regulator of fetal growth and development (16). Proper fetal growth and development can be compromised due to poor maternal nutrition and metabolism, maternal disease, or altered placental function. They are all critical for adequate delivery and utilization of oxygen and nutrients by the fetus. The growth tempo of the human fetus is relatively homogenous up to around 16 weeks of gestation, after which fetal growth differences become evident (17). This observation is considered to be explained by the increased influence of genetic factors early in pregnancy and the subsequent increase in importance of the fetal environment for optimal growth, but also because over 90% of fetal growth occurs in the second half of pregnancy (15). Fetal development begins early in life, with each organ and system having a specific timing for growth and cell differentiation. As a result, environmental insults acting during the prenatal period will differentially impact fetal
growth, development, and long-term organ function, depending on the period during gestation when they occur, and the type, severity, and duration of the insult. The identification of these environmental factors and a better understanding of their functional impact can be useful for developing interventions aimed at achieving optimal fetal growth and development, and promoting long-term health.

1.1 Genetic determinants of fetal growth

Genetic abnormalities can cause deformations in the developing fetus and alter fetal growth; however, these are infrequent and, therefore, are an uncommon cause of fetal growth retardation. Of greater importance is the effect environmental factors have on the expression of genes involved in fetal growth. There is a wealth of evidence supporting the role of epigenetics in the regulation of fetal growth and development, and also long-term health outcomes (18). The term epigenetics refers to processes by which chemical compounds are added to the genome which regulates gene expression without affecting the gene sequence. Of particular interest are imprinted genes, a subset (<1%) of the human genome affected by epigenetic processes (19).

Genomic imprinting is characterized by an epigenetic modification process affecting genomic expression selectively, according to the parental origin of the allele, typically caused by methylation of cytosine bases in particular DNA regions known as imprinting control regions (20). These imprints are established in the germ line, remain as cells divide during development, and can be inherited through generations. It has been postulated that the conflict between maternal and paternal genome in the way maternal resources are allocated to the developing embryo is the evolutionary explanation of imprinting (21). It is believed that imprinted genes affect the growth, morphology, and transfer capacity of the placenta, controlling the nutrient supply to the fetus and, hence, affecting fetal growth (20). Of particular interest is the imprinting of the Igf2 gene which has been directly involved in modulating placental development, placental nutrient transfer capacity, and embryonic growth; all of which affect intrauterine growth (20). It is...
hypothesized that the maternal genome limits the supply of nutrients to the offspring as a way to save resources for future pregnancies, whereas the paternal genome promotes nutrient supply to the fetus as a way to benefit its offspring at the expense of potential future offspring from a different paternal genomic makeup (22, 23). Therefore, fetal growth can be seen as the result of the conflict between maternal imprinted genes tending to inhibit fetal growth and paternal imprinted genes having a tendency to favor fetal growth.

1.2 Nutritional environment and prenatal growth

The prenatal nutritional environment is a main regulator of fetal growth, development, and survival. Sufficient availability of energy and an adequate and balanced supply of macro- and micro-nutrients are required to meet fetal nutritional needs (24). Maternal nutritional and metabolic status prior to conception and during pregnancy, together with placental function, determine, to a great extent, nutrient availability to the fetus and its metabolic and endocrine status, therefore influencing fetal growth and development, perinatal survival, and well-being (16). To be able to sustain pregnancy and meet increased energy requirements, several behavioral and physiologic adjustments occur in the mother to transition her metabolism into an anabolic state. The basal metabolic rate is reduced, and there is an increase in food consumption and a decrease in physical activity (25). In addition, adjustment in nutrient metabolism takes place. There is an increase in the absorption and deposition of specific nutrients, and nutrient redistribution among maternal tissues takes place (26). This is achieved by modifying either the absorption or excretion of nutrients as a response to hormonal changes, fetal requirements, and maternal supply. There is, however, a restricted capacity to adjust nutrient metabolism; therefore, if surpassed, fetal growth and development will be affected. Because particular nutrients are involved in the growth and development of each of the different fetal organs and systems, deficiency of a specific nutrient will affect the development, growth, and long-term function of the unique organ systems in which the specific nutrient is involved. Further, because fetal growth
and the development of the various organs occur at distinct points of time during gestation, impairment of fetal growth and organ function will be dependent on the timing at which nutritional insults occur.

The source of nutrition for the fetus is dependent on the stage of pregnancy. In early pregnancy, nutrition is histiotrophic, with the main nutritional sources coming from the phagocytosis of oviductal and uterine carbohydrate-rich secretions by the trophoblast (27). Once the placental circulation is established as a result of the physical apposition between maternal and fetal tissues, there is a gradual progression to the hemotrophic pathway for nutrition which will remain for the remainder of pregnancy. In the human fetus, there is evidence of this transition occurring between 6-10 weeks of gestation (28). Initially, lactate and pyruvate are the main energy substrates to the fetus. With progression of pregnancy, glucose becomes the main energy source for the developing fetus (27).

**Maternal nutritional history**

Maternal height is considered a measure of past nutritional exposure. Maternal stunting can limit blood flow and growth of the uteroplacental organs and the fetus. Maternal height at gestation is directly associated with placental size and birth weight (29). Maternal stunting is also associated with increased risk of perinatal mortality, due, in part, to the disparity in size between the head of the fetus and the pelvic size of the mother resulting in the extreme with obstructed labor and asphyxia at birth (30). Maternal pre-pregnancy weight also influences fetal growth. It has been estimated that there is an increase of 9.5 g in birth weight per each kg increase in maternal pre-pregnancy weight, and an 84% increase in the risk of IUGR associated with low maternal pre-pregnancy weight (<49.5 kg) (30).

**Maternal weight gain during pregnancy**
Although specific recommendations for weight gain have changed over the years, there is agreement as to the usefulness of gestational weight gain as an indicator of energy sufficiency during pregnancy (31). Observational studies have shown a consistent association between poor maternal weight gain and IUGR (32-34). It is likely that the timing of weight gain during pregnancy (and possibly changes in body composition) have different effects on fetal growth, although results are mixed. Based on a combined analysis of two studies conducted in the U.S., it has been estimated that, in women with a normal pre-pregnancy body mass index (BMI), for each kilogram (kg) gained throughout pregnancy (total gestational weight) there is an average increase in weight at birth of 20 grams (30). It is possible that these estimates vary according to the timing at which gestational weight gain is measured. A study conducted in Guatemala estimated that for each kilogram of maternal weight gain in the second trimester, there was a mean increase in weight at birth of 62 g (p<0.001), whereas in the third trimester it was 26 g (p<0.05) (35). In this same study, a positive association was seen between maternal weight gain in the second trimester of pregnancy only and length (0.24 cm/kg, p<0.01) and head circumference at birth (0.14 cm/kg, p<0.001). These results are in agreement with another study also conducted in Guatemala, but on a younger generation, which found that maternal weight gain from the first to the second trimester of pregnancy was positively associated with fetal femur and tibia lengths at 17 and 30 weeks of gestation, and with length at birth; none of these associations were modified by maternal pre-pregnancy weight or height (33). In contrast with these findings, a study conducted in Malawi showed that a decreased rate of maternal weight gain in late pregnancy was associated with shorter length at birth (36). However, in this study no significant associations were observed between gestational weight gain from the second to the third trimester of pregnancy and fetal linear growth or length at birth. Less information is available on the effect of weight gain on fetal growth in the first trimester of pregnancy. An analysis of over 10,000 U.S. women taking part in the National Collaborative Perinatal Project (NCPP) and the Child Health and Development Study (CHDS) reported no association between low gestational weight gain in the first trimester
and intrauterine growth retardation (IUGR <2500 g) (32). However, in agreement with previously mentioned studies, an association between low gestational weight gain in the second or third trimester and increased risk of IUGR was reported; the strength of the associations was similar for both trimesters within each cohort, but stronger in the CHDS (NCPP cohort – second trimester RR: 1.8, 95%CI: 1.3, 2.6 and third trimester RR: 1.7, 95%CI: 1.3, 2.3. CHDS cohort – second trimester RR: 2.6, 95%CI: 1.6, 4.1 and third trimester RR: 2.5, 95%CI: 1.7, 3.8). A study conducted in Bangladesh reported a positive association between maternal weight and fat-free mass at 10 weeks of gestation and gains between 20-32 weeks of gestation, and higher placental and birth weight (p ≤ 0.01) (29). Maternal weight gain from 10-20 weeks of gestation was also associated with birth weight (p <0.05).

**Maternal energy and macronutrient intake**

Adenosine triphosphate (ATP) is the main form of energy source for human metabolism; in fetal life it is required for a wide variety of fetal biological processes including tissue synthesis, cellular transportation, and maintenance of physiologic and metabolic processes (37). Maternal dietary macronutrients constitute energy substrates for fetal growth and development, and serve as molecules (or precursors) for physiologic functions to adequately operate. Dietary macronutrients are transferred from the mother to the fetus through the placenta, and in general, there is very limited fetal endogenous production. Because the majority of macronutrients that become available to the fetus come from maternal sources, it is expected that maternal diet influences fetal growth and development. Glucose is the main energy substrate during fetal life, but also serves as a cofactor for enzymes involved in metabolic processes (37). Most fetal glucose is the product of metabolism of maternal dietary carbohydrates, although some glucose is generated from maternal non-carbohydrate sources, such as amino acids, glycerol, or glycogen (38). Amino acids serve as building blocks for proteins, are precursors for the synthesis of DNA, hormones, and neurotransmitters, and are involved in the regulation process of various metabolic
pathways (39). The majority of the amino acid supply to the fetus is from maternal dietary sources, yet some nutritionally nonessential amino acids can be synthesized from those nutritionally essential, provided adequate maternal supply (40). Fetal lipids can be synthesized from glucose and amino acids in fetal tissues, but the majority are transferred from the mother through the placenta (41). Maternal dietary triglycerides are hydrolyzed in the placenta into fatty acids and glycerol, in which form they can be permeable to the placental barrier. The main role of lipids in the fetal period is to contribute to energy storage in fetal adipose tissue, particularly in the last stage of pregnancy (41). They also serve as building blocks for phospholipids and therefore, have an important role in building and maintaining cellular membranes, and are precursors of molecules involved in immunological responses such as prostaglandins and leukotrienes (41, 42).

Achieving appropriate maternal energy intake and intake of specific nutrients is essential for meeting maternal and fetal nutritional requirements and, therefore, sustaining a healthy pregnancy (31). Maternal energy and nutrient intake will likely impact intrauterine growth, and because growth and development of specific fetal organs and systems occur during specific periods in gestation, it is possible that it is dependent on the timing during pregnancy when dietary intake is measured.

Some of what is known about the effect of differences in maternal nutrition on fetal growth at varying stages of pregnancy has arisen from the study of the Dutch famine occurring in the 1940s (43). The Dutch famine is considered a natural experiment of restricted prenatal nutrition in a population which was previously well-nourished. In 1944, as a result of Germans restricting food transportation and an extremely severe winter, there was a brief shortage of food supply in the Netherlands. Food rations for the general adult population decreased from an average of 1800 kcals in December 1943 to 1400 kcals in October 1944, to under 1000 kcals in November 1944, and to 400-800 kcals in April 1945. By June 1945, rations had risen to over 2000 kcals. Despite this period of hunger, women continued getting pregnant and delivering
babies. Because obstetric care was provided to pregnant women and detailed records were kept throughout pregnancy (including size and health of babies at birth), researchers had the ability to study the effect of restricted maternal nutrition at different stages of pregnancy on the offspring. In addition, because birth records were kept, researchers were able to locate individuals who were exposed to the famine in utero, when they were around 50 years of age, which allowed them to evaluate the effects of famine on long-term health outcomes. Fetuses exposed to famine early in pregnancy did not have lower birth weights, whereas those exposed in late pregnancy had lower birth weights (44, 45).

Although some studies have found no association between maternal macronutrient intake and intrauterine growth, others have found a positive association for protein intake and an inverse association with carbohydrate and fat intake (34, 46-48). An observational study conducted in ~500 pregnant women in the United Kingdom found that placental and birth weights were inversely associated with maternal carbohydrate intake in early pregnancy and directly associated with maternal protein intake in late pregnancy (47). In contrast, another observational study conducted in ~550 women in Australia found that placental and birth weights were directly associated with the proportion of energy derived from protein in early pregnancy and inversely associated with the proportion of energy derived from carbohydrates in early and late pregnancy (48).

Nutrition interventions manipulating maternal dietary composition look promising in promoting intrauterine growth, especially those using food supplements or fortified foods. Based on a systematic review which included 6 trials and over 4000 pregnant women, mainly from developing countries, it was estimated that balanced protein-energy supplementation (supplements in which protein provides less than 25% of the total energy content) reduces the risk of being small for gestational age by 30% (49). A second systematic review, including 12 trials conducted in populations from both developed and developing countries, found that food and fortified food products were effective in increasing birth weight by an average of 125 grams and
reducing the incidence of low birth weight (<2500 g) by 27% (50). A subanalysis of studies focused on interventions altering the macronutrient composition (including balanced protein-energy supplementation) were found to be effective in increasing birth weight (~72 g) and birth length (~0.2 cm), and reducing the incidence of low birth weight by 24%; the size of the effects was greater in studies conducted in undernourished (or nutritionally at-risk) and low-income populations. No significant effects of food supplementation have been reported on other neonatal or infant outcomes.

Maternal micronutrient status

Several micronutrients affect fetal growth and development, and micronutrient deficiencies have been associated with adverse pregnancy and birth outcomes (51, 52). Minerals and vitamins are required in humans in small amounts and for very specialized functions. Except for vitamin D and niacin, they cannot be synthesized by human tissues, and hence need to be provided in the diet. In the fetus, micronutrients become available from maternal sources; therefore, the effect of maternal micronutrient status on pregnancy and infant outcomes has been of particular interest. Observational studies have shown associations between almost every micronutrient deficiency in pregnant women and fetal growth restriction or some other adverse pregnancy outcome, yet results from interventional studies are less consistent (51). Following, the roles of folate, iron, and zinc are described. Other micronutrients, such as vitamin A, vitamins B6 and B12, iodine, vitamin D and calcium are also considered essential for normal fetal human growth and development, but are not included in this review.

Folate

Folate is critical for the developing fetus. It is required for the synthesis of DNA bases, amino acids, and erythrocytes (53, 54). It is also essential for cellular division, tissue growth, DNA methylation, and the development of immune and neurological systems (54). Folate
deficiency is associated with a decrease in the concentration of folate in serum and erythrocytes, and an increase in serum homocysteine (53). During pregnancy, folate requirements increase to support fetal and placental growth. Folate insufficiency during pregnancy has been shown to be associated with an increased risk of developing megaloblastic anemia, pre-eclampsia, intrauterine growth retardation, and neural tube defects in the offspring (55-58). Periconceptional folic acid supplementation has been shown to be effective in improving pregnancy outcomes, and, therefore, is widely recommended (59). Based on a systematic review, including five randomized clinical trials, it has been estimated that periconceptional supplementation with folic acid reduces the risk of development of neural tube defects by 72% (RR: 0.28, 95%CI: 0.15, 0.52) and results in a 68% reduction in recurrence (RR: 0.32, 95%CI: 0.17, 0.60) (56). A second review including 31 trials (mainly from developed countries) estimated that prenatal supplementation with folic acid was associated with an average reduction in the incidence of megaloblastic anemia of 79% (RR: 0.21, 95%CI: 0.11, 0.38) and a mean increase in birth weight of 136 g (95%CI: 47.85, 223.68) (60). No conclusive evidence exists of other pregnancy benefits of prenatal folic acid supplementation (59).

Iron

Iron is a constituent of many proteins, particularly those involved in the metabolism and transport of oxygen (61). During pregnancy, iron requirements increase to expand the number of erythrocytes, and to allow the growth of the placenta and fetus (62). To compensate for the increased iron requirements during pregnancy, iron absorption progressively increases throughout pregnancy (62). In addition to this compensatory mechanism, sufficient iron stores during the preconception period and pregnancy, and adequate maternal iron intakes during pregnancy, are also essential for the fetus to have an adequate iron supply (63). Maternal iron deficiency during pregnancy can result in anemia, hypoxia, suboptimal fetal growth, and possibly preterm birth (64, 65). Prenatal supplementation of iron (alone or in conjunction with other micronutrients) has been
shown to be effective in improving pregnancy outcomes. A systematic review, including 43 randomized clinical trials and over 27,000 women, estimated that prenatal iron supplementation was associated with a 19% reduction (RR: 0.81, 95%CI: 0.68-0.97) in low birth weight (<2500 g), an average increase of 20g in weight at birth (30.81, 95%CI: 5.94, 55.68), a 70% reduction of maternal anemia at term (RR: 0.30, 95%CI: 0.19, 0.46), and a 57% reduction in iron deficiency at term (RR: 0.43, 95%CI: 0.27, 0.66) (66). In this same systematic review, women receiving iron supplements prenatally were less likely to deliver premature infants, although the association did not reach statistical significance (RR: 0.88, 95%CI: 0.77, 1.01).

Zinc

Zinc is a transition metal and a component of over 200 enzymes. Zinc-dependent enzymatic systems exert more than 20 distinct biological functions, including structural, catalytic, and regulatory (67). In the human, there is no known storage site for zinc; therefore, zinc metabolism and status change rapidly in response to variations in dietary intake or requirements (68). Similar to previously mentioned micronutrients, zinc requirements increase during pregnancy, mainly because of fetal zinc requirements (69).

Although the role of folate and iron and the effects of those micronutrient deficiencies in pregnancy and infant outcomes have been widely studied, the importance of zinc during gestation is less well-known (70). Zinc has been implicated in fetal growth and the development of the fetal neurological and immune function (71). Understanding these associations is relevant because it has been estimated that 17% of the world’s population is at risk of zinc deficiency and that 82% of pregnant women in the world have insufficient zinc intakes and, therefore, a large proportion of women and their offspring can potentially suffer from any adverse health effects associated with zinc deficiency (72, 73).

For several reasons it is plausible to argue the essentiality of zinc for normal human fetal growth: 1) the gene expression of insulin growth factor 1 (IGF-1) and growth hormone (GH)
receptors – both hormones being involved in the fetal growth process – is regulated by zinc (74); 2) several zinc transporters are expressed in the placenta, suggestive of maternal-fetal zinc exchange (75); 3) studies conducted in pregnant women with acrodermatitis enteropathica – a rare condition resulting from a defect in a zinc transporter – have shown an association between severe zinc deficiency and pregnancy complications (76); 4) studies manipulating dietary zinc intake in animals indicate that mild and moderate zinc deficiency during pregnancy can cause intrauterine growth retardation (77, 78); and 5) observational studies have reported an association between poor maternal zinc status and impaired fetal growth and development of the immune and neurological function (71).

Despite the evidence presented above, suggesting biological plausibility of the role of zinc on fetal growth, interventional studies evaluating the effect of prenatal zinc supplementation on pregnancy and infant outcomes in humans are not conclusive. A systematic review and meta-analysis of 17 randomized clinical trials found no significant effect of prenatal zinc supplementation on weight at birth, in general, or after stratifying by zinc status (79). Another review including 20 trials evaluated the effect of prenatal zinc supplementation on pregnancy and infant outcomes (80). The only significant effect of prenatal zinc supplements was a 14% reduction in preterm birth (RR: 0.86, 95%CI: 0.76, 0.97). No significant effect of prenatal zinc supplementation was observed for other maternal or infant outcomes, including low birth weight. It has been hypothesized that the discrepancy between results coming from observational studies and those from clinical trials is related to the presence of confounding or a decreased bioavailability of the supplement (79). It is possible that the effect of prenatal zinc on fetal growth is modest or is specific to certain body proportions and weight at birth is not able to capture those differences; a prenatal zinc supplementation trial conducted in Peru showed a positive effect in fetal femur diaphysis length starting at 24 weeks of gestation, but no significant effects on length or weight at birth (81).
There is evidence supporting the role of zinc in fetal neural development. Studies in animals show that zinc deficiency early in pregnancy results in an increased risk of congenital malformations of the nervous system and zinc deficiency in later pregnancy can alter growth and development of neurons and the synaptic cleft (82). An observational study conducted in Egyptian pregnant women showed a positive association between maternal intakes of bioavailable zinc and neonatal habituation behavior, a sign of infant neural development (83). In addition, 2 separate trials in Peru have found that prenatal zinc supplementation is associated with a decrease in fetal heart rate (FHR) and an increase in fetal heart rate variability (HRV), both suggestive of improvement in fetal neurobehavioral development (84, 85).

Due to the lack of conclusive evidence, to date prenatal zinc supplementation is not a recommended intervention to improve maternal and child health outcomes. Yet it has been suggested that more studies are needed (86).

Maternal multiple micronutrient supplementation

Most of the studies linking maternal micronutrient status and fetal growth and development are focused on single micronutrients (51). The factors that increase the risk of malnutrition in developing countries are common for most micronutrient deficiencies; therefore, deficiencies of more than one micronutrient usually coexist in these populations. Due to increased requirements, micronutrient deficiencies are exacerbated during pregnancy (26). Maternal multiple micronutrient supplementation (MMS, supplements containing 3+ micronutrients) is seen as an attractive strategy to improve pregnancy and birth outcomes. Based on current available evidence, it has been suggested that prenatal iron-folate supplements be replaced by MMS (86). A systematic review including 21 clinical trials and over 75,000 women from developed and developing countries, found that MMS was associated with an 11% reduction in low birth weight infants (RR: 0.89, 95%CI: 0.83, 0.94) and a 13% reduction in small-for-gestational age infants (RR: 0.87, 95%CI: 0.81, 0.95) (87). No significant associations were
found with other maternal and pregnancy outcomes, including adverse effects on maternal
mortality, stillbirths, or perinatal mortality. A recent study conducted in rural Bangladesh,
confirmed the lack of effect of maternal MMS on infant mortality at 6 months (RR: 0.95, 95%CI:
0.86, 1.06) and in the reduction in low birth weight (RR: 0.88, 95%CI: 0.85, 0.91); however, also
reported a reduction in stillbirths (RR: 0.89, 95%CI: 0.81, 0.99) and in preterm births (RR: 0.85,
95%CI: 0.80, 0.91) (88).

1.3 Placental function and fetal growth

The placenta is a metabolically active organ that connects the maternal uterine wall with
the developing fetus. It serves as the site for exchange of gases, nutrients and waste substances
between mother and fetus, and produces placental hormones to maintain pregnancy; therefore,
adequate placental growth and function are critical for proper fetal growth and development.

Trophoblast invasion and placental vascular changes

The placenta begins its development with the implantation of the blastocyst into the
maternal uterine endometrium; the most external layer of the blastocyst becomes the trophoblast.
Adequate trophoblast invasion is required for proper fetal growth and development. During the
first 10-12 weeks of gestation, the trophoblast induces changes in the uterine spiral arteries to
adapt for the delivery of the increased blood supply required during the last two trimesters of
pregnancy. Spiral arteries become elongated and trophoblasts invade their wall cells, causing a
replacement of their muscular and elastic walls by fibrinoid layer cells (89, 90). Trophoblasts also
form plugs to occlude spiral arteries, preventing maternal blood from entering the placenta and,
hence, creating a physiological hypoxic state in the placenta and fetus (91). During the first
trimester of pregnancy, the placenta and fetus need to remain in a hypoxic state because there is
limited protection against oxidative damage given that antioxidative enzymes are not expressed
until later in pregnancy (8-9 weeks of gestation) (92). Near the end of the first trimester, the
trophoblast plugs loosen, and by the end of the first trimester, the maternoplacental circulation is established (93). Between 8-10 weeks of pregnancy, the partial pressure of oxygen in the placenta has been estimated to be about half that of the endometrium, and, at around weeks 12-13, they even out (93). Although the mechanisms behind these processes are not fully understood, it has been hypothesized that trophoblasts sense oxygen pressure through pathways utilizing transcription factors which promote gene expression resulting in trophoblast survival and differentiation (94).

Alterations in factors associated with oxidative stress or oxygen sensing pathways likely affect trophoblast differentiation and function and, hence, affect fetal survival, growth, and development. Associations between altered spiral arteries remodeling, altered uteroplacental perfusion, and intrauterine growth restriction have been reported (95). Incomplete physiological conversion of spiral arteries is associated with decreased blood flow to the placenta in later gestation and possibly with poor plugging of vessels and early onset of maternal blood flow (91). Once the maternal arterial circulation is activated, the concentration of oxygen in the placenta is tripled (93, 96). An early onset of maternal circulation can expose the developing fetus to oxidative stress and affect the development of the placenta, due to excessive loss of placental villous tissue (96). Utero-placental malperfusion secondary to impaired spiral artery conversion is an important cause of IUGR, especially in developed countries (27). Placental development follows different trajectories in healthy pregnancies and those with fetal growth retardation. In pregnancies with IUGR the placenta is typically smaller at 12 weeks of gestation, but has a normal growth rate thereafter, unless an additional condition, such as pre-eclampsia, affects the pregnancy later on (97, 98).

With pregnancy progression, an increased uterine blood flow becomes essential to meet the metabolic demands of the uteroplacental organs and the growing fetus; there is an estimated three-fold increase in the blood flow of the uterine artery and new blood vessels develop in the uterus (99, 100). Reduced uteroplacental blood flow during the last two trimesters of pregnancy
can result in oxygen and nutrient deprivation to the fetus, which can lead to fetal growth restriction. The uteroplacental blood flow is reduced in pre-eclampsia and in high-altitude pregnancies; both situations create hypoxic states and are well-established risk factors for fetal growth restriction (101).

**Transport of nutrients**

Around half of the oxygen and glucose reaching the fetus is delivered by the uterine circulation (102). The remaining nutrients are transported across the placenta by passive (oxygen, carbon dioxide, urea, fatty acids) or facilitated (glucose and lactate) diffusion, active transport (amino acids, fatty acids), endocytosis, or exocytosis (14). Alterations in the transport capacity and diffusion will affect delivery of gases and nutrients, and have been associated with fetal growth restriction (14).

**1.4 Hormonal regulation of growth in utero**

The mother, the fetus, and the placenta are all hormonally active and work in synchrony with each other to regulate fetal growth. A few weeks after the human placenta is formed, it starts producing hormones which are, in part, responsible for the physiological changes in the mother in preparation for pregnancy (and later on lactation). Hormones also regulate the signaling between the mother and the fetus for proper exchange of nutrients and waste products, and, therefore, play an important role in fetal growth (14).

During gestation, the human placenta secretes several hormones which are involved in fetal growth regulation, including human growth hormone variant (hGH-V), human placental lactogen (hPL), and insulin-like growth factors (IGFs). Contrary to postnatal growth, GH is not the main regulator of growth in utero. GH receptors are expressed at low levels in fetal tissues and are increased postpartum (103). The somatotrophic axis seems to have the major regulating role in fetal growth (104). IGF-2 is the main hormone influencing embryonic growth and IGF-1 is
the dominant growth factor in later gestation. When IGF-2 binds to the IGF-1 receptor, it promotes growth, while binding to the IGF-2 receptor (considered a clearance receptor) reduces circulating IGF-2 (17). IGF-1 is affected by maternal nutrition, with maternal undernutrition being associated with reduced IGF-1 concentrations and alterations in IGF binding proteins (105). Fetal insulin is the primary promoter of fetal IGF-1 (106).

Human PL and GH-V modulate maternal metabolism to increase availability of nutrients to the fetus and stimulate the production of maternal and fetal IGFs, and fetal insulin, thus promoting fetal growth (14). A study assessing the association between serial serum hPL and serial ultrasound examinations during pregnancy found that the rate of change of hPL was positively correlated with an increase in growth velocity \(r = 0.34\) and birth weight \(r = 0.32\) (107). It is possible that this association is mediated by the production of insulin and IGF-I, both growth-promoting hormones (108). In the human pregnancy, the secretion of maternal growth hormone (GH) by the pituitary gland is inhibited and a variant secreted by the placenta (hGH-V) becomes the predominant source of GH in the mother. Human GH-V promotes growth of maternal tissues and induces maternal insulin resistance, facilitating the mobilization of nutrients from the mother to the fetus by stimulating maternal lipolysis and gluconeogenesis (109, 110).

1.5 Other factors influencing fetal growth

Along with the previously described major factors affecting fetal growth and development, additional maternal, constitutional, behavioral, and socioeconomic factors exist that can influence the developing fetus (30). Studies have consistently reported sex-differences in fetal growth; on average, male infants are born heavier and taller than their female counterparts. Based on a review of 66 studies, it was estimated that males are born 93.1-126.4 g heavier than their female counterparts, and that females had a 19% increased risk of being born IUGR (RR: 1.19) as compared to males (31). International standards for newborn size have recently been developed as part of INTERGROWTH-21\textsuperscript{st}, a population-based, multiethnic, and multi-country project that
assessed fetal growth and newborn size and which included over 20,000 women (111, 112). These international standards confirm previously reported sex-differences in fetal growth; after taking gestational age into account, males tend to be born heavier and taller, and with a larger head circumference than females (112).

Maternal age is known to affect fetal growth. Adolescent women are at increased risk of developing adverse maternal and fetal outcomes, including intrauterine growth restriction (113, 114). The mechanism proposed for the observed association is direct competition between the (still growing) mother and the developing fetus for the available nutrients (115).

Increased exposure to glucocorticoids during the fetal period has also been associated with intrauterine growth retardation and detrimental effects in fetal brain development (116). Up to a certain point, the placenta protects the fetus from maternal glucocorticoid exposure by the action of 11β-hydroxysteroid dehydrogenase, which inactivates glucocorticoids (117). However, excessive levels of glucocorticoids can by-pass the placental barrier. Numerous studies in animals have shown an inverse association between maternal serum cortisol concentrations (or manipulations to increase maternal glucocorticoid levels) in pregnancy and birth weight of the offspring (118). Similarly, studies in humans have also shown this inverse association quite consistently (119). Interestingly, most studies showing a relationship between maternal cortisol levels and fetal growth have shown associations with cortisol measurements in early- and mid-gestation only (120). It is possible that the fetus is more susceptible to changes in glucocorticoids earlier in gestation, when the hypothalamic-pituitary-adrenal axis is not fully developed. It has been posited that this could be the mechanism by which maternal psychological stress and extreme maternal malnutrition increase the risk of fetal growth retardation (121).

Other maternal factors that have been shown to impair fetal growth and development are poor maternal socioeconomic and education status, maternal inflammatory disease during pregnancy (i.e. malaria, genitourinary tract infections), pre-eclampsia, primiparity, and maternal smoking, among others (30).
2. Early life exposures and long-term cardiometabolic risk

Over two decades ago, Barker and colleagues emphasized the importance of environmental factors acting in the prenatal and early postnatal periods on the risk of long-term disease. They reported a geographical association between infant mortality rates in 1921-25 and ischaemic heart disease mortality rates in 1968-78 in the U.K., and suggested that childhood influences the risk of adult heart disease (122). A few years later, the same working group reported an inverse association between birth weight and death from ischaemic heart disease and impaired glucose tolerance (7, 123). In an attempt to explain these observations, they put together the “thrifty phenotype hypothesis”, which proposed that poor nutrition in early life affects the development of certain organs and tissues with long-lasting functional consequences (124).

Since the thrifty phenotype hypothesis was first proposed, a large number of studies have supported the epidemiological associations that brought it forward, and together with additional evidence from observational and experimental studies, it evolved into the concept of the “developmental origins of health and disease” (DOHaD). The DOHaD paradigm proposed that environmental factors acting in the fetal and early childhood periods alter the trajectory of the organism through developmental plasticity and/or epigenetic modification, and affect the manner and capacity of the organism to cope with environmental influences throughout life (125). Due to the overwhelming evidence supporting this theory, DOHaD is now considered accepted biology (126).

2.1 Prenatal nutrition and long-term cardiometabolic outcomes

It is now widely accepted that the risk for developing cardiovascular and metabolic diseases in adulthood can have its origins before birth. Gestation and early infancy are periods of great susceptibility to programming – a phenomenon occurring when environmental exposures during a sensitive period of development can result in changes in the structure of organ systems in the organism, with subsequent alterations in long-term physiological function. Although some
environmental chemicals have been implicated in the programming of disease, nutritional exposures are of most interest (127). Nutritional insults during the prenatal period can have permanent effects on the cardiometabolic function of an individual that are not evident at birth and act independently from (or interact with) other risk factors present later in life. The fetus responds to these nutritional insults with variations in hormonal production or tissue-specific hormonal sensitivity and metabolic changes, affecting the normal development of certain hormonal systems and organs. These changes have been called fetal adaptive responses and are thought to be responsible for the increased risk of cardiometabolic conditions observed among individuals suffering from nutritional deprivation during early life (128).

Although fetal growth per se is not responsible for the programming of long-term metabolic function, the great majority of DOHaD studies rely on anthropometric measures of body size (usually weight at birth) to reflect the fetal nutritional environment. There are numerous reports of epidemiologic studies showing an inverse association between birth weight and an increased susceptibility to later coronary heart disease and type 2 diabetes, or risk factors for those diseases, such as high blood pressure, dyslipidemia, and insulin resistance (129). Fewer studies have reported similar associations between placental size or biometric measures of intrauterine growth and early markers of cardiometabolic risk (130, 131). There are also increasing numbers of animal studies evaluating the effect of differences in energy and macronutrient balance, which have replicated many of the associations linking maternal undernutrition with cardiovascular and metabolic outcomes observed in epidemiological studies. Maternal restriction of protein-caloric dietary intake during pregnancy has been associated with a reduction in the offspring size, increased blood pressure, and glucose intolerance (132, 133). Findings from natural experiments on exposure to famine also support the theory that prenatal nutrition restriction affects the long-term cardiometabolic risk of individuals, and that it is dependent on the timing of exposure during gestation. When compared to their unexposed counterparts, those exposed, in early gestation, to nutritional restriction imposed by famine had a
more atherogenic lipid profile, higher incidence of coronary heart disease, greater stress
responsiveness, and more obesity; those exposed in mid-gestation had an increased risk of
microalbuminuria; and those exposed at any stage of pregnancy had an increased risk of
developing glucose intolerance in adulthood (43). It has also been postulated that maternal
micronutrient status during pregnancy affects fetal growth, organogenesis, and differentiation,
leading to differences in cardiometabolic functional outcomes throughout life (134). Roles for
vitamin A, vitamin B12, folate, iron, and zinc in the development of renal, cardiovascular, and
pancreatic function, as well as in aspects of body composition, have been described (134).

Potential role of zinc in the development of long-term cardiovascular and metabolic function

Global recognition of the importance of zinc deficiency during pregnancy has expanded
over recent years, due to its association with adverse pregnancy and birth outcomes (135). Half of
the world’s population is estimated to be at risk for low zinc intake, and approximately one-third
of the population is zinc deficient (73, 136). The global prevalence of zinc deficiency among
pregnant women is unknown, but an estimated 82% of women worldwide do not consume the
recommended intake of zinc during pregnancy (72). In most low- or middle-income countries, the
average zinc intake of pregnant women is below the Estimated Average Requirement (EAR)
(137). Maternal zinc deficiency has been associated with preterm delivery, prolonged labor,
premature rupture of membranes, post-partum hemorrhage, and fetal growth restriction (72). The
long-term effects of inadequate zinc status during pregnancy have been less explored. Based on
the available evidence, it seems plausible to hypothesize that zinc is involved in the pathogenesis
of cardiovascular and metabolic diseases. First, animal studies have demonstrated that prenatal
zinc restriction is associated with decreased 1) number and size of nephrons, 2) glomerular
filtration rate, 3) lean body mass, and, 4) insulin response to glucose, and with increased 1)
systolic blood pressure, and, 2) body fat (138, 139). Second, observational studies in humans
have shown that lower serum zinc concentrations and lower consumption of dietary zinc are
associated with an increased prevalence of hypertension, hypertriglyceridemia, coronary artery
disease, and diabetes (140, 141). Third, results from interventional studies suggest a beneficial
effect of zinc in glucose control and lipid metabolism, but mainly among adults with conditions
known to influence zinc metabolism (142-144). Fourth, prenatal zinc supplementation has been
associated with changes in heart rate (HR) measures consistent with greater parasympathetic
control of cardiac function during the fetal period which continue into childhood up to 4.5 years
of age, and also with a reduction in the risk of microalbuminuria and peripheral adiposity at 8
years of age (84, 85, 145-147).

2.2 Methodological challenges in DOHaD studies

Despite active research on the developmental origins of health and disease, for more than
two decades, there are still methodological limitations in the design, conduct, and analysis of
DOHaD studies that leave the details controversial.

One important challenge is to provide accurate measures of the fetal nutritional
environment, which is hypothesized to be the main determinant of the changes going on in the
developing fetus (16). Most human studies are limited to using fetal size as a proxy indicator of
fetal environment. Even though size at birth correlates well with the nutritional fetal environment
and provides information about the size of the fetus at the culmination of the prenatal period, it is
limited in characterizing intrauterine growth trajectories or in identifying critical periods of fetal
growth and development where nutritional insults could result in the programming of postnatal
conditions. The appropriateness of the use of size at birth when studying the DOHaD has been
questioned for: 1) the DOHaD concept is based on fetal responses to its environment, not on the
size of the child at birth (125); 2) size at birth is dependent not only on the adequacy of the pre-
natal environmental but also on the innate individual growth potential (23); 3) fetal development
might be impaired without affecting size at birth (43); and 4) insults at different stages of
pregnancy can affect fetal growth and development in different ways, and size at birth may not be
able to distinguish among them (45). With increased use of ultrasound examination as part of routine antenatal care monitoring – mainly in developed countries – it has been possible to evaluate the associations between intrauterine measures of fetal growth and certain nutritional and health outcomes later in life. A recent systematic review including 29 studies summarized these findings (130). A total of 20 studies had follow-up during infancy, 5 in childhood, 3 in adolescence, and 1 in adulthood. This review indicated: 1) an inverse association between estimated fetal weight (EFW) and adiposity in childhood evidenced as early as mid-pregnancy; 2) positive associations between the growth of abdominal circumference (AC), femur diaphysis length (FL), and EFW, and postnatal bone size, which strengthens with advancing gestational age; 3) a positive association between FL and length/height in infancy, which was apparent as early as the first trimester; 4) a positive association between mean abdominal diameter in the third trimester and childhood BMI; 5) an inverse association between fetal growth and systolic blood pressure up to adolescence; and, 6) an inverse association between EFW in the third trimester and thickening of the aortic abdominal wall as early as 18 months of age. Based on the studies included in this review, the association between fetal growth and glucose metabolism or lipid profiles was inconclusive. Although this review included a total of 26 studies, which were classified to be on average of high methodological quality, the number of studies for specific outcomes was very limited. Other limitations identified as part of this review were that most studies included fetal measures at one or two points of time during the third trimester only. To better understand the relationship between the fetal nutritional environment and its long-term health consequences, it is important to more thoroughly characterize fetal growth, using measures of fetal dimensions earlier in pregnancy, as well as including measures at more points of time throughout gestation. This would allow for more complex modelling of intrauterine growth and could help identify whether any specific periods during gestation are critical for programming later body size and composition.
Another challenge in DOHaD studies is to characterize fetal organ and system development. This type of study can enhance our insight of fetal adaptation to its environment, especially if measurements are taken throughout pregnancy. Several human studies have focused on assessing the effects of IUGR on organ development, but very few have been conducted within the context of DOHaD. Changes in the cardiovascular system have been the most studied in the human. Fetal cardiovascular status is usually assessed with fetal ultrasound, and although it is technically challenging, it has shown that IUGR fetuses have a lower systolic function, as well as lower left and right ventricular diastolic filling, when compared to normal fetuses (148).

Functioning of some other organs or systems is difficult to evaluate in utero; however, other alternative approaches, such as studying stillbirths or measuring postnatal markers can be used. Post-mortem analysis of kidneys from stillbirths has shown that IUGR fetuses have a reduced number of nephrons compared to that non-IUGR fetuses (148, 149).

Another equally important factor to take into account is that DOHaD focuses on long-term disease risk. This can be challenging because studies of stable cohorts are needed, with participants followed from before birth until late adulthood when chronic outcomes will present. Also, multiple measures are needed throughout the life course, ideally with very few missing data or loss to follow-up. Evidently, not only the logistics in terms of time and avoiding missing data are complicated, but also securing funding for such a long period. As a result, most studies in this area use surrogate end-points of disease as outcomes, and rely on previously recorded data at birth to characterize exposure. Very few studies have used CVD morbidity or mortality as the outcome measure. One of the most representative studies which used a hard outcome is a longitudinal study conducted in Helsinki in which death and hospitalizations for coronary heart disease and type 2 diabetes were outcomes (150). However, the study was limited to use of size at birth as the summary measure for the quality of the fetal environment.

There is one additional issue, related mainly to the setting or population studied, that needs to be overcome in new DOHaD studies. Most of the research conducted in this area is in
populations from developed countries. It is particularly relevant to study these associations among populations from developing countries, who are exposed to greater nutritional stress, and who therefore are more likely to suffer from prenatal growth impairment and in which transition to a westernized lifestyle is becoming an increasing concern. A few years ago, available long-term data from 5 large cohorts from low- and middle-income countries was brought together with the aim of studying the long-term consequences of maternal and child undernutrition; this collaborative network has been named COHORTS (Consortium of Health-Oriented Research in Transitioning Societies) (151). Much of the recent evidence in DOHaD from developing societies has been generated by the COHORTS group. Among the most important findings of special relevance for developing countries was the reported association between higher birth weight and faster linear growth, and reduced risk of short adult stature and of not completing secondary school (152). Although higher birth weight, faster linear growth, and faster weight gain were all associated with an increased risk of becoming overweight (BMI>25 kg/m2), the increased BMI was mainly attributed to a higher lean mass. Similarly, faster linear growth and weight gain were both associated with a slightly increased risk of developing elevated blood pressure. Higher birth weight (but not gains in height or weight) was inversely associated with dysglycaemia. The reductions on short stature, of not completing secondary school, and of dysglycaemia outweighed the risks of adverse health outcomes. Further analyses have revealed that the greater weight gain at any age is positively related to an elevated blood pressure in adulthood; these results have been interpreted to mean that childhood weight gain is expected to increase the risk of high blood pressure to the extent that it predicts adult body size (153).

3. The neurovisceral approach to understanding cardiometabolic risk

A wide range of factors have been identified which increase the risk of CVD. Environmental factors have been widely studied because they are potentially modifiable. Among them, smoking, certain dietary patterns, and decreased physical activity are the most
representative. Some other factors such as dyslipidemia, high blood pressure, and glucose impairment, have also gained interest because they are early markers of cardiometabolic disease. These factors can be present early in childhood, and if they are not addressed, they tend to persist later in life and increase the risk of chronic diseases (154). High BMI in childhood predicts high BMI and increased risk of hypertension later in life (155, 156), serum lipoprotein concentrations track from childhood into early adulthood (157), and BMI and serum lipoprotein concentrations during childhood predict carotid intima media thickness – a predictive measure of clinical coronary atherosclerosis in young adults (158). These factors are interrelated and usually coexist in the same individual (154); therefore, it is essential to investigate unified models that could explain this clustering. Under this logic, based on the extensive evidence from animal and human studies, showing that neural activity modulates cardiovascular and metabolic function, a neurovisceral model is proposed as an approach to better understand the etiology of cardiometabolic outcomes (159).

The autonomic nervous system (ANS) is the part of the nervous system responsible for internal allostasis; it has 2 divisions: 1) the parasympathetic nervous system (PNS); and, 2) the sympathetic nervous system (SNS). The PNS, via acetylcholine release, promotes anabolic functions related to day-to-day living, such as food digestion and absorption. The SNS is activated under certain environmental stimuli and prepares the individual to cope with emergency situations, via the release of norepinephrine. In a physiological state both branches are in a dynamic balance. If one dominates the other, the organism enters autonomic imbalance – a state which has been associated with disease (160). The importance of the ANS in health and disease has been known for many years; however, its role in the etiology of cardiometabolic diseases has very recently gained interest because it is still not completely understood. There is ample evidence, generated mainly from observational studies in adults, suggesting that a predominance of sympathetic over parasympathetic activity is associated with all major risk factors for CVD, including but not limited to obesity, high blood pressure, type 2 diabetes, and dyslipidemia (159).
More importantly, this predominance has also been associated with all-cause mortality, including that related to CVD (159).

Assessing the balance between the sympathetic and parasympathetic branches of the ANS, or autonomic control, is not easy due to the variety of systems the ANS controls and the difficulty of measuring visceral effects. Markers of autonomic control related to cardiac function are the most widely used because they can be measured noninvasively and at a low cost. Among them, baseline HR and HRV are the most widely used (161).

The heart is innervated by both the SNS and PNS. At rest, it is mainly controlled by the inhibitory effects of the PNS. Under certain environmental stressors, the SNS will increase its activity and overcome the parasympathetic inhibition (161). By modulating its parasympathetic and sympathetic dominances, the ANS has multiple effects in cardiac physiology. It controls the rate of depolarization in the sinoatrial node (chronotropic effect), the velocity of the spread of action potentials through the heart (dromotropic effect), and the intensity of cardiac myocyte contractility (inotropic effect). Measurement of HR and its variation are good indicators of the chronotropic effect of the ANS on the heart. Baseline HR is the simplest cardiac measure of autonomic control and corresponds to the number of beats per minute at which the heart completes one cardiac cycle at rest. It is considered a key determinant of myocardial oxygen consumption, and, therefore, it is an indirect estimate of the rate of myocardial energy use (162). Even though several factors, such as temperature, hypoxia, or exercise can affect the intrinsic HR (or HR without any autonomic effects), the ANS is the main determinant of the actual baseline HR that is routinely measured (163). Evidence is consistent in showing that baseline HR has a positive linear association with all-cause mortality, including that related to CVD (157). HRV measures the oscillations in the intervals between two heart beats (164). The use of HRV as a cardiac measure of AC relies upon the fact that variations in HR occur constantly in response to physiological perturbations, and static levels in the HR are a manifestation of lack of adaptability to environmental influences (165). Both HR and HRV can also be measured under physical or
psychological stress, and can give further insight into how the body copes with stress.

Independently of the markers of autonomic function used, most studies in this area have shown that predominance of sympathetic over parasympathetic control is associated with an increased risk of CVD in adults. Specifically, several studies have shown that high resting HR, and decreased HRV are associated with hypertension, type 2 diabetes, increased total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations, low high-density lipoprotein (HDL) cholesterol concentrations, and obesity (159).

3.1 The autonomic nervous system within the context of the Developmental Origins of Health and Disease (DOHaD)

Although it is now widely accepted that a nutritionally adverse fetal environment increases the risk for cardiovascular and metabolic diseases later in life, the mechanisms by which this takes place remain controversial. It has been proposed that homeostatic systems mediating stress can be affected by early nutritional insults, predisposing individuals to long-term disease. The hypothalamic-pituitary-adrenal (HPA) axis has been the most widely studied, and it is known that its development can be affected by an adverse prenatal environment (166). Fewer studies have focused on assessing the impact of an impaired development of the fetal autonomic nervous system; however, their results consistently suggest that it is affected under a nutritionally restricted prenatal environment. Low birth weight newborns have higher HR and lower HRV as infants (167), and higher resting pulse rate as adults (168). A prenatal zinc supplementation trial showed that fetuses of zinc-supplemented mothers have lower HR, greater HRV and more HR accelerations than their non-zinc-supplemented counterparts, and these differences continue into childhood (85, 145).

The development of the ANS begins during the fetal period and continues later in life, with neural cellular proliferation occurring early in the prenatal period, and axonal growth and dendritic arborization continuing well after birth. The period between 28 and 32 weeks gestation
seems to be critical for the development of the fetal ANS. During this period, there is an increase in the duration of FHR accelerations as well as the highest peak of HRV (169, 170). At about 32 weeks, the fetus has completed the majority of its neural development, and by the end of gestation, the neurodevelopment of the fetus is almost identical to that of the newborn infant. Although environmental influences occurring during critical periods of ANS development can cause functional alterations, which may persist later in life with long-lasting consequences (171), stability of cardiac measures of autonomic control from the prenatal to postnatal period has been previously documented (172-175). There is evidence of autonomic control tracking from the prenatal (as early as 20 weeks gestation) to the postnatal period. Three previous studies have shown stability of HRV from the prenatal into the postnatal period up to 1 y of age (172-174), and one of them also reported an inverse association between fetal HRV and HR at 1 year (172). These studies have all been conducted in U.S. children. No studies of this type have been conducted in populations from developing countries. Because the level and trajectories of HR patterns during gestation, and the neurobehavioral development during infancy, are known to differ between populations from different geographical, ethnic, and cultural backgrounds, it is of importance to understand if these associations generalize to populations from developing countries exposed to greater environmental disadvantages than those from developed countries (176-178).

In view of the facts presented above, it seems plausible to hypothesize that prenatal nutrition is crucial for the development of the fetal ANS, that differences in fetal autonomic control persist into the postnatal period, and that these differences can be translated into differences on cardiometabolic risk.

4. Previous findings
4.1 Effect of maternal zinc supplementation trials on fetal growth and neurobehavioral development in Peru

Dr Caulfield and collaborators have been investigating the effect of zinc on fetal growth and neurobehavioral development for several years. An initial randomized clinical trial was conducted in a periurban area of Lima, Peru, to examine the effect of maternal zinc supplementation on maternal and perinatal outcomes (179). A total of 1295 pregnant women were randomized (by parity and week of gestation) between gestation weeks 10 and 24, to receive a daily prenatal supplement containing 60 mg iron (ferrous sulfate) and 250 μg of folic acid, with or without 15 mg of zinc (zinc sulfate). Although maternal and neonatal zinc concentrations remained lower than those reported in pregnant women from well-nourished populations, maternal zinc supplementation was associated with an increase in maternal serum (0.21-0.23 SD) and urinary zinc concentrations (0.16-0.21 SD) at 28-30 and 37-38 weeks of gestation and cord zinc concentrations (0.23-0.27 SD) in the neonate (180). No effect of maternal zinc supplementation was observed on the duration of pregnancy or size and body composition of the infant at birth (weight, head circumference, crown-heel length, chest circumference, mid-upper arm circumference, calf circumference, or their skinfold thickness) (179). To evaluate the effect of maternal zinc supplementation on fetal neurobehavioral outcomes, a subsample of mothers participating in this trial was selected (from now on called the pilot study). Fetal neurobehavioral development was assessed at 32 and 36 weeks gestation by means of electronic fetal monitoring (EFM), assessing HR, and fetal movement patterns. With advancing gestational age, fetuses from zinc-supplemented mothers showed an increase in the variability of their HR and in the number of movement bouts and time spent moving; both findings are consistent with an improvement in fetal neurobehavioral development according to normal developmental trends (84, 181).

To further understand the effect of zinc on fetal development, a second maternal zinc supplementation trial was conducted in the same study area (from now on called the pregnancy
study) (81, 85). A total of 242 women were randomized (by parity and week of gestation) to receive 60 mg iron (ferrous sulphate) and 250 μg of folic acid, with or without 25 mg of zinc (zinc sulfate). Several methodological improvements were made in this second trial. First, because serum and urinary concentrations of zinc in women from the previous study were below those reported for women with adequate zinc intakes (even after being supplemented throughout pregnancy), the level of supplemental zinc in the intervention group was increased from 15 to 25 mg. Second, recruitment and zinc supplementation started earlier in pregnancy (from 10-24 to 10-16 weeks gestation), which, together with an increased level of zinc in the supplements, allowed for a greater time of exposure to zinc supplementation. Third, ultrasound examinations and EFM were conducted at 20, 24, 28, 32, 36, and 38 weeks of gestation, which allowed for the assessment of intrauterine growth in a prospective and more accurate manner and improved the assessment of fetal movement and HR parameters during the second half of pregnancy. Fourth, sophisticated EFM hardware and software which were developed by one of the study investigators – Dr. Janet DiPietro – were used, which allowed them to then derive additional and more accurate measures of fetal movements and HR patterns (GESTATE, James Long Company, Caroga Lake, NY). The methodology used to collect and analyze fetal movement and HR data was similar to that used by Dr DiPietro in a study being conducted at the same time in Baltimore, Maryland, US. This permitted a comparison of the neurodevelopment of two large cohorts of fetuses developing in two distinct environments (176).

In this second study, data from 195 of the 242 subjects initially enrolled in the study were used in the final analysis. Results from this study confirmed findings from the pilot study regarding greater variability in their HR, but not those related to fetal movements (85). Further, the inclusion of measures of fetal movement and HR at various points in time allowed observing for the first time the declining trend of baseline FHR and increasing trend in HRV, with the evolution of pregnancy (figure 2.1 and figure 2.2). Both were steeper in fetuses from zinc supplemented mothers. These results suggest an increase in parasympathetic control with the
evolution of pregnancy, stronger with higher maternal zinc intakes. Fetal baseline HR was consistently lower in the zinc-supplemented group compared to the non-zinc-supplemented group throughout pregnancy (figure 2.1). Differences in fetal HRV and the number of accelerations became evident in the zinc-supplemented group starting at 28 weeks of gestation and progressing after that (figure 2.2 and figure 2.3). Results from previous studies suggest that the period between 28 and 30 weeks of gestation is critical for many components of neural development. Between 28 and 30 weeks of gestation, there is an increase in the duration of FHR accelerations as well as the highest peak in pregnancy of HRV (167, 168). These findings suggest that during this period there is a switch in autonomic control from the medulla oblongata to the cortex (176).

In addition to the evaluations of fetal movement and HR patterns, an autonomic assessment in the mother – using measurements on the cardiorespiratory status and electrodermal activity— were also conducted at 20, 24, 28, 32, 36, and 38 weeks of gestation. Anthropometric measures of maternal size and body composition (weight, height, and the circumference and skinfold thickness of upper and lower extremities), and the concentration of hemoglobin, hematocrit, and plasma zinc were also evaluated at enrollment (10-16 weeks of gestation), and at 28 and 36 weeks of gestation. Analyses of these data showed that maternal baseline HR and mean arterial blood pressure (MAP) increased with pregnancy progression, whereas respiratory sinus arrhythmia (RSA), skin conductance level (SCL), and respiratory rate progressively decreased with gestation (182). Although no effect of zinc supplementation on any of the measures of autonomic control were observed in the mother, a low hemoglobin concentration (<10.5 g/dL) at 28 weeks of gestation was associated with higher HR and respiratory frequency, and lower RSA, and the change in hemoglobin from enrollment to week 36 of gestation was associated with a lower SCL. In addition, RSA was inversely associated with changes in calf muscle throughout gestation, and changes in calf fat area were directly associated with MAP and SCL. Lower HR and MAP suggest greater parasympathetic control. RSA is a cardiorespiratory phenomenon resulting from the interaction of the cardiovascular and respiratory systems; higher RSA implies a
greater fluctuation in the HR in association with variations in respiratory frequency, and is suggestive of an increased parasympathetic control of the heart (181). SCL is derived from sweat gland activity and is commonly used as an index of sympathetic activity (182). These findings indicate a withdrawal of both sympathetic and parasympathetic influences with pregnancy progression and that these changes are associated with the nutritional status of the mother.

Results from fetal biometric measures assessed by ultrasonography revealed a positive effect of maternal zinc supplementation on fetal growth (81). Femur diaphysis length was greater in fetuses of mothers receiving zinc-containing supplements starting in mid-pregnancy, and the difference increased with progression of pregnancy. At 38 weeks of gestation, the effect of zinc supplementation on fetal femur diaphysis length was estimated to represent ~0.25 SD of the reference. No significant effect of zinc was noted on other fetal biometric measures (head circumference, biparietal diameter, and abdominal circumference), or on length of pregnancy or size at birth (weight, length, and head circumference). These findings suggest that maternal zinc status could have an essential role in fetal bone growth.

Because results from the pregnancy study indicated a positive effect of maternal zinc supplementation on fetal growth and neurobehavioral outcomes, a follow-up study was conducted to examine if these associations continued into childhood (from now on called the follow-up study) (145). The 195 participants included in the formal analysis, and 10 of the 27 excluded participants (who completed the protocol and whose babies survived the neonatal period and were free of congenital malformations) were identified for follow up. A total of 184 children (90%) were located and participated in the follow-up study at ~4.5 years of age (54 months). Due to incomplete electrocardiogram data, the final analytic sample was 165 children (79 children of zinc-supplemented mothers and 86 on non-zinc-supplemented mothers). Children of mothers receiving the zinc-containing supplements were significantly better-off regarding cardiac measures of autonomic control (145). Compared with children from mothers receiving the supplement without zinc, children from zinc-supplemented mothers had significantly greater
heart period – HP (lower HR) and greater variability in the HP (higher standard deviation of the HP – HP-SD, higher mean square of successive differences – MSSD, wider range of the HP, and higher vagal tone – \( V \)). HP-SD is a time-independent measure of HRV, MSSD is a time-dependent measure of HRV, and \( V \) is a measure of parasympathetic control. These differences were present at rest and under mental challenges (\( p < 0.05 \) except for range of HP and \( V \) which neared significance, \( p = 0.08 \)). Further, children from zinc-supplemented mothers had lower systolic blood pressure, which also neared statistical significance (\( p < 0.10 \)). No differences according to supplement type were observed in any of the anthropometric measures of body size (weight, height, BMI).

Based on the positive effect of prenatal zinc supplementation on fetal neurobehavioral outcomes of the pregnancy study and previous findings indicating an association between fetal movement and HR patterns and developmental outcomes in childhood, the effect of prenatal zinc supplementation on the cognitive, social, and behavioral development of participants at age 4.5 years was also assessed. As part of the follow-up study, children underwent an evaluation of their cognitive, social, and behavioral development. Standardized tests of intelligence, language and number skills, representational ability, interpersonal understanding, and adaptive behavior and behavioral adjustment were administered to the children by a two Peruvian trained and standardized Peruvian psychologists. There was no significant improvement in any of the developmental outcomes associated with prenatal zinc supplementation (185).
Figure 2.1 Variations in fetal heart rate between 20 and 38 weeks of gestation, by supplement type

Figure 2.2 Variations in fetal heart rate variability between 20 and 38 weeks of gestation, by supplement type
4.2 Stability and continuity of cardiac measures of autonomic control from the prenatal to the postnatal period

Dr. DiPietro and collaborators have been studying the prenatal determinants of postnatal neural development for many years. They have conducted various studies assessing fetal neural development throughout pregnancy using EFM. Findings from these studies have provided evidence of neural organization during pregnancy and the stability and continuity of autonomic control from the prenatal into the postnatal period.

In a first study, EFM was used in 31 U.S. pregnant women with uncomplicated clinical courses at 20, 24, 28, 32, 36, and 38-39 weeks gestation to measure FHR and fetal movement patterns (186). This study showed that FHR decreased linearly from 20 weeks through term and that fetal HRV increased throughout gestation at a higher rate between 20 and 32 weeks (average increase of 5% per week) and at a lower rate from 32 weeks through term (average increase of 1.1% per week).
A second study was conducted to better understand the progression of prenatal neural development to the postnatal period (172). A total of 52 US women with normal pregnancies went over EFM monitoring at 24, 30, and 36 weeks gestation and FHR and fetal movement patterns were derived for each time point. Measures of HR were also conducted during the neonatal period (2 weeks after birth) and in infancy (1 year of age), to assess the stability of HR measures from the prenatal period into infancy. Results from this study showed the following: 1) stability of FHR and fetal HRV during the last half of gestation ($r = 0.30-0.72$); 2) stability between fetal and neonatal HR and HRV ($r > 0.27$); and, 3) consistency of fetal and infant (1 year) HR and HRV after adjusting for relevant maternal variables.

A third study was conducted to better understand the fetal trajectories of cardiac measures of autonomic control throughout pregnancy and how they may differ in dissimilar populations (176). A parallel study was implemented using the exact same data collection procedures in Baltimore, Maryland, U.S. and Lima, Peru (85). Data from the study in Peruvian women corresponds to the pilot study described in the previous sub-section. The main methodological improvements in this study were the increase in sample size and the inclusion of a culturally different study sample that will serve to externally validate their findings. Data from 137 U.S. and 101 Peruvian pregnant women with normal clinical courses showed the following: 1) similar trends in progression of FHR measures throughout pregnancy (decrease of HR and increase of HRV and number of HR accelerations, steeper between 28-32 weeks); and, 2) higher HRV, greater number of accelerations, and lower HR in U.S. fetuses when compared to Peruvian fetuses. Results from this study, regarding progression of HR measures, were concordant with those from the first study. Additionally, an obvious deceleration in the neural development of Peruvian fetuses was identified, as assessed by all HR measures derived. Similarities in HR and HRV trends have been attributed to increased parasympathetic control due to neural maturation with the transition of neural control from the medulla oblongata to the cortex (176). Differences in HR and HRV between the two populations of fetuses could not be explained by relevant
maternal and demographic characteristics. Although the possibility of ethnic differences in autonomic control cannot be ruled out, it is also possible that unmeasured confounders, such as maternal nutritional status (including micronutrient status), maternal stress, or utero-placental insufficiency, could be responsible for the differences observed.

Results from these studies confirm earlier observations on the trajectories of HR and HRV throughout pregnancy, and confirm earlier observations suggesting that between 28 and 32 weeks gestation critical changes take place with respect to the neural fetal development, with fetuses reaching a level of neural maturity that allows them to survive in the extrauterine environment from the neurological standpoint. Further, these studies support that the development of the autonomic nervous system has its origins during the fetal period and that prenatal individual differences in autonomic control continue in to the postnatal period.

5.3 Significance of preliminary results

Summarized here are the results from previous work conducted by researchers involved in this study, regarding the effect of prenatal zinc supplementation on fetal growth, as well as the autonomic function in the pregnant mother, the fetus, and at a follow-up in childhood. We have also summarized results related to the trajectories of cardiac measures of autonomic control during the fetal period and its continuity into early childhood. Results from these studies suggest that the development of the autonomic nervous system begins during the prenatal period, that prenatal zinc status seems to influence fetal growth and the development of the fetal autonomic nervous system, and that the zinc effect on autonomic control continues into the postnatal period. The long-term effects of differences in fetal autonomic control or prenatal zinc supplementation on long-term health outcomes still need to be elucidated. In adulthood, zinc status, as well as an autonomic profile characterized by predominance of the sympathetic system, has been associated with incidence of cardiometabolic diseases and their associated risk factors (138, 139, 157). The overall goal of the present study was to evaluate whether intrauterine growth, prenatal zinc, and
the development of the fetal autonomic nervous system influence the risk of long-term health outcomes, with emphasis in cardiometabolic disease. Specifically evaluated are the association between fetal growth and body size and composition in childhood, the effect of maternal zinc supplementation during pregnancy on cardiometabolic risk in childhood, and the association between cardiac measures of autonomic control during the fetal period and corresponding measures in childhood.
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CHAPTER 3: STUDY OVERVIEW AND METHODS

1. Study overview

To address the aims of this study, data were used from pregnant women participating in a maternal zinc supplementation trial (previously referred as the pregnancy study), which took place in Lima, Peru, and a follow-up (previously referred as the follow-up study) of their offspring when they were approximately 4.5 years of age. Results from these studies have helped to understand the role of prenatal zinc in fetal growth and development of the autonomic nervous system. In the present study, the effects of intrauterine growth, prenatal zinc, and the development of the fetal autonomic nervous system on the risk of long-term health outcomes, with emphasis in cardiometabolic disease (from now on referred as the cardiometabolic programming study), are studied.

2. The pregnancy study

2.1 Study design and participants

Between 1998 and 2000, a prenatal zinc supplementation trial was conducted in periurban Lima, Peru. This was a collaborative study between Johns Hopkins Bloomberg School of Public Health (JHSPH) and the Instituto de Investigacion Nutricional (IIN) in Lima, Peru (1, 2). It was conducted in Villa el Salvador, a large urban shantytown in Lima, Peru, with a population of more than 300,000 inhabitants. A study clinic was built inside the Hospital Materno Infantil San Jose, a maternal-child hospital that takes care of low-risk pregnancies. Women were recruited at the prenatal care clinic in this hospital. There, the study protocol was explained and informed written consent was obtained. Women were eligible for the study if they were carrying a singleton fetus, considered low risk (eligible for vaginal delivery), receiving care at the Hospital...
Materno Infantil San Jose, residents of Villa el Salvador, and had been living in coastal Peru for a minimum of 6 months before becoming pregnant. Individuals were enrolled between 10 and 16 weeks of gestation; duration of pregnancy was determined at enrollment by date of last menses and verified by ultrasonography. The average woman recruited (during pregnancy) was ~ 23 years of age, had an average BMI of ~ 23.5 kg/m², was pregnant with her first (~58%) or second (~25%) child, and had some high school education (<10% had primary or less education). Food insecurity in these women is not a major constraint; however, there is inadequate micronutrient intake, particularly of iron (93% inadequacy), zinc (88% inadequacy), folate (87% inadequacy), and calcium (86% inadequacy) (3). Women were randomized within parity (primipara/multipara) and week of gestation (10-13/14-16 wk) strata to receiving 60 mg iron (ferrous sulfate) and 250 µg folic acid, with or without 25 mg of zinc (zinc sulfate) daily throughout pregnancy.

A total of 242 women were enrolled in the trial (121 in each study arm), 20 were lost to follow-up due to change of address, travel or declination of participation, and 27 were excluded from the final analysis due to significant obstetrical or medical complications (miscarriage, pre-term delivery, congenital malformations); therefore, the final analytic sample consisted of 195 women. Loss to follow up and exclusions did not significantly alter the composition of both arms of the study: 94 received zinc supplement and 101 did not (figure 3.1). There were no obvious differences in the distributions of obstetrical or medical complications by supplement type.

2.2 Data collection

The key measurements and methods for data collection used in the pregnancy study are briefly described below (table 3.1).

**Maternal sociodemographic, nutritional, and pregnancy measurements**

Maternal socioeconomic status and education level, as well as health and obstetric history were collected at enrollment, using a questionnaire. Maternal anthropometric status (weight,
height, waist circumference, and upper and lower limb circumferences and skinfold thicknesses),
dietary intake (using the 24-hour recall method), and iron and zinc status (hemoglobin,
 hematocrit, plasma ferritin, plasma and urinary zinc, and erythrocyte methallothein) were
evaluated at enrollment (10-16 weeks of gestation), and at 28 and 36 weeks of gestation.
Evolution of pregnancy and supplement adherence were assessed at every programmed visit from
enrollment until delivery.

**Fetal neurobehavioral development**

Electronic fetal monitoring (EFM) was conducted at 20, 24, 28, 32, 36, and 38 weeks of
gestation. Women were monitored for 50-min, while resting comfortably in a semirecumbent,
left-lateral position, using standard cardiotocography (Toitu, MT 320, Wayne, Pa). A transducer
was attached to the fetal actocardiograph to record simultaneously FHR and fetal movement. This
duration was selected to exceed the 30-40 min recording time recommended to ensure intrafetal
consistency (4, 5). Testing took place between 9 a.m. to 6 p.m., and women were instructed to eat
1 ½ hours prior to testing but not thereafter. No systematic differences in FHR have been found
when testing during diurnal hours (6).

The signals were registered on polygraphic paper and concurrently digitized and analyzed
off-line using customized software (GESTATE; James Long Company, NY). The data underwent
error rejection procedures based on moving averages of acceptable values, as needed. Variable
extraction included measures of rate and variability computed in 1-minute epochs and averaged
over the 50-min recording. Baseline FHR was quantified in beats per minute (bpm), and the
following measures of heart rate variability were computed: 1) the standard deviation of FHR
(FHR-SD); 2) the range of FHR; and, 3) the number of FHR accelerations, defined as any
increase above the baseline FHR of 10 bpm or more, lasting for at least 15 seconds (NICHD
1997). Slow FHR (FHR < 110 bpm), and the number, duration, and magnitude of each FHR
deceleration (< 15 bpm below baseline for 15 seconds) were also quantified, as well as the % of FHR rejected as artifact.

The fetal movement signal is produced by the actocardiograph. The output is in arbitrary units (a.u.s.), which range between 0 and 100. A movement was defined as an actograph signal, which attained 15 a.u.s. and lasted until the signal fell below 15 a.u.s for at least 10 seconds. According to this threshold, the fetal movement parameters quantified by the software were: 1) the number of movement bouts; 2) the mean amplitude of the movement bouts; and, 3) the mean duration of the movement bouts and activity level, which was calculated by multiplying the total number of movement bouts times the mean movement duration, and which reflects the total time the fetus is moving during the monitoring session.

Fetal movement-FHR coupling occurs when the FHR accelerates in conjunction with fetal movements. It indicates neural integration somatic and cardiac activity. Cross-correlation ($p$) functions between fetal movements and FHR are computed at each lag of +/- 50 seconds, to detect when the two occur in a related fashion (7).

Fetal biometry

At 20, 24, 28, 32, 36, and 38 weeks of gestation (+/- 0.5 weeks), women underwent ultrasonography to evaluate fetal growth. At each ultrasonography session, head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC), and femur diaphysis length (FL) were measured according to published techniques (8). Circumferences were calculated by using the largest transverse and longitudinal dimensions of the head and abdomen. Fetal biometry examinations were conducted by two obstetricians who had been trained and standardized by personnel at the Johns Hopkins Fetal Assessment Center. Details of the training and standardization procedures are published elsewhere (1). Intraobserver and interobserver measurement errors were assessed according to a published protocol. Briefly, each examiner obtained two images of each fetal anatomical parameter under study at ~5 minutes apart.
Differences between the two measurements were expressed as the proportion of the measurement obtained from the technically best image of the two available. The differences in the proportions were used to take into account the increase in the dimensions of the fetal anatomical parameters with progression of pregnancy. Proportion differences for the two examiners were averaged and the mean values compared to zero and to each other using \( t \)-test analysis. The margin of error was within expected limits and comparable with published data (1, 9). Further, measurement error was evaluated by calculating intra-class correlation coefficients (ICC) between the pair of repeated measurements; all ICC were \( > \) than 0.998 and comparable with previously published data from a similar Peruvian population (10).

In total, 1142 biometric evaluations were conducted. A total of 168 women had all 6 evaluations, 26 women had 5 evaluations (19 had no 38-week evaluation, 1 had no 24-week evaluation, and 6 had no 20-week evaluation), and 1 woman had 4 evaluations (no 24 week and 28-week evaluations).

**Fetal and infant measurements**

Iron and zinc status were evaluated in the fetus (cord blood) and at birth using the following: hemoglobin, hematocrit, plasma ferritin, plasma zinc, urinary zinc, placental zinc, and erythrocyte and placental metallothionein. Weight and length at birth were measured by hospital personnel, and head circumference was measured by study personnel within 12 hours of birth. Infant health status was assessed by a neonatologist at birth. All newborns included in the study were generally healthy.

3. **Follow-up study**

3.1 **Study design and participants**
In 2003, children whose mothers participated in the pregnancy study took part in follow-up assessments. The 195 participants included in the formal analysis, and 10 of the 27 excluded participants (who completed the protocol and whose babies survived the neonatal period and were free of congenital malformations) were identified for follow up. In all, 184 children (90%) were located, contacted and evaluated (figure 3.1).

The evaluation was completed by trained study health professionals over two visits. The protocol included interviews with the caregiver and review of clinical records to collect information on socioeconomic conditions of the family, and the health, nutritional, and developmental history of the child, a health exam, a nutritional evaluation, and behavioral and developmental testing.

3.2 Data collection

The key measurements and methods for data collection used in the follow-up study are briefly described below (table 3.1).

**Child health and nutritional evaluation**

A health evaluation was conducted by the study physician by physical examination and by interviews of the mothers. Blood pressure was measured by the study physician with the child in a seated position in triplicate, at 1-minute intervals, using a pediatric sphygmomanometer (Reister, Jungingen, Germany). Anthropometric measures were conducted by a trained anthropometrist following standard procedures (22). Standing height of the child was measured to the nearest 0.1 cm using a stadiometer, and weight was recorded on a digital scale with 0.1 kg precision (Seca, Hamburg, Germany). Circumferences (waist, chest, mid-upper arm, calf) were measured to nearest 0.1 cm with a measuring tape (Seca, Hamburg, Germany). Skinfold thicknesses (biceps, triceps, calf, and subscapular) were measured to the nearest 0.5mm with Lange precision calipers (Cambridge Scientific Instruments, Cambridge, MD).
A child’s feeding history was collected, gathering information by maternal recall about the child’s diet from birth, including breastfeeding, introduction to solids, age at final weaning and transition from complementary food to the family diet. In addition, child dietary intake was measured by a Peruvian trained nutritionist. 24-hour dietary recalls were conducted interviewing the child and the mother. The methods were similar to those used in mothers during pregnancy.

Children were asked to fast overnight and on the following morning they were picked up from their homes and brought to the study clinic, where the study phlebotomist collected venous blood samples. Blood samples were drawn into tubes containing heparin, and within 30 minutes the samples were centrifuged at 600 g for 10 minutes for separation of plasma. Plasma samples were frozen at -20° C until micronutrient analyses were conducted.

**Child health and development history**

Relevant growth and developmental evaluations were collected by abstraction of clinical records from birth to 4.5 years. These included general health history, growth monitoring data, and developmental evaluations from the “CRED” program (crecimiento y desarrollo, growth and development program). They were performed within child-well visits at 2, 4, 6, 9, 12, 18, 24, 36 and 48 months, and include serial measures of weight, length, and head circumference, immunizations, significant illnesses and treatment, and any relevant diagnoses made from birth. Assessment of cognitive development included concept formation, event representation, intelligence, language skills, mechanical ability, number concepts, and problem solving ability. Assessment of social and emotional development included adaptive behavior and adjustment.

**Child cardiac monitoring**

Cardiac monitoring was conducted by electrocardiography (ECG), once children were familiar with testing procedures. A belt with two embedded pediatric electrodes was positioned on the child’s chest under a shirt. ECG data were collected with the children at rest for
approximately 5 minutes. To reduce movement artifact while collecting measures at rest, children
remained in a seated position and were exposed to interesting visual stimuli to maintain
quiescence. R-waves were collected, amplified, and timed by a standard, commercially available,
apparatus (Mini-Logger 2000, Mini Mitter, Bend, OR).

ECG data were transferred to a computer, manually edited for artifact, and processed
using MXedit software (Delta Biometrics, Bethesda MD). To derive cardiac measures, each
tracing was divided into 30-second epochs, each epoch was analyzed separately, and arithmetic
means were computed for each cardiac measure. Baseline heart period (HP) is the mean interval
(ms) between successive R-R waves; this metric is essentially the inverse of heart rate. The
following measures of HP variability were computed: 1) the standard deviation of HP (HP-SD);
2) the range of HP per 30-minute epoch; and, 3) the mean square of successive differences
(MSSD), a time-dependent method of analyzing variations in successive HP. Vagal tone ($V$), the
natural logarithm of the extracted variance calculated using methods developed by Porges, which
is a measure of respiratory sinus arrhythmia, was also collected (11). In addition HR data were
also collected under effortful attention. This is an increasingly common procedure in studies of
developmental function because of the innervation of the vagus nerve during periods of stress. It
includes cognitive challenges, varies across individuals, and has been suggested as a marker for
physiologic integrity (12). Thus, these data provide an additional source of information regarding
responsivity of the nervous system during effortful cognitive processing. The onset and offset of
each task was marked by a button press device that is part of the Mini-Logger system, which
provides time-linked data as output, which allows computing each cardiac measure within
specific task durations.

Missing ECG data were typically due to signal artifact. ECG data were available for 165
(80%) out of the 184 children at follow-up, 79 from the zinc group and 87 from the control group
(figure 3.1). Of these, 3 in the zinc group and 4 in the control group were not included in the
original analysis (2).
4. Cardiometabolic programming study

For the cardiometabolic programming study, additional measurements were conducted using cryopreserved plasma samples from the follow-up study. In 2011, the plasma samples were thawed to conduct the analyses presented here. A Cholestech LDX analyzer (Cholestech Corporation, Hayward, California) was used to measure total and HDL-cholesterol, triglycerides, and glucose concentrations. LDL-cholesterol concentration was calculated using the Friedewald equation (26). Plasma insulin concentration was measured using an ultrasensitive immunoassay (Alpco Diagnostics, Salem, New Hampshire). Insulin resistance was estimated by using the homeostasis model assessment, HOMA-IR = fasting plasma insulin concentration (mU/L) X fasting plasma glucose (mmol/L) / 22.5 (27).

Of the 184 participants located for follow-up (98 in the control group and 86 in the treatment group), 20 had no frozen plasma samples to conduct biochemical measurements, and 5 had missing information on blood pressure (figure 3.1). For some cases, plasma samples were missing due to refusal of the blood draw at follow-up or due to insufficient volume to conduct these biochemical analyses. The present study is, therefore, restricted to 159 children with complete biochemical and blood pressure data, 86 (85.1%) from the control group and 73 (77.7%) from the treatment group. Missing data between treatment groups were non-differential (p=0.67).

5. Ethical considerations

The pregnancy study, the follow-up study, and the cardiometabolic programming study were all reviewed and approved by the Institutional Review Board of the Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland and the Ethics Committee of the Instituto de Investigacion Nutritional (IIN) in Lima, Peru.
Figure 3.1 Participants included in the pregnancy study, the follow-up study, and the cardiometabolic programming study, by prenatal supplement type.
Table 3.1 Summary of measurements taken in the pregnancy study, in the follow-up study, and cardiometabolic programming study

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Pregnancy study (weeks of gestation)</th>
<th>Follow-up study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal background data and health history</td>
<td>10-16 20 24 28 32 36 38 Birth</td>
<td>4.5 y New data</td>
</tr>
<tr>
<td>Maternal anthropometry</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Maternal dietary intake</td>
<td>X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Maternal zinc and iron status</td>
<td>X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Evolution of pregnancy</td>
<td>X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Supplemental adherence</td>
<td>X X X X X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Electronic fetal monitoring</td>
<td>X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Fetal biometry</td>
<td>X X X X X X</td>
<td></td>
</tr>
<tr>
<td>Child anthropometry</td>
<td></td>
<td>X X</td>
</tr>
<tr>
<td>Child health status</td>
<td></td>
<td>X X</td>
</tr>
<tr>
<td>Fetal/child iron and zinc status</td>
<td></td>
<td>X X</td>
</tr>
<tr>
<td>Child developmental history</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Child feeding history and dietary intake</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Cardiac monitoring</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Biochemical cardiometabolic markers in plasma</td>
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REFERENCES


ABSTRACT

Introduction: Childhood body size and composition have important short- and long-term implications for health and educational and economic performance, especially in low- and middle-income countries. Growth and body composition during childhood are influenced to some degree by growth during the prenatal period.

Aims: This study examines associations between fetal size and growth (change of size in time) in the second half of pregnancy, and body size and composition at 4.5 years of age.

Subjects: One hundred and eighty-four Peruvian children whose mothers were part of a prenatal zinc supplementation trial.

Study design: Biometric measures of fetal growth (head circumference, abdominal circumference, fetal femur length, and estimated fetal weight) were estimated by ultrasonography at 20, 24, 28, 32, and 36 weeks of gestation, and anthropometric measures of body size and composition at 4.5 years of age.

Outcome measures: Height, weight, head circumference, body mass index, fat mass index, sum of skinfolds, waist circumference, subscapular/triceps skinfold ratio, and fat-free mass index.

Results: Fetal size and growth, particularly between 28-32 weeks of gestation, were positively associated with child size and adiposity and inversely associated with a measure of trunk-relative-to-extremity adipose tissue among males and females, and positively associated with lean mass among females only.
Conclusions: These results provide evidence of the fetal origins of child size and body composition, with the period between 28-32 weeks of gestation being critical.

INTRODUCTION

Childhood body size and composition have important short- and long-term implications for health and educational and economic performance, especially in low- and middle-income countries (LMICs). Childhood stunting is associated with reduced stature and fat free mass (1, 2), lower achieved schooling (3, 4), decreased economic productivity (4), adverse maternal outcomes (5), and increased risk of cardiometabolic diseases later in life (6). Increased weight and body fat during childhood have been associated with higher adult fat mass and also with an increased risk of developing cardiometabolic diseases (7, 8).

Growth and body composition during childhood are influenced to some degree by growth during the prenatal period. Size at birth has been positively associated with later height, weight, and fat-free mass (and to lesser extent with fat mass), and negatively associated with abdominal adiposity (9). Low birth weight (<2500g) or small-for-gestational age (SGA, weight for gestational age < 10th percentile) increases the odds of becoming wasted, stunted, or underweight during childhood on the magnitude of 2.5-3.5-fold (10).

Most investigations of fetal programming of growth and body composition rely on weight at birth as a measure of the adequacy of fetal growth and, hence, of the prenatal nutritional environment (9, 11). Birth weight provides information about the size of the fetus at the culmination of the prenatal period; however, it is limited in characterizing intrauterine growth trajectories or in identifying critical periods of fetal growth and development, where nutritional insults could result in the programming of postnatal conditions.

With increased use of ultrasound examination as part of routine antenatal care monitoring – mainly in developed countries – it has been possible to evaluate the associations between intrauterine measures of fetal growth and nutritional and health outcomes later in life. A recent
review summarized these findings; positive associations were identified between fetal biometric dimensions (femur length, head circumference /diameter, abdominal circumference/diameter) and estimated fetal weight, and height, weight, and BMI in childhood (12). The number of studies identified was limited and most included fetal measures at one or two points of time during the third trimester only; none have been conducted in populations from LMICs. To better understand the relationship between fetal and child growth, it is important to more thoroughly characterize fetal growth, using measures of fetal dimensions earlier in pregnancy, as well as including measures at more timepoints during gestation. This would allow for more complex modelling of intrauterine growth and may help identify whether any specific periods during gestation are critical for programming later body size and composition. It is particularly relevant to study these associations among populations from developing countries, in whom growth failure in early life is still prevalent and in whom the prevalence of obesity is increasing at an alarming rate.

In this study, we used ultrasound measures of fetal dimensions to evaluate whether fetal size and growth, in the second half of gestation, are associated with body size and composition in a sample of Peruvian children at 4.5 years of age. We hypothesized that greater fetal growth would be positively associated with overall body size and lean mass, and inversely associated with adiposity.

METHODS

Study design and participants

This study is an observational analysis of participants in a prenatal zinc supplementation trial conducted in Lima, Peru between 1998 and 2000. In 2003, when the children were approximately 4.5 years of age, a follow-up study was conducted. The original study design, details of recruitment, enrollment, and results relating to zinc supplementation during the fetal period and childhood have been previously published (13-15). A total of 242 pregnant women receiving antenatal care at a local hospital in periurban Lima, who were classified with a low-risk
pregnancy, carrying a singleton fetus, and who had lived on the coast of Peru for at least 6 months before pregnancy, were enrolled in the supplementation trial. Of those, 20 were lost to follow-up and 27 were excluded from the original analyses due to significant obstetrical or medical complications; therefore, 195 were included in the formal analysis and targeted for follow-up. Of those, 177 (91%) were located and evaluated, and are the focus of the current report.

**Prenatal measures**

At enrollment (10-16 weeks of gestation), women were interviewed to obtain sociodemographic information, and their health and nutritional status were evaluated. At 20, 24, 28, 32, 36, and 38 weeks of gestation (+/- 0.5 weeks), the women underwent ultrasonography to evaluate fetal growth. At each ultrasonography session, head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur diaphysis length (FL) were measured, according to published techniques (16). Circumferences were calculated by using the largest transverse and longitudinal dimensions of the head and abdomen. Fetal biometry examinations were conducted by two obstetricians who had been trained and standardized by personnel at the Johns Hopkins Fetal Assessment Center. Details of the training and standardization procedures are published elsewhere (13). Intraobserver and interobserver measurement errors were assessed according to a published protocol; margin of error was within expected limits and comparable with published data (13, 17). All evaluations occurred in the study clinic, and all deliveries took place in the hospital. Weight and length at birth were measured by hospital personnel.

**Child anthropometric measures (4.5 y)**

All follow-up assessments were completed by trained study health professionals. Anthropometric measures were conducted by a trained and standardized anthropometrist, following published protocols (18). Standing height of the child was measured to the nearest 0.1 cm using a stadiometer, and weight was recorded on a digital scale with 0.1 kg precision (Seca,
Hamburg, Germany). Circumferences (head, chest, waist, mid-upper arm, calf) were measured to nearest 0.1 cm, with a plastic inextensible measuring tape (Seca, Hamburg, Germany). Skinfold thicknesses (biceps, triceps, calf, and subscapular) were measured to the nearest 0.5 mm, with Lange precision calipers (Cambridge Scientific Instruments, Cambridge, MD).

**Anthropometric assessment of body size and composition.**

Height, weight, head circumference, and body mass index (BMI = weight (kg) / height (m)\(^2\)) were used as measures of child body size. Fat mass index (FMI) and the sum of the four skinfold thicknesses (biceps, triceps, subscapular, and calf) were used as measures of overall fatness. Waist circumference (WC) and the ratio of the subscapular and triceps skinfold thickness (SS/Tri SF ratio) were used as measures of regional adiposity. WC is considered a measure of abdominal visceral adiposity, whereas the SS/Tri SF ratio captures central distribution of subcutaneous adipose tissue relative to the extremities; both have been associated with cardiometabolic conditions in children (19). Fat free mass index (FFMI) was used as a measure of overall lean mass. FMI and FFMI were calculated by dividing fat mass (FM, kg) and fat free mass (FFM, kg) by height (m)\(^2\), and represent total adiposity and leanness adjusted for height. FM and FFM were estimated from total body water (FM = TBW + Fat free mass) from an equation developed in Peruvian children (TBW = 0.276*weight (kg) + 0.105*height (cm) + 0.051* chest circumference (cm) – 0.319 (sex, female = 1) – 6.134) and validated using \(^{18}\)O dilution (20).

**Statistical considerations**

Maternal and birth characteristics of participants and non-participants in the follow-up study were compared using t test or chi-square analysis; no statistically significant differences were observed (data not shown). Maternal, birth, and child characteristics were evaluated by sex, using t test, Mann-Whitney test, or chi-square analysis. Statistical significance was defined as P
< 0.05; all data analyses were conducted using Stata 12.0 (Stata Corporation, College Station, Texas).

**Fetal growth modeling**

HC was used as a measure of fetal head size, AC as a measure of fetal trunk size, FL as a measure of fetal length, and estimated fetal weight (EFW) as a measure of total fetal size. EFW was calculated using the “Hadlock 4” formula ($\log_{10}\text{EFW} = 1.3596 - 0.00386 \text{AC} \times \text{FL} + 0.0064 \text{HC} + 0.00061 \text{BPD} \times \text{AC} + 0.0424 \text{AC} + 0.174 \text{FL}$) at each time point during pregnancy (21). To remove some of the statistical issues of modeling highly correlated individual fetal biometric data, and to evaluate the separate contribution of fetal growth at each specific period of gestation and over the entire pregnancy, we created “conditional fetal size” variables at different gestational ages (2, 3, 22). We developed sex- and prenatal zinc supplement-specific linear regression models for each fetal size measure (HC, AC, FL, EFW) at 24, 28, 32, and 36 weeks gestation as outcomes, and any prior measures of the same fetal size variable as predictors, and predicted their standardized residuals (observed – predicted value). For example, conditional HC at 36 weeks is the standardized residual from HC at 36 weeks regressed on HC at 32, 28, 24, and 20 weeks. These standardized residuals can be interpreted as the deviation of an individual’s fetal size at a specific gestational age from its expected value, given his or her prior measures of the same fetal size measure.

**Effect size models**

To evaluate the association between fetal growth and child anthropometric status at 4.5 years, we developed two sets of sex-specific models for each of the nine child anthropometric outcomes (height, weight, HC, BMI, FMI, Sum of SF, WC, SS/Triceps SF ratio, and FFMI). With the first set of models we used each of the fetal size measures (HC, AC, FL, or EFW) at each gestational age time point (20, 24, 28, 32 or 36 weeks of gestation) and weight at birth as
predictors, whereas with the second set of models, we analyzed fetal growth dynamically by including the fetal size variable (HC, AC, FL, or EFW) at 20 weeks, together with the corresponding conditional fetal size variables at 24, 28, 32, and 36 weeks, as predictors. Models for each outcome include the largest sample with complete data. Of the 177 participants included in this report, 18 did not have a 38-week visit; therefore, the last fetal size measures included in the analysis, correspond to the 36-week visit. The main reason for missing the last scheduled visit was delivery prior to the visit, although only 2 were born preterm; one participant was born at 40 weeks, 7 at 38 weeks, 7 at 37 weeks, and 2 at 36 weeks. On average, participants missing the last scheduled visit were born to younger and less educated mothers and were born earlier and smaller, than participants who attended the visit ($P<0.05$); there were no significant differences in child anthropometric measures at 4.5 years between them ($P>0.05$, data not shown). All the analyses in this report were also carried out excluding these 18 participants. The results were similar in the direction and magnitude of the association; therefore, we report the most comprehensive models.

To compare the strength of associations among diverse fetal growth measures at different time points during gestation and their association with child outcome measures, we used standardized regression coefficients, as opposed to absolute regression coefficients; in regression analysis, these types of models are known as effect size models (23). Standardized coefficients can be interpreted as the expected change in SD units in the outcome variable per sample-specific 1-SD increments in the predictor variable, and allow us to compare relative effects of predictors and outcomes with different units. Standardized regression coefficients of 0.1 are considered low, 0.3 are considered moderate, and 0.5 are considered strong (23). All models were adjusted for prenatal zinc supplement, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity. Inclusion of these variables in the model did not significantly alter the associations between fetal growth and child size and body composition. Interaction terms...
between prenatal zinc and fetal biometric measures were tested; none were significant and, hence, are not included in the final models.

In the first set of models, we developed sex-specific linear regression models to evaluate the association of fetal size status (HC, AC, FL, and EFW) at each point in time during pregnancy (20, 24, 28, 32, or 36 weeks) and weight at birth with each of the nine child anthropometric status measures at 4.5 years. Because of the large number of fetal biometric predictors and child anthropometric outcomes, we present results from models using EFW and weight at birth only. Models using EFW were selected because results in these models were the most salient and because results from models using HC, AC, and FL were, in general, consistent with them. As mentioned earlier, EFW is a composite variable estimated utilizing the different fetal size measures and is, therefore, considered a measure of overall fetal size (16). Results from HC, AC, and FL are available in the appendix.

In the second set of models, we developed sex-specific linear regression models to evaluate the association between fetal growth (change of HC, AC, FL, and EFW over time) and each of the nine child anthropometric status measures at 4.5 years. Predictors in each model included the fetal biometric measure at 20 weeks of gestation, representing fetal size at mid-pregnancy, and the different conditional fetal size variables, representing measures of fetal growth at each 4-week interval; these conditional variables are independent of the prior measure. For the same reasons mentioned earlier, we present results from models using EFW only, and graphically present regression coefficients (and 95% CIs) from conditional fetal HC, AC and FL at 32 weeks only; detailed tables with other regression coefficients from these models are available in the appendix. The 32-week time point was selected as the most representative because fetal growth in the 28-to-32-week period consistently showed the strongest association with all child outcomes.
The design and methods of the pregnancy and the follow-up studies were approved by the Institutional Review Boards of the Instituto de Investigación Nutricional, Lima, Peru, and The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.

RESULTS

Characteristics of study participants

Characteristics of the study participants are summarized in tables 4.1 and 4.2. There were no differences in maternal characteristics, except that, compared to females, males were born to relatively older mothers (~1.5 years). At any given gestational age, males tended to be larger than females, overall, on head size, trunk size, and femur length; these differences tended to continue after birth. At 4.5 years, on average children were shorter (height for age Z-score = -0.85, SD = 0.79) and heavier (BMI for age Z-score = 0.77, SD = 0.95) than children of their same sex and age, when using the WHO growth standards, and females had greater adiposity, as compared to males.

Associations of fetal size with child size and body composition

The relative differences (effect sizes) in body size and composition at 4.5 years associated with EFW during the prenatal period (20, 24, 28, 32, or 36 weeks of gestation) or at birth (weight), are presented in table 4.3. After adjusting for prenatal zinc supplement, gestational age at delivery, and selected maternal characteristics, EFW was positively associated ($P<0.05$) with child height, weight, HC, FMI, sum of SF, and WC, in males and females. Relations between EFW and child BMI and FFMI were also positive, but were only statistically significant among females. EFW was inversely associated with child SS/Triceps SF ratio, but this association was statistically significant among males only. All associations tended to be strongest at 32 weeks and effect sizes corresponded generally to moderate relationships. In general, associations of EFW with measures of child size were stronger for males, whereas associations
between EFW and measures of child body composition were stronger for females. Similar associations were observed for weight at birth. The direction of the associations between HC, AC, or FL and measures of child size and body composition were similar (tables S4.1-S4.3, supplemental tables), although associations with FL tend to be weaker, and were only significant for height and HC in males, and for weight, BMI, FMI, sum of SF, WC, and FFMI in females.

Associations of fetal growth with child size and body composition

The mean effect sizes (and 95% CIs) for relative differences in child anthropometric outcomes associated with 1-SD increase in conditional EFW are presented in table 4.4, and the mean effect sizes (and 95% CIs) for relative differences in child anthropometric outcomes associated with 1-SD increase in conditional fetal HC, AC, and FL at 32 weeks of gestation are presented in figures 4.1A-C. Detailed tables showing regression coefficients for fetal HC, AC, and FL at 20 weeks, and their corresponding conditional fetal size measures at 24, 28, and 36 weeks, are presented as supplemental tables (tables S4.4-S4.6, supplemental). After adjusting for prenatal zinc supplement, gestational age at delivery, and selected maternal characteristics, conditional EFW at 32 weeks was positively associated ($P<0.05$) with child height, weight, HC, BMI, FMI, and sum of SF in males and females, and with child FFMI in females only, and was inversely associated ($P<0.05$) with child SS/Tri SF ratio in males only. Conditional EFW at 24 weeks was associated with child HC in males and females, and with child height, weight, and BMI in females only. All effect sizes correspond to moderate or low associations; effect sizes for measures of child size were greater for males, whereas effect sizes for child body composition were greater in females.

Conditional HC at 32 weeks was positively associated ($P<0.05$) with child height, weight, HC, BMI, and FMI among females only. Positive associations ($P<0.05$) between conditional HC at other gestational ages and child height, weight, HC, and WC, and an inverse
association between conditional HC at 24 weeks and child SS/Tri SF ratio, were observed among males.

Conditional AC at 32 weeks was positively associated ($P<0.05$) with child height, weight, HC, FMI, sum of SF, and WC among males and females. Conditional AC at 32 weeks was positively associated ($P<0.05$) with child BMI in females, and showed a positive association in males, although it did not reach significance. Positive associations ($P<0.05$) were also observed between conditional AC at 24 weeks and child height, weight, and HC in females, and conditional AC at 28 weeks and child HC and WC in males. Conditional AC at 32 weeks and at 28 weeks were inversely associated ($P<0.05$) with child SS/Tri SF ratio in males and females, respectively.

Conditional FL at 32 weeks was positively ($P<0.05$) associated with child HC in males, and with child weight, BMI and FMI in females. Conditional FL at 20 weeks in males and at 24 and 36 weeks in females were associated ($P<0.05$) with child height. Conditional FL at 28 weeks was inversely associated ($P<0.05$) with child SS/Tri SF ratio in females only.

**DISCUSSION**

Our analysis evaluated the associations between measured dimensions of intrauterine growth (20-36 weeks of gestation) and child size and body composition at 4.5 years in a sample of Peruvian children. Despite some differences in these associations according to sex and the fetal dimension used, consistencies in our findings emerged. We found that both fetal size and fetal growth, especially between 28-32 weeks of gestation, were positively associated with child size and adiposity and inversely associated with a measure of trunk-relative-to-extremity adipose tissue among males and females, and positively associated with lean mass among females only. To our knowledge, this is the first study linking fetal biometric measures throughout the second half of pregnancy with measures of body size and composition in childhood in a sample of children from a developing country. Further, to our knowledge, this is the first study using
complex statistical modelling techniques to characterize fetal growth from fetal biometric measurements at 5-fixed time-points during the second half of pregnancy, using various individual fetal dimensions (HC, AC, and FL) as well as a composite measure of total fetal size (EFW). Our findings are generally consistent with those previously reported and add significantly to prior studies conducted in developed country populations, suggesting that fetal growth may influence long-term regulation of energy balance (12).

We have shown that fetal size and growth (HC, AC, FL, and EFW) moderately predict child height, weight, HC, and BMI, with some differences, according to sex and the time-point in gestation, when associations become evident. Both fetal size and growth were positively associated with child height and HC; these relationships were apparent as early as 20 weeks of gestation for child height in males, and 24 weeks of gestation for child height in females, and HC in both sexes. Although both fetal size and growth were also positively associated with child weight and BMI, these relationships were not evident until later in pregnancy. Fetal size and growth predicted child weight and BMI in females, starting at 24-32 weeks of gestation, and child weight in males, starting at 28-32 weeks of gestation; fetal measures did not consistently predict child BMI among males.

These results are in alignment with findings from prior studies tracking intrauterine measures of body size from the prenatal to the postnatal period. Probably the most consistent relationship reported in the literature is the positive association observed between fetal length (FL or crown-rump length, CRL) or fetal linear growth (deviations in FL or CRL in pregnancy) and child length/height (24-27). This is not surprising, since fetal FL is correlated with length at birth ($r = 0.7$) (28), and length during the first year of life is an established predictor of final attained height (on average, 1-cm increase in birth length is associated with 1-cm increase in attained adult height) (29, 30). In general, studies showing positive relationships between FL and child height report associations between fetal measures in the second or third trimesters of pregnancy up to 8 years in the childhood period, although one study found a significant association noticeable
beginning in the first trimester up to 14 months of age only (27). In our study, both fetal FL size
and growth were positively associated with child height, although there were pronounced
discrepancies according to sex. In males, fetal FL size was positively associated with child height,
starting at 20 weeks, and this association became stronger with progression of the pregnancy. On
the other hand, in the model with conditional fetal FL variables, only FL at 20 weeks – and not
conditional FL variables – was associated with child height, suggesting that fetal FL size
throughout pregnancy is associated with child height, to the extent that it is determined by prior
measures and possibly due to a stronger genetic influence in height. In females, a positive
association between fetal FL and child height was also observed, but reached significance only at
36 weeks of gestation. Conversely, in the model with conditional fetal FL variables, conditional
FL at 24 and 36 weeks was associated with child height. These sex differences are difficult to
interpret, especially because previously published studies have not stratified analyses according to
sex. However, because similar sex differences were observed when using other fetal dimensions
as predictors, we believe it deserves further investigation. Less information is available on the
relationship between fetal size and growth and child HC, although a positive association between
fetal size (CRL) during the first trimester and HC up to 14 months of age has been reported (27).
Consistent with this report, we found positive associations between both fetal size and growth
(HC, AC, FL, and EFW) and child HC; these associations were similar in both sexes, observed as
early as 24 weeks of gestation and, as expected, were stronger and more consistent when using
the fetal HC measures as predictors.

Positive associations between both fetal size (AC, FL or crown-rump length, or EFW) and
fetal growth and child weight or BMI have also been previously reported; however, these
associations are weaker than for height, less consistent during early pregnancy, and have been
reported up to 5 years of age only (25-27, 31, 32). Our results on child weight and BMI are in
agreement with these prior findings, although they were typically evident earlier and more
consistently for females (24 weeks) than males (28 weeks). Despite the previously mentioned
sex- and gestational age-differences, the consistent positive relationship observed in our study between fetal size and growth – characterized by diverse fetal dimensions – and child size, and the alignment of our results with previous reports, strongly support the conclusion that prenatal growth influences child growth several years later.

Our findings related to body composition indicate that greater fetal growth is associated with greater overall fat mass, fatness relative to height, lean mass, and adiposity in the abdominal area, and lower trunk fat, relative to the extremities in childhood. We found that both fetal size and growth were positively associated with FMI, the sum of SF, WC, and FFMI (among females only) starting, in general, at 28-32 weeks of gestation. The associations were somewhat stronger for FMI and the sum of SF than for FFMI, both in males and females, and were stronger for fetal AC or EFW as predictors. On the other hand, we found an inverse association between fetal size and growth and SS/Tri SF ratio, a measure of trunk fat relative to the extremities; this relationship was strongest at 32 weeks gestation in males and at 28 weeks gestation in females.

Our results are somewhat consistent with regard to the regional distribution of fat, but contradictory in relation to total adiposity, which challenges interpretation. Previous studies conducted in developed country populations have typically shown an inverse association between intrauterine growth, as measured by fetal biometry, and total adiposity or central to peripheral adiposity in early childhood up to 6 years of age (33-36). It is commonly thought that individuals suffering from intrauterine growth restriction will compensate in terms of growth in early infancy, and, therefore, experience greater fat accumulation (9). It is possible that in our population certain factors contributing to growth differences during the prenatal period, such as nutritional deficiencies, continue to be present after birth with compensatory growth being limited, and, therefore, the programming effects only being evident using a measure of relative central fatness rather than measures of overall fatness; the location of adipose tissue on the body is a more important predictor of metabolic risk than total adiposity (37). Although we were unable to find studies relating measures of intrauterine growth with postnatal lean body mass, several studies
have shown a positive association between size at birth (weight, length) and postnatal lean body mass (9); our results are in agreement with previous findings for females only. Our findings, together with previous reports, strongly suggest that prenatal growth patterns are associated with later body composition. The reason why the association observed between fetal growth and two measures of abdominal adiposity is opposite, is not clear either; it can potentially be related to the fact that they measure somewhat different aspects of body fat distribution. The WC is considered a measure of visceral and subcutaneous fat whereas the SS/Tri SF ratio reflects subcutaneous fat on the upper trunk (relative to that on the extremities) (38). Because both measures have been associated with increased cardiometabolic risk in children, makes our findings worthy of further investigation (19).

Our study has several strengths that support the validity of our results. To the best of our knowledge, this is the first study to measure intrauterine growth and relate it to child growth in a sample of children in a developing country. Although the associations described do not prove causality, the longitudinal, prospective design of our study increases certainty of the temporal associations observed, and the consistency of the reported relationships utilizing various dimensions of fetal size and child size and body composition suggest that our findings are compelling. As part of our protocol, assessment of fetal dimensions and child anthropometric measures was conducted by trained and standardized operators, which increases the validity of the measures. We used various measures of child size and body composition, which allowed us to characterize child growth in a comprehensive way. Child height and head circumference served as measures of bone growth, and child weight and BMI served as measures of overall size, including bone mass and soft-tissue mass. Body composition was characterized in several ways: skinfold measures allowed us to estimate body fat (sum) and fat distribution (SS/Tri SF ratio), and FMI and FFMI provided estimates of fat and fat-free mass, adjusted for height. Another strength of our study is that we conducted fetal biometric measurements at 4-weeks intervals, beginning at mid-pregnancy. Because measurements were taken at regular gestational age
intervals, it allowed comparability of measurements across study participants and the use of complex statistical techniques to model fetal growth in the second half of pregnancy, and identification of critical periods of fetal growth programming child anthropometric outcomes. We expressed associations using standardized regression coefficients (size effects), which allowed us to compare the relative effects of fetal growth, at different gestational ages, on body size and composition outcomes, including those with different units. In addition, we were able to characterize overall fetal growth and growth of specific fetal dimensions (head, trunk, leg), and their effects on child body size, total and regional adiposity, and lean mass. Considering that there was no contact with participants of the prenatal study until 4.5 years after pregnancy completion, the follow-up rate was relatively high; ~90% of eligible participants were located and included in this analysis.

Despite the strengths of our study, there are some limitations to it. Because participants of the pregnancy study were not contacted until they were 4.5 years of age, information is missing on environmental factors such as infant diet and early postnatal growth that could have influenced their postnatal growth; attained size and body composition in childhood result from the combination of genetic potential, dietary quality during pregnancy, infancy and early childhood, and exposure to infections in early life (39). Paternal height was not collected as part of the protocol; therefore, parental influences on child growth were not completely controlled for. Our sample size is smaller than those of some previous studies assessing the relationship between intrauterine growth and child body composition, yet all those studies come from the same cohort in the Netherlands, and only one of them has a longer follow-up (6 y) (33, 35, 36). Our fetal protocol included measurements starting at 20 weeks of gestation; therefore, our results can only be extrapolated to the second half of pregnancy. Fetal development rates in early pregnancy are high and previous studies have shown that differences in growth in the first trimester can influence postnatal growth (33, 35, 36). Ultrasound examinations are prone to measurement error; however, as mentioned earlier, all measurements were conducted by trained and standardized
obstetricians, and intraobserver and interobserver measurement errors were within expected limits (13, 17). Our child body composition outcomes relied on indirect estimates of body fat and fat-free mass using skinfold measures and circumference measurements; therefore, error in our estimates can be present. To reduce error as much as possible, we used equations developed in Peruvian children and validated using $^{18}$O (20), and included several skinfolds in our indices.

Prenatal growth and child growth are related, and disentangling genetics from shared prenatal and postnatal environmental conditions influencing child growth can be challenging. Yet there is compelling evidence showing that growth patterns in the prenatal period, at least in part, affect the course of growth during childhood. Because most studies use weight at birth as a measure of prenatal growth, it is still unclear how variations in specific fetal dimensions throughout pregnancy, or differences in growth at critical periods during gestation, influence this process. Moreover, the few studies using measures of intrauterine growth are heterogeneous in their methodology – including the measures of fetal and child growth selected, the number and timing of the prenatal and postnatal measurements conducted, and the analytical methods used – which make comparisons and, therefore, precise conclusions difficult. Despite these challenges, our findings, together with those previously published, suggest that interventions focused in supporting optimal fetal growth will likely result in improvements in child nutrition and health outcomes. Adequate fetal growth will likely result in greater attained height – which has been associated with improved educational and health outcomes (3) and reduced subcutaneous fat deposited in the trunk than on the limbs – which is correlated with a decreased metabolic risk (19).
### TABLE 4.1 Selected characteristics of 177 Peruvian children by sex

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Males (n=88)</th>
<th>Females (n=89)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maternal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>24.2 ± 5.3</td>
<td>22.6 ± 4.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Height, cm</td>
<td>152.1 ± 4.9</td>
<td>152.7 ± 5.7</td>
<td>0.51</td>
</tr>
<tr>
<td>Complete secondary or higher education, %</td>
<td>70.5</td>
<td>60.7</td>
<td>0.21</td>
</tr>
<tr>
<td>Primiparity, %</td>
<td>52.3</td>
<td>64.0</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at delivery, wk</td>
<td>39.2 ± 1.1</td>
<td>39.4 ± 1.1</td>
<td>0.20</td>
</tr>
<tr>
<td>Small for gestational weight, %</td>
<td>12.5</td>
<td>14.6</td>
<td>0.68</td>
</tr>
<tr>
<td>Weight at birth, g</td>
<td>3313.2 ± 416.4</td>
<td>3240.3 ± 404.7</td>
<td>0.24</td>
</tr>
<tr>
<td>Length at birth, cm</td>
<td>50.3 ± 2.0</td>
<td>49.4 ± 1.9</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>4.5 y</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>103.2 ± 3.7</td>
<td>102.0 ± 3.5</td>
<td>0.03</td>
</tr>
<tr>
<td>Height-for-age, Z-score</td>
<td>-0.79 ± 0.83</td>
<td>-0.93 ± 0.77</td>
<td>0.25</td>
</tr>
<tr>
<td>Stunted (HAZ≤2), %</td>
<td>4.6 (4)</td>
<td>6.7 (6)</td>
<td>0.53</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>17.6 ± 2.3</td>
<td>17.1 ± 2.3</td>
<td>0.10</td>
</tr>
<tr>
<td>HC, cm</td>
<td>50.2 ± 1.3</td>
<td>49.3 ± 1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>16.5 ± 1.4</td>
<td>16.3 ± 1.5</td>
<td>0.42</td>
</tr>
<tr>
<td>BMI-for-age, Z-score</td>
<td>0.86 ± 0.94</td>
<td>0.64 ± 0.91</td>
<td>0.12</td>
</tr>
<tr>
<td>Obese, (BAZ&gt;2), %</td>
<td>11.4 (10)</td>
<td>9.0 (8)</td>
<td>0.60</td>
</tr>
<tr>
<td>FMI, kg/m2</td>
<td>5.8 ± 1.2</td>
<td>6.1 ± 1.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Sum of SF, mm</td>
<td>26 (24, 31)</td>
<td>30 (26, 33)</td>
<td>0.002</td>
</tr>
<tr>
<td>WC, cm</td>
<td>55.0 ± 3.6</td>
<td>54.7 ± 4.6</td>
<td>0.63</td>
</tr>
<tr>
<td>SS/Triceps SF ratio</td>
<td>0.64 ± 0.14</td>
<td>0.65 ± 0.11</td>
<td>0.57</td>
</tr>
<tr>
<td>FFMI , kg/m2</td>
<td>10.7 ± 0.29</td>
<td>10.2 ± 0.31</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are mean ± SD or % (n). Sum of SF is median (IQR). Continuous variables were compared using t-test, and categorical variables using chi-square analysis. Sum of SF were compared using Mann-Whitney test. HAZ = height-for-age Z-score, BAZ = body mass index-for-age Z-score, HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular, FFMI = fat free mass index.
### Table 4.2 Intrauterine biometric measures at 20, 24, 28, 32, 36, and 38 weeks of gestation in 177 Peruvian fetuses by sex

<table>
<thead>
<tr>
<th>Fetal biometric measure</th>
<th>Weeks of gestation</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 (n=84)</td>
<td>24 (n=87)</td>
<td>28 (n=87)</td>
<td>32 (n=88)</td>
<td>36 (n=88)</td>
<td>38 (n=78)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head circumference, mm</td>
<td>175.0 ± 6.3</td>
<td>220.0 ± 5.9</td>
<td>261.5 ± 6.2</td>
<td>291.9 ± 8.1</td>
<td>314.6 ± 7.7</td>
<td>321.2 ± 8.7</td>
</tr>
<tr>
<td>Abdominal circumference, mm</td>
<td>152.8 ± 7.1</td>
<td>198.5 ± 7.3</td>
<td>243.5 ± 8.6</td>
<td>282.8 ± 10.1</td>
<td>316.0 ± 11.7</td>
<td>329.3 ± 12.7</td>
</tr>
<tr>
<td>Femur diaphysis length, mm</td>
<td>33.2 ± 1.3</td>
<td>44.0 ± 1.3</td>
<td>53.5 ± 1.5</td>
<td>61.9 ± 1.8</td>
<td>68.5 ± 2.1</td>
<td>71.0 ± 2.0</td>
</tr>
<tr>
<td>Estimated fetal weight, g</td>
<td>355.5 ± 31.3</td>
<td>707.1 ± 54.5</td>
<td>1255.7 ± 101.1</td>
<td>1954.5 ± 171.0</td>
<td>2696.4 ± 232.2</td>
<td>3019.9 ± 272.8</td>
</tr>
<tr>
<td>Females</td>
<td>(n=88)</td>
<td>(n=88)</td>
<td>(n=89)</td>
<td>(n=89)</td>
<td>(n=89)</td>
<td>(n=81)</td>
</tr>
<tr>
<td>Head circumference, mm</td>
<td>171.8 ± 6.3</td>
<td>215.3 ± 6.0</td>
<td>256.7 ± 7.1</td>
<td>288.0 ± 7.5</td>
<td>309.8 ± 9.1</td>
<td>317.6 ± 9.7</td>
</tr>
<tr>
<td>Abdominal circumference, mm</td>
<td>150.3 ± 7.1</td>
<td>194.4 ± 6.8</td>
<td>238.7 ± 9.5</td>
<td>279.4 ± 10.0</td>
<td>313.4 ± 12.1</td>
<td>327.6 ± 13.8</td>
</tr>
<tr>
<td>Femur diaphysis length, mm</td>
<td>32.9 ± 1.4</td>
<td>43.9 ± 1.4</td>
<td>53.4 ± 1.5</td>
<td>61.8 ± 1.6</td>
<td>68.5 ± 1.9</td>
<td>70.8 ± 2.0</td>
</tr>
<tr>
<td>Estimated fetal weight, g</td>
<td>344.1 ± 31.7</td>
<td>676.0 ± 51.9</td>
<td>1200.5 ± 105.2</td>
<td>1891.2 ± 156.5</td>
<td>2628.9 ± 226.7</td>
<td>2958.5 ± 278.1</td>
</tr>
</tbody>
</table>
### Table 4.3 Effect sizes for estimated fetal weight (EFW) at 20, 24, 28, 32, or 36 weeks gestation or weight at birth, and body size and composition at 4.5 y by sex

<table>
<thead>
<tr>
<th>Anthropometric Measure (4.5 y)</th>
<th>20</th>
<th>24</th>
<th>28</th>
<th>32</th>
<th>36</th>
<th>Weight at birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males (n=84)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.20</td>
<td>0.19</td>
<td>0.22</td>
<td>0.37</td>
<td>0.35</td>
<td>0.21</td>
</tr>
<tr>
<td>Weight</td>
<td>0.13</td>
<td>0.10</td>
<td>0.20</td>
<td>0.35</td>
<td>0.28</td>
<td>0.22</td>
</tr>
<tr>
<td>HC</td>
<td>0.13</td>
<td>0.20</td>
<td>0.34</td>
<td>0.48</td>
<td>0.41</td>
<td>0.29</td>
</tr>
<tr>
<td>BMI</td>
<td>0.02</td>
<td>-0.03</td>
<td>0.10</td>
<td>0.21</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>FMI</td>
<td>0.03</td>
<td>-0.01</td>
<td>0.11</td>
<td>0.23</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Sum of SF</td>
<td>0.14</td>
<td>0.12</td>
<td>0.15</td>
<td>0.33</td>
<td>0.26</td>
<td>0.29</td>
</tr>
<tr>
<td>WC</td>
<td>0.07</td>
<td>0.06</td>
<td>0.19</td>
<td>0.31</td>
<td>0.23</td>
<td>0.14</td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
<td>-0.04</td>
<td>-0.10</td>
<td>-0.09</td>
<td>-0.32</td>
<td>-0.29</td>
<td>-0.47</td>
</tr>
<tr>
<td>FFMI</td>
<td>-0.04</td>
<td>-0.08</td>
<td>0.04</td>
<td>0.07</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>Females (n=88)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.004</td>
<td>0.23</td>
<td>0.25</td>
<td>0.29</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.11</td>
<td>0.27</td>
<td>0.43</td>
<td>0.40</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>0.17</td>
<td>0.33</td>
<td>0.27</td>
<td>0.41</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.16</td>
<td>0.22</td>
<td>0.43</td>
<td>0.43</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>FMI</td>
<td>0.14</td>
<td>0.21</td>
<td>0.43</td>
<td>0.43</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Sum of SF</td>
<td>0.07</td>
<td>0.10</td>
<td>0.17</td>
<td>0.31</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>0.16</td>
<td>0.22</td>
<td>0.20</td>
<td>0.43</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
<td>0.18</td>
<td>0.17</td>
<td>-0.09</td>
<td>-0.002</td>
<td>-0.13</td>
<td></td>
</tr>
<tr>
<td>FFMI</td>
<td>0.18</td>
<td>0.15</td>
<td>0.33</td>
<td>0.33</td>
<td>0.29</td>
<td></td>
</tr>
</tbody>
</table>

All are sample-specific standardized variables. All models are adjusted for prenatal zinc, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity.

EFW = estimated fetal weight, HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular Tri = triceps, FFMI = fat free mass index. †P: 0.05-0.10, *P<0.05, **P<0.01, ***P<0.001.
# Table 4.4 Effect sizes of fetal weight gain (EFW) between 20 and 36 weeks of gestation on body size and composition at 4.5 y by sex

<table>
<thead>
<tr>
<th>Anthropometric measure (4.5 y)</th>
<th>Males (n=83)</th>
<th>Females (n=87)</th>
<th>EFW (weeks of gestation)</th>
<th>20</th>
<th>c24</th>
<th>c28</th>
<th>c32</th>
<th>c36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>0.18 (-0.02, 0.38)†</td>
<td>-0.09 (-0.30, 0.11)</td>
<td>0.15 (-0.07, 0.36)</td>
<td>0.17 (-0.03, 0.38)</td>
<td>0.19 (0.04, 0.34)*</td>
<td>1.0 (-1.0, 0.30)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.16 (-0.05, 0.38)</td>
<td>0.03 (-0.19, 0.26)</td>
<td>0.24 (0.03, 0.46)*</td>
<td>0.25 (0.09, 0.41)**</td>
<td>-0.01 (-0.22, 0.20)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>HC</td>
<td>0.18 (-0.03, 0.38)†</td>
<td>0.22 (0.001, 0.43)*</td>
<td>0.41 (0.21, 0.62)***</td>
<td>0.29 (0.14, 0.44)***</td>
<td>0.10 (-0.10, 0.30)</td>
<td></td>
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</tr>
<tr>
<td>BMI</td>
<td>0.08 (-0.16, 0.31)</td>
<td>-0.06 (-0.32, 0.19)</td>
<td>0.21 (-0.03, 0.45)†</td>
<td>0.21 (0.03, 0.39)*</td>
<td>-0.06 (-0.30, 0.17)</td>
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</tr>
<tr>
<td>FMI</td>
<td>0.09 (-0.15, 0.33)</td>
<td>-0.04 (-0.29, 0.21)</td>
<td>0.21 (-0.03, 0.45)†</td>
<td>0.22 (0.04, 0.41)*</td>
<td>-0.06 (-0.29, 0.18)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sum of SF</td>
<td>0.16 (-0.05, 0.38)</td>
<td>0.08 (-0.15, 0.31)</td>
<td>0.13 (-0.09, 0.35)</td>
<td>0.25 (0.08, 0.41)***</td>
<td>-0.03 (-0.24, 0.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>0.11 (-0.08, 0.31)</td>
<td>-0.01 (-0.21, 0.20)</td>
<td>0.24 (0.05, 0.44)*</td>
<td>0.22 (0.07, 0.36)**</td>
<td>-0.04 (-0.23, 0.16)</td>
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<tr>
<td>SS/Tri SF ratio</td>
<td>-0.02 (-0.28, 0.24)</td>
<td>-0.21 (-0.49, 0.06)</td>
<td>-0.18 (-0.44, 0.08)</td>
<td>-0.25 (-0.45, -0.06)*</td>
<td>-0.11 (-0.36, 0.15)</td>
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<td></td>
</tr>
<tr>
<td>FFMI</td>
<td>0.01 (-0.18, 0.21)</td>
<td>-0.12 (-0.32, 0.09)</td>
<td>0.14 (-0.05, 0.34)</td>
<td>0.11 (-0.04, 0.26)</td>
<td>-0.07 (-0.26, 0.12)</td>
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</table>

All are sample-specific standardized variables. All models are adjusted for prenatal zinc, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity.

c24, c28, c32, and c36 = conditional EFW at 24, 28, 32, and 36 weeks of gestation, HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular, FFMI = fat free mass index. †P: 0.05-0.10, *P<0.05, **P<0.01, ***P<0.001.
CHAPTER 4: INTRAUTERINE GROWTH AND BODY SIZE AND COMPOSITION

A

Change in anthropometric measure (SD) at 4.5y per sample-specific 1-SD increase in conditional HC at 32 weeks gestation

B

Change in anthropometric measure (SD) at 4.5y per sample-specific 1-SD increase in conditional AC at 32 weeks gestation
Figure 4.1 Mean effect sizes (and 95% CIs) for anthropometric measures for height, weight, head circumference (HC), body mass index (BMI), fat mass index (FMI), sum of skinfolds (SF), waist circumference (WC), subscapular (SS) / Triceps (Tri) SF ratio, and fat free mass index (FFMI) per sample-specific 1-SD increase in conditional HC (A), AC (B), and FL (C) at 32 weeks of gestation by sex (males = 83, females = 87). All models include HC at 20 weeks of gestation, and conditional HC at 24, 28, and 36 weeks of gestation, and are adjusted for prenatal zinc supplement, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity.
REFERENCES


### SUPPLEMENTAL TABLES

**Table S4.1** Effect sizes for fetal head circumference (HC) at 20, 24, 28, 32, or 36 weeks gestation, and body size and composition at 4.5 y by sex

<table>
<thead>
<tr>
<th>Anthropometric measure (4.5 y)</th>
<th>20</th>
<th>24</th>
<th>28</th>
<th>32</th>
<th>36</th>
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<tbody>
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<td>(n=84)</td>
<td>(n=87)</td>
<td>(n=87)</td>
<td>(n=88)</td>
<td>(n=88)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.23</td>
<td>0.21</td>
<td>0.41</td>
<td>0.28</td>
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</tr>
<tr>
<td></td>
<td>(0.04,0.42)*</td>
<td>(0.03,0.40)*</td>
<td>(0.23, 0.58)***</td>
<td>(0.09, 0.47)**</td>
<td>(0.16, 0.55)**</td>
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<tr>
<td>Weight</td>
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<td>0.12</td>
<td>0.28</td>
<td>0.30</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>(-0.01,0.40)†</td>
<td>(-0.08, 0.33)</td>
<td>(0.08, 0.47)**</td>
<td>(0.10, 0.50)**</td>
<td>(0.11, 0.53)**</td>
</tr>
<tr>
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<td>0.18</td>
<td>0.54</td>
<td>0.53</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
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<td>(-0.04, 0.39)</td>
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<td>(0.33, 0.73)***</td>
<td>(0.34, 0.76)***</td>
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<td>0.01</td>
<td>0.06</td>
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<td>0.20</td>
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<td>(-0.21, 0.23)</td>
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<td>(-0.01, 0.44)†</td>
<td>(-0.04, 0.43)</td>
</tr>
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<td>0.11</td>
<td>0.03</td>
<td>0.24</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
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<td>(-0.19, 0.25)</td>
<td>(0.01, 0.46)*</td>
<td>(0.05, 0.43)*</td>
<td>(0.07, 0.46)**</td>
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<tr>
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<td>0.19</td>
<td>0.23</td>
<td>0.23</td>
</tr>
<tr>
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<td>(-0.07, 0.34)</td>
<td>(-0.02, 0.39)†</td>
<td>(0.02, 0.45)*</td>
<td>(0.01, 0.46)*</td>
</tr>
<tr>
<td>WC</td>
<td>0.08</td>
<td>0.10</td>
<td>0.24</td>
<td>0.24</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>(-0.17,0.34)</td>
<td>(-0.09, 0.29)</td>
<td>(0.06, 0.42)*</td>
<td>(0.05, 0.43)*</td>
<td>(0.07, 0.46)**</td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
<td>-0.03</td>
<td>-0.17</td>
<td>-0.13</td>
<td>-0.32</td>
<td>-0.23</td>
</tr>
<tr>
<td></td>
<td>(-0.27,0.22)</td>
<td>(-0.42, 0.07)</td>
<td>(-0.38, 0.11)</td>
<td>(-0.56, -0.07)*</td>
<td>(-0.49, 0.03)†</td>
</tr>
<tr>
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<td>-0.02</td>
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<td>0.07</td>
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<td>(-0.20, 0.16)</td>
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<td>(-0.12, 0.26)</td>
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<td>Females</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
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<td>0.05</td>
<td>-0.01</td>
<td>0.22</td>
<td>0.12</td>
</tr>
<tr>
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<td>(-0.19, 0.18)</td>
<td>(0.04, 0.40)†</td>
<td>(-0.07, 0.31)</td>
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<tr>
<td>Weight</td>
<td>0.07</td>
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<td>0.16</td>
<td>0.35</td>
<td>0.28</td>
</tr>
<tr>
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<td>(-0.05, 0.35)</td>
<td>(-0.04, 0.36)</td>
<td>(0.16, 0.54)***</td>
<td>(0.09, 0.48)**</td>
</tr>
<tr>
<td>HC</td>
<td>0.13</td>
<td>0.36</td>
<td>0.28</td>
<td>0.39</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>(-0.08,0.33)</td>
<td>(0.19, 0.54)***</td>
<td>(0.10, 0.46)**</td>
<td>(0.22, 0.56)***</td>
<td>(0.18, 0.53)***</td>
</tr>
<tr>
<td>BMI</td>
<td>0.13</td>
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<td>0.24</td>
<td>0.35</td>
<td>0.33</td>
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<td>(-0.10,0.36)</td>
<td>(-0.03, 0.40)†</td>
<td>(0.03, 0.45)*</td>
<td>(0.45, 0.55)***</td>
<td>(0.12, 0.53)***</td>
</tr>
<tr>
<td>FMI</td>
<td>0.13</td>
<td>0.18</td>
<td>0.24</td>
<td>0.35</td>
<td>0.33</td>
</tr>
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<td>(-0.10,0.35)</td>
<td>(-0.03, 0.39)†</td>
<td>(0.03, 0.44)*</td>
<td>(0.16, 0.55)***</td>
<td>(0.12, 0.53)***</td>
</tr>
<tr>
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<td>0.10</td>
<td>0.15</td>
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<td>(-0.25, 0.19)</td>
<td>(-0.17, 0.26)</td>
<td>(-0.12, 0.31)</td>
<td>(-0.06, 0.37)</td>
</tr>
<tr>
<td>WC</td>
<td>-0.07</td>
<td>0.18</td>
<td>0.14</td>
<td>0.26</td>
<td>0.33</td>
</tr>
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<td>(-0.32,0.18)</td>
<td>(-0.06, 0.42)</td>
<td>(-0.10, 0.37)</td>
<td>(0.03, 0.49)†</td>
<td>(0.10, 0.56)**</td>
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<tr>
<td>SS/Tri SF ratio</td>
<td>0.28</td>
<td>0.07</td>
<td>0.01</td>
<td>-0.06</td>
<td>-0.11</td>
</tr>
<tr>
<td></td>
<td>(0.08,0.47)</td>
<td>(-0.13, 0.26)</td>
<td>(-0.18, 0.20)</td>
<td>(-0.25, 0.13)</td>
<td>(-0.30, 0.07)</td>
</tr>
<tr>
<td>FFMI</td>
<td>0.11</td>
<td>0.15</td>
<td>0.17</td>
<td>0.24</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>(-0.09,0.31)</td>
<td>(-0.04, 0.34)</td>
<td>(-0.02, 0.35)†</td>
<td>(0.06, 0.42)*</td>
<td>(0.06, 0.41)*</td>
</tr>
</tbody>
</table>

All are sample-specific standardized variables. All models are adjusted for prenatal zinc, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity at 13 weeks of gestation. HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular, FFMI = fat free mass index. †P: 0.05-0.10, *P<0.05, **P<0.01, ***P<0.001.
Table S4.2 Effect sizes for fetal abdominal circumference (AC) at 20, 24, 28, 32, or 36 weeks gestation, and body size and composition at 4.5 y by sex

<table>
<thead>
<tr>
<th>Anthropometric measure (4.5 y)</th>
<th>20</th>
<th>24</th>
<th>28</th>
<th>32</th>
<th>36</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>(n=84)</td>
<td>(n=87)</td>
<td>(n=87)</td>
<td>(n=88)</td>
<td>(n=88)</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.15 (-0.04, 0.35)</td>
<td>0.12 (-0.07, 0.32)</td>
<td>0.11 (-0.08, 0.30)</td>
<td>0.29 (0.11, 0.48)**</td>
<td>0.25 (0.06, 0.45)*</td>
</tr>
<tr>
<td>Weight</td>
<td>0.12 (-0.08, 0.33)</td>
<td>0.09 (-0.12, 0.30)</td>
<td>0.16 (-0.04, 0.37)</td>
<td>0.31 (0.11, 0.50)**</td>
<td>0.24 (0.03, 0.44)*</td>
</tr>
<tr>
<td>HC</td>
<td>0.15 (-0.07, 0.36)</td>
<td>0.20 (-0.02, 0.42)†</td>
<td>0.32 (0.11, 0.53)**</td>
<td>0.38 (0.17, 0.59)**</td>
<td>0.35 (0.14, 0.57)**</td>
</tr>
<tr>
<td>BMI</td>
<td>0.04 (-0.19, 0.26)</td>
<td>0.02 (-0.20, 0.24)</td>
<td>0.14 (-0.09, 0.36)</td>
<td>0.20 (-0.02, 0.42)†</td>
<td>0.14 (-0.08, 0.37)</td>
</tr>
<tr>
<td>FMI</td>
<td>0.05 (-0.18, 0.28)</td>
<td>0.03 (-0.19, 0.25)</td>
<td>0.14 (-0.08, 0.36)</td>
<td>0.22 (0.01, 0.44)*</td>
<td>0.16 (-0.06, 0.39)</td>
</tr>
<tr>
<td>Sum of SF</td>
<td>0.13 (-0.08, 0.33)</td>
<td>0.10 (-0.11, 0.31)</td>
<td>0.14 (-0.07, 0.35)</td>
<td>0.31 (0.11, 0.51)**</td>
<td>0.21 (0.01, 0.42)*</td>
</tr>
<tr>
<td>WC</td>
<td>0.08 (-0.11, 0.27)</td>
<td>0.09 (-0.10, 0.28)</td>
<td>0.19 (0.002, 0.37)*</td>
<td>0.30 (0.12, 0.48)**</td>
<td>0.20 (0.01, 0.39)*</td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
<td>-0.04 (-0.29, 0.20)</td>
<td>-0.09 (-0.34, 0.15)</td>
<td>-0.06 (-0.31, 0.18)</td>
<td>-0.34 (-0.57, -0.11)**</td>
<td>-0.31 (-0.55, -0.07)*</td>
</tr>
<tr>
<td>FFMI</td>
<td>-0.01 (-0.19, 0.17)</td>
<td>-0.02 (-0.20, 0.16)</td>
<td>0.08 (-0.10, 0.26)</td>
<td>0.07 (-0.10, 0.25)</td>
<td>0.03 (-0.14, 0.22)</td>
</tr>
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<td>Females</td>
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<td>(n=89)</td>
<td>(n=89)</td>
<td>(n=89)</td>
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<tr>
<td>Height</td>
<td>0.05 (-0.15, 0.25)</td>
<td>0.22 (0.03, 0.41)*</td>
<td>0.12 (-0.07, 0.31)</td>
<td>0.26 (0.08, 0.44)**</td>
<td>0.27 (0.09, 0.45)**</td>
</tr>
<tr>
<td>Weight</td>
<td>0.11 (-0.10, 0.32)</td>
<td>0.28 (0.08, 0.48)**</td>
<td>0.20 (-0.005, 0.40)†</td>
<td>0.41 (0.23, 0.59)***</td>
<td>0.37 (0.19, 0.56)***</td>
</tr>
<tr>
<td>HC</td>
<td>0.18 (-0.02, 0.37)†</td>
<td>0.33 (0.15, 0.51)**</td>
<td>0.24 (0.06, 0.43)*</td>
<td>0.38 (0.21, 0.56)***</td>
<td>0.31 (0.13, 0.49)**</td>
</tr>
<tr>
<td>BMI</td>
<td>0.13 (-0.10, 0.36)</td>
<td>0.24 (0.02, 0.46)*</td>
<td>0.20 (-0.02, 0.42)†</td>
<td>0.41 (0.21, 0.61)***</td>
<td>0.34 (0.14, 0.55)***</td>
</tr>
<tr>
<td>FMI</td>
<td>0.11 (-0.11, 0.33)</td>
<td>0.23 (0.02, 0.44)*</td>
<td>0.20 (-0.01, 0.41)†</td>
<td>0.40 (0.21, 0.59)***</td>
<td>0.35 (0.15, 0.54)***</td>
</tr>
<tr>
<td>Sum of SF</td>
<td>0.06 (-0.17, 0.28)</td>
<td>0.13 (-0.09, 0.35)</td>
<td>0.15 (-0.06, 0.37)</td>
<td>0.29 (0.09, 0.50)**</td>
<td>0.27 (0.07, 0.48)*</td>
</tr>
<tr>
<td>WC</td>
<td>0.14 (-0.10, 0.39)</td>
<td>0.22 (-0.02, 0.45)†</td>
<td>0.18 (-0.06, 0.41)</td>
<td>0.41 (0.19, 0.63)***</td>
<td>0.30 (0.08, 0.53)***</td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
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<td>0.17 (-0.02, 0.37)†</td>
<td>-0.11 (-0.31, 0.08)</td>
<td>-0.02 (-0.22, 0.17)</td>
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</tr>
<tr>
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<td>0.21 (0.02, 0.40)*</td>
<td>0.13 (-0.05, 0.32)</td>
<td>0.32 (0.14, 0.49)***</td>
<td>0.23 (0.05, 0.41)*</td>
</tr>
</tbody>
</table>

All are sample-specific standardized variables. All models are adjusted for prenatal zinc, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity at 13 weeks of gestation.

HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular, FFMI = fat free mass index. †P: 0.05-0.10, *P<0.05, **P<0.01, ***P<0.001.
### Table S4.3 Effect sizes for fetal femur diaphysis length (FL) at 20, 24, 28, 32, or 36 weeks gestation, and body size and composition 4.5 y by sex

<table>
<thead>
<tr>
<th>Anthropometric Measure (4.5 y)</th>
<th>FL (weeks of gestation)</th>
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<th>24</th>
<th>28</th>
<th>32</th>
<th>36</th>
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</thead>
<tbody>
<tr>
<td>Males</td>
<td>(n=84)</td>
<td>(n=87)</td>
<td>(n=87)</td>
<td>(n=88)</td>
<td>(n=88)</td>
<td></td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Males</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.06 (-0.15, 0.27)</td>
<td>0.02 (-0.19, 0.23)</td>
<td>0.06 (-0.16, 0.28)</td>
<td>0.17 (-0.04, 0.38)</td>
<td>0.17 (-0.06, 0.40)</td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>0.02 (-0.20, 0.24)</td>
<td>0.09 (-0.13, 0.32)</td>
<td>0.11 (-0.13, 0.34)</td>
<td>0.31 (0.09, 0.53)**</td>
<td>0.21 (-0.04, 0.46)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.06 (-0.30, 0.15)</td>
<td>-0.13 (-0.35, 0.10)</td>
<td>-0.07 (-0.31, 0.17)</td>
<td>-0.01 (-0.24, 0.22)</td>
<td>-0.02 (-0.27, 0.24)</td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>0.08 (-0.13, 0.29)</td>
<td>0.05 (-0.16, 0.26)</td>
<td>-0.001 (-0.23, 0.22)</td>
<td>0.11 (-0.11, 0.32)</td>
<td>0.17 (-0.07, 0.40)</td>
<td></td>
</tr>
<tr>
<td>WC</td>
<td>-0.01 (-0.20, 0.18)</td>
<td>-0.07 (-0.26, 0.12)</td>
<td>-0.03 (-0.23, 0.18)</td>
<td>0.06 (-0.13, 0.26)</td>
<td>0.09 (-0.12, 0.55)*</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.06 (-0.29, 0.17)</td>
<td>-0.10 (-0.33, 0.12)</td>
<td>-0.07 (-0.31, 0.17)</td>
<td>0.01 (-0.22, 0.24)</td>
<td>-0.0001 (-0.25, 0.25)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.06 (-0.29, 0.17)</td>
<td>-0.10 (-0.33, 0.12)</td>
<td>-0.07 (-0.31, 0.17)</td>
<td>0.01 (-0.22, 0.24)</td>
<td>-0.0001 (-0.25, 0.25)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>-0.10 (-0.28, 0.09)</td>
<td>-0.17 (-0.35, 0.004)**</td>
<td>-0.07 (-0.26, 0.12)</td>
<td>-0.06 (-0.25, 0.12)</td>
<td>-0.07 (-0.27, 0.13)</td>
<td></td>
</tr>
</tbody>
</table>

| Females                        | (n=88)                  | (n=88)      | (n=89)      | (n=89)      | (n=89)      |             |
| **Height**                     |                         |             |             |             |             |             |
| Weight                         | 0.13 (-0.11, 0.37)      | 0.12 (-0.10, 0.34) | 0.12 (-0.10, 0.33) | 0.28 (0.07, 0.49)** | 0.25 (0.04, 0.46)* |           |
| HC                             | 0.10 (-0.13, 0.33)      | 0.18 (-0.02, 0.39)** | 0.18 (-0.02, 0.37)** | 0.16 (-0.04, 0.36) | 0.12 (-0.08, 0.33) |           |
| BMI                            | 0.21 (-0.04, 0.46)      | 0.05 (-0.18, 0.29) | 0.13 (-0.10, 0.35) | 0.28 (0.06, 0.50)* | 0.14 (-0.10, 0.37) |           |
| BMI                            | 0.19 (-0.06, 0.44)      | 0.06 (-0.17, 0.29) | 0.12 (-0.11, 0.34) | 0.27 (0.05, 0.49)* | 0.15 (-0.08, 0.38) |           |
| BMI                            | 0.17 (-0.08, 0.43)      | 0.05 (-0.18, 0.29) | 0.24 (0.02, 0.46)* | 0.27 (0.05, 0.49)* | 0.17 (-0.06, 0.40) |           |
| **BMI**                        |                         |             |             |             |             |             |
| BMI                            | 0.19 (-0.09, 0.47)      | 0.14 (-0.12, 0.39) | 0.20 (-0.05, 0.45) | 0.32 (0.08, 0.56)* | 0.30 (0.05, 0.55)* |           |
| BMI                            | 0.23 (0.001, 0.49)*     | 0.10 (-0.11, 0.31) | -0.05 (-0.25, 0.15) | 0.05 (-0.15, 0.25) | -0.06 (-0.27, 0.15) |           |
| **BMI**                        |                         |             |             |             |             |             |
| SS/Tri SF ratio                | 0.21 (-0.003, 0.43)**   | 0.03 (-0.18, 0.23) | 0.11 (-0.08, 0.31) | 0.22 (0.03, 0.41)* | 0.07 (-0.13, 0.27) |           |

All are sample-specific standardized variables. All models are adjusted for prenatal zinc, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity at 13 weeks of gestation.

HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular, FFMI = fat free mass index. $\dagger P$: 0.05-0.10, *$P$<0.05, **$P$<0.01, ***$P$<0.001.
### Table S4.4 Effect sizes of fetal head circumference (HC) growth between 20 and 36 weeks gestation on body size and composition at 4.5 y by sex

<table>
<thead>
<tr>
<th>Anthropometric measure (4.5 y)</th>
<th>HC (weeks of gestation)</th>
<th>Males (n=83)</th>
<th>Females (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>c24</td>
<td>c28</td>
</tr>
<tr>
<td>Height</td>
<td>-0.10 (-0.30, 0.09)</td>
<td>0.08 (-0.10, 0.26)</td>
<td>0.02 (-0.13, 0.18)</td>
</tr>
<tr>
<td>Weight</td>
<td>0.004 (-0.20, 0.21)</td>
<td>0.15 (-0.04, 0.34)</td>
<td>0.14 (-0.03, 0.30)</td>
</tr>
<tr>
<td>HC</td>
<td>0.08 (-0.11, 0.26)</td>
<td>0.37 (0.20, 0.54)***</td>
<td>0.14 (-0.01, 0.28)†</td>
</tr>
<tr>
<td>BMI</td>
<td>0.07 (-0.16, 0.30)</td>
<td>0.17 (-0.04, 0.37)</td>
<td>0.19 (0.005, 0.37)*</td>
</tr>
<tr>
<td>FMI</td>
<td>0.07 (-0.16, 0.29)</td>
<td>0.17 (-0.04, 0.37)</td>
<td>0.19 (0.01, 0.37)</td>
</tr>
<tr>
<td>Sum of SF</td>
<td>-0.04 (-0.28, 0.20)</td>
<td>-0.02 (-0.25, 0.19)</td>
<td>0.07 (-0.12, 0.26)</td>
</tr>
<tr>
<td>WC</td>
<td>0.02 (-0.24, 0.27)</td>
<td>0.17 (-0.06, 0.40)</td>
<td>0.08 (-0.12, 0.29)</td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
<td>0.30 (0.09, 0.51)**</td>
<td>-0.09 (-0.28, 0.10)</td>
<td>-0.09 (-0.25, 0.08)</td>
</tr>
<tr>
<td>FFMI</td>
<td>0.07 (-0.13, 0.27)</td>
<td>0.13 (-0.05, 0.32)</td>
<td>0.12 (-0.04, 0.28)</td>
</tr>
</tbody>
</table>

All are sample-specific standardized variables. All models are adjusted for prenatal zinc, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity at 13 weeks of gestation.

c24, c28, c32, and c36 = conditional EFW at 24, 28, 32, and 36 weeks of gestation, HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular, FFMI = fat free mass index. †P: 0.05-0.10, *P<0.05, **P<0.01, ***P<0.001.
# Table S4.5

Effect sizes of fetal abdominal circumference (AC) growth between 20 and 36 weeks gestation on body size and composition at 4.5 y by sex

<table>
<thead>
<tr>
<th>Anthropometric Measure (4.5 y)</th>
<th>AC (weeks of gestation)</th>
<th>Males (n=83)</th>
<th>Females (n=87)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>c24</td>
<td>c28</td>
</tr>
<tr>
<td><strong>Height</strong></td>
<td>0.13 (-0.08, 0.34)</td>
<td>0.06 (-0.16, 0.29)</td>
<td>0.05 (-0.16, 0.26)</td>
</tr>
<tr>
<td><strong>Weight</strong></td>
<td>0.14 (-0.08, 0.36)</td>
<td>0.02 (-0.22, 0.26)</td>
<td>0.18 (-0.04, 0.41)</td>
</tr>
<tr>
<td><strong>HC</strong></td>
<td>0.13 (-0.08, 0.35)</td>
<td>0.18 (-0.05, 0.42)</td>
<td>0.32 (0.10, 0.54)**</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>0.08 (-0.16, 0.33)</td>
<td>-0.03 (-0.30, 0.24)</td>
<td>0.22 (-0.03, 0.47)†</td>
</tr>
<tr>
<td><strong>FMI</strong></td>
<td>0.09 (-0.15, 0.33)</td>
<td>-0.02 (-0.29, 0.25)</td>
<td>0.21 (-0.04, 0.47)†</td>
</tr>
<tr>
<td><strong>Sum of SF</strong></td>
<td>0.15 (-0.07, 0.37)</td>
<td>0.04 (-0.20, 0.28)</td>
<td>0.14 (-0.09, 0.36)</td>
</tr>
<tr>
<td><strong>WC</strong></td>
<td>0.10 (-0.09, 0.30)</td>
<td>0.02 (-0.19, 0.24)</td>
<td>0.23 (0.03, 0.43)*</td>
</tr>
<tr>
<td><strong>SS/Tri SF ratio</strong></td>
<td>-0.01 (-0.27, 0.25)</td>
<td>-0.15 (-0.44, 0.13)</td>
<td>-0.12 (-0.38, 0.15)</td>
</tr>
<tr>
<td><strong>FFMI</strong></td>
<td>0.03 (-0.17, 0.23)</td>
<td>-0.05 (-0.27, 0.17)</td>
<td>0.16 (-0.04, 0.37)</td>
</tr>
</tbody>
</table>

All are sample-specific standardized variables. All models are adjusted for prenatal zinc, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity at 13 weeks of gestation.

c24, c28, c32, and c36 = conditional EFW at 24, 28, 32, and 36 weeks of gestation, HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular, FFMI = fat free mass index. †P: 0.05-0.10, *P<0.05, **P<0.01, ***P<0.001.
### Table S4.6: Effect sizes of fetal femur diaphysis length (FL) growth between 20 and 36 weeks gestation on body size and composition at 4.5 y by sex

<table>
<thead>
<tr>
<th>Anthropometric Measure (4.5 y)</th>
<th>FL (weeks of gestation)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
<td>c24</td>
<td>c28</td>
<td>c32</td>
<td>c36</td>
</tr>
<tr>
<td><strong>Males (n=83)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.25 (0.04, 0.46)*</td>
<td>0.16 (-0.01, 0.34)†</td>
<td>0.03 (-0.15, 0.22)</td>
<td>0.18 (-0.005, 0.37)†</td>
<td>0.11 (-0.09, 0.32)</td>
</tr>
<tr>
<td>Weight</td>
<td>0.09 (-0.15, 0.32)</td>
<td>0.03 (-0.17, 0.23)</td>
<td>0.02 (-0.19, 0.23)</td>
<td>0.16 (-0.05, 0.37)</td>
<td>0.04 (-0.19, 0.27)</td>
</tr>
<tr>
<td>HC</td>
<td>0.12 (-0.11, 0.34)</td>
<td>0.19 (-0.002, 0.38)†</td>
<td>-0.02 (-0.22, 0.18)</td>
<td>0.35 (0.14, 0.55)**</td>
<td>0.03 (-0.20, 0.25)</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.08 (-0.33, 0.18)</td>
<td>-0.08 (-0.29, 0.14)</td>
<td>-0.005 (-0.23, 0.22)</td>
<td>0.09 (-0.14, 0.32)</td>
<td>-0.02 (-0.27, 0.24)</td>
</tr>
<tr>
<td>FMI</td>
<td>-0.06 (-0.32, 0.19)</td>
<td>-0.05 (-0.27, 0.17)</td>
<td>-0.02 (-0.25, 0.20)</td>
<td>0.11 (-0.12, 0.34)</td>
<td>-0.01 (-0.27, 0.24)</td>
</tr>
<tr>
<td>Sum of SF</td>
<td>0.09 (-0.14, 0.33)</td>
<td>0.06 (-0.14, 0.26)</td>
<td>-0.07 (-0.28, 0.14)</td>
<td>0.11 (-0.10, 0.32)</td>
<td>0.09 (-0.14, 0.32)</td>
</tr>
<tr>
<td>WC</td>
<td>-0.01 (-0.23, 0.20)</td>
<td>-0.06 (-0.24, 0.12)</td>
<td>0.02 (-0.17, 0.21)</td>
<td>0.08 (-0.12, 0.27)</td>
<td>0.06 (-0.15, 0.27)</td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
<td>-0.04 (-0.33, 0.24)</td>
<td>-0.09 (-0.32, 0.15)</td>
<td>0.004 (-0.25, 0.25)</td>
<td>-0.01 (-0.27, 0.24)</td>
<td>-0.05 (-0.33, 0.24)</td>
</tr>
<tr>
<td>FFMI</td>
<td>-0.11 (-0.31, 0.09)</td>
<td>-0.14 (-0.31, 0.03)</td>
<td>0.05 (-0.12, 0.23)</td>
<td>0.02 (-0.16, 0.20)</td>
<td>-0.02 (-0.23, 0.18)</td>
</tr>
<tr>
<td><strong>Females (n=87)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>-0.03 (-0.24, 0.19)</td>
<td>0.24 (0.06, 0.41)**</td>
<td>0.01 (-0.17, 0.19)</td>
<td>0.11 (-0.05, 0.27)</td>
<td>0.22 (0.03, 0.40)*</td>
</tr>
<tr>
<td>Weight</td>
<td>0.12 (-0.11, 0.36)</td>
<td>0.13 (-0.06, 0.33)</td>
<td>0.02 (-0.18, 0.21)</td>
<td>0.21 (0.03, 0.38)*</td>
<td>0.10 (-0.10, 0.30)</td>
</tr>
<tr>
<td>HC</td>
<td>0.11 (-0.12, 0.33)</td>
<td>0.15 (-0.04, 0.34)</td>
<td>0.06 (-0.13, 0.25)</td>
<td>0.04 (-0.13, 0.25)</td>
<td>-0.01 (-0.20, 0.19)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.19 (-0.07, 0.44)</td>
<td>0.02 (-0.19, 0.23)</td>
<td>0.01 (-0.20, 0.23)</td>
<td>0.21 (0.02, 0.40)*</td>
<td>-0.03 (-0.25, 0.19)</td>
</tr>
<tr>
<td>FMI</td>
<td>0.17 (-0.08, 0.42)</td>
<td>0.04 (-0.17, 0.24)</td>
<td>0.01 (-0.20, 0.22)</td>
<td>0.21 (0.02, 0.39)*</td>
<td>-0.01 (-0.22, 0.20)</td>
</tr>
<tr>
<td>Sum of SF</td>
<td>0.16 (-0.09, 0.41)</td>
<td>-0.02 (-0.22, 0.19)</td>
<td>0.19 (-0.02, 0.40)†</td>
<td>0.15 (-0.03, 0.34)</td>
<td>0.002 (-0.21, 0.22)</td>
</tr>
<tr>
<td>WC</td>
<td>0.19 (-0.09, 0.46)</td>
<td>0.10 (-0.13, 0.33)</td>
<td>0.10 (-0.14, 0.33)†</td>
<td>0.19 (-0.01, 0.40)</td>
<td>0.12 (-0.12, 0.35)</td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
<td>0.22 (-0.005, 0.44)†</td>
<td>0.03 (-0.15, 0.22)</td>
<td>-0.26 (-0.44, -0.07)**</td>
<td>0.03 (-0.13, 0.20)</td>
<td>-0.14 (-0.32, 0.05)</td>
</tr>
<tr>
<td>FFMI</td>
<td>0.19 (-0.03, 0.41)</td>
<td>-0.04 (-0.22, 0.14)</td>
<td>0.02 (-0.16, 0.20)</td>
<td>0.16 (-0.003, 0.32)†</td>
<td>-0.08 (-0.26, 0.11)</td>
</tr>
</tbody>
</table>

All are sample-specific standardized variables. All models are adjusted for prenatal zinc, gestational age at delivery, maternal age, maternal height, maternal education, and maternal parity at 13 weeks of gestation.

c24, c28, c32, and c36 = conditional EFW at 24, 28, 32, and 36 weeks of gestation, HC = head circumference, BMI = body mass index, FMI = fat mass index, SF = skinfold thickness, WC = waist circumference, SS = subscapular, FFMI = fat free mass index. †P: 0.05-0.10, *P<0.05, **P<0.01, ***P<0.001.
CHAPTER 5: EFFECT OF MATERNAL ZINC SUPPLEMENTATION ON THE CARDIOMETABOLIC PROFILE OF PERUVIAN CHILDREN AT 4.5 YEARS: RESULTS FROM A PRENATAL RANDOMIZED CLINICAL TRIAL

ABSTRACT

Introduction: Maternal zinc insufficiency has been associated with poor renal function, altered insulin response to glucose, and increased systolic blood pressure, as well as aspects of body composition.

Aims: This study evaluated the effect of maternal zinc supplementation during pregnancy on the cardiometabolic profile in childhood.

Study design: Pregnant women were randomly assigned to receive a daily supplement containing iron + folic acid, with or without zinc, and a follow-up study when children of participating mothers were 4.5 years of age.

Subjects: One hundred and fifty-nine Peruvian children whose mothers were part of a prenatal zinc supplementation trial.

Outcome measures: Cardiometabolic profile at 4.5 years of age, including anthropometric measures of body size and composition, blood pressure, lipid profile, and insulin resistance.

Results: No difference in measures of child cardiometabolic risk at 4.5 years depending on whether mothers received supplemental zinc during pregnancy.

Conclusions: Our results do not support the hypothesis that maternal zinc supplementation reduces the risk of offspring cardiometabolic disease.
INTRODUCTION

There is increasing evidence that nutritional insufficiency in early life is one additional risk factor for cardiometabolic diseases in adulthood (1, 2). Size at birth is inversely associated with high blood pressure, type 2 diabetes, and ischaemic heart disease in adulthood, but the specific mechanisms explaining these associations are not fully understood (3-5). It has been proposed that maternal micronutrient status during pregnancy affects fetal growth, organogenesis, and differentiation, leading to differences in cardiometabolic functional outcomes throughout life (6). Roles for vitamin A, vitamin B12, folate, iron, and zinc in the development of renal, cardiovascular, and pancreatic function, as well as in aspects of body composition, have been described (6). Of particular interest is zinc. Studies in animals have demonstrated that prenatal zinc restriction is associated with decreased number and size of nephrons, glomerular filtration rate, lean body mass, and altered insulin response to glucose, and increased systolic blood pressure and body fat (7, 8). In humans, the prenatal effect of zinc deficiency on the risk of cardiometabolic diseases later in life is less well studied, although prenatal zinc supplementation has been reported to result in a reduction in the risk of microalbuminuria and peripheral adiposity at 8 years of age (9, 10).

Half of the world’s population is estimated to be at risk for low zinc intake, and approximately one-third of the population is zinc deficient (11, 12). The global prevalence of zinc deficiency among pregnant women is unknown, but an estimated 82% of women worldwide do not consume the recommended intake of zinc during pregnancy (13). In most low- or middle-income countries, the average zinc intake of pregnant women is below the Estimated Average Requirements (EAR) (14).

In Peru, the burden due to obesity, cardiovascular disease, and diabetes is on the rise. Cardiovascular disease is the top cause of mortality (15.4% of all deaths), and it has been estimated that around 15% of the adult population has high blood pressure, 7% has raised blood sugar, 20% has dyslipidemia, and 20% is obese (15-17). In addition, it has been estimated that 80-
88% of pregnant women consume inadequate amounts of zinc (18). In this context, where zinc requirements during pregnancy are usually not met and chronic diseases are increasing, it is important to evaluate the effects of maternal zinc nutrition on cardiometabolic disease in offspring. We previously reported that prenatal supplementation with zinc (in addition to iron and folic acid) is associated with changes in heart rate (HR) measures during the fetal period, which continue into early childhood (19, 20). We showed that fetuses from mothers who received prenatal zinc supplementation (25 mg/d) had a steeper increase in fetal HRV and steeper decrease in fetal heart rate (FHR) throughout pregnancy, as compared to fetuses of mothers who did not receive supplemental zinc (19). These differences persisted at 4.5 years of age (n = 165) (20). Lower HR and greater HRV are interpreted as evidence of greater parasympathetic control of cardiac function. Both low HR and increased HRV have been associated with lower risk of obesity, high blood pressure, dyslipidemia, and type 2 diabetes, and with all-cause mortality including cardiovascular disease in adults (21). To further understand the role of zinc in the development of cardiometabolic diseases, in this report we examine the effects of prenatal zinc supplementation on the risk of metabolic syndrome and its individual components in early childhood (4.5 years). These are known to increase the risk of cardiovascular disease and diabetes in adults, and track from childhood to adulthood (22).

**METHODS**

**Study design and participants**

Between 1998 and 2000, a double-blind, randomized clinical trial was conducted in 242 pregnant women in a periurban area of Lima, Peru, to assess the effect of prenatal zinc supplementation on fetal neural development and growth. When the children were approximately 4.5 years of age, they participated in a follow-up study to evaluate the long-term effect of supplementation on health, nutritional, and developmental outcomes. Results for these primary
outcomes have been previously reported (19, 20, 23). The focus of this report is the effect of prenatal zinc supplementation on the cardiometabolic profile of these children at 4.5 years.

Details of recruitment, enrollment, and supplementation are provided in earlier reports (19, 20, 23). Briefly, women who were receiving care at the Hospital Materno Infantil San José (Villa el Salvador, Lima, Peru), who were classified with a low-risk pregnancy, carrying a singleton fetus, and had lived on the Coast of Peru for at least 6 months before pregnancy were eligible for the study. Participants were enrolled between 10-16 weeks of gestation, and randomized within parity (primipara/multipara) and week of gestation (10-13/14-16 wk) strata to receiving 60 mg iron (ferrous sulfate) and 250 µg folic acid, with or without 25 mg of zinc (zinc sulfate), daily throughout pregnancy.

Follow-up assessments

In 2003, children whose mothers participated in the prenatal supplementation trial were invited to take part in follow-up assessments. The evaluation was completed by trained study health professionals over two visits. The protocol included interviews with the caregiver, review of clinical records to collect information on socioeconomic conditions of the family, and the health, nutritional, and developmental history of the child, as well as a health exam, a nutritional evaluation, and behavioral and developmental testing. Anthropometric measures were conducted by a trained anthropometrist following standard procedures (24). Standing height of the child was measured to the nearest 0.1 cm, using a stadiometer, and weight was recorded on a digital scale with 0.1 kg precision (Seca, Hamburg, Germany). Body Mass Index was calculated as BMI = \[\text{weight (kg) / height (m)}^2\]. BMI-for-age Z-scores were calculated, using the World Health Organization (WHO) 2006 growth standards (25). Circumferences (waist, chest, mid-upper arm, calf) were measured to the nearest 0.1 cm with a measuring tape (Seca, Hamburg, Germany). Fat mass (FM) was estimated from total body water (FM = TBW + Fat free mass) from an equation developed in Peruvian children (TBW = 0.276*weight (kg) + 0.105*height (cm) + 0.051* chest
circumference (cm) – 0.319 (sex, female = 1) – 6.134) and validated using $^{18}$O dilution (26). Fat mass index was calculated as FMI = [FM (kg) / height (m) $^2$]. Blood pressure was measured, in triplicate, by the study physician with the child in a seated position at 1-min intervals using a pediatric sphygmomanometer (Reister, Jungingen, Germany). For the analyses, we used the mean value of the three measures. Mean arterial blood pressure was calculated as MAP = [diastolic pressure + (systolic pressure – diastolic pressure) / 3]. Blood pressure percentiles were calculated using the U.S. reference population, according to sex, age, and height-for-age percentile of the child (27). Height-for-age percentiles were calculated using the CDC 2000 growth reference, which were then used to estimate age- and sex-appropriate blood pressure percentiles, using the U.S. blood pressure reference curves (27).

Children were asked to fast overnight and on the following morning they were picked up from their home and brought to the study clinic where the study phlebotomist collected venous blood samples. Blood samples were drawn into tubes containing heparin, and within 30 minutes the samples were centrifuged at 600 g for 10 min for separation of plasma. Plasma samples were frozen at -20° C until micronutrient analyses were conducted. In 2011, the remaining frozen plasma samples were thawed to conduct the analyses presented here. A Cholestech LDX analyzer (Cholestech Corporation, Hayward, California) was used to measure total and HDL-cholesterol, triglycerides, and glucose concentrations. LDL cholesterol concentration was calculated using the Friedewald equation (28). Plasma insulin concentration was measured using an ultrasensitive immunoassay (Alpco Diagnostics, Salem, New Hampshire). Insulin resistance was estimated by using the homeostasis model assessment, HOMA-IR = fasting plasma insulin concentration (mU/L) X fasting plasma glucose (mmol/L) / 22.5 (29).

Data analyses

Data analyses were conducted using Stata 12.0 (Stata Corporation, College Station, Texas). Selected maternal characteristics, including maternal age, anthropometry during
pregnancy, parity, and education, and child characteristics at birth, including gestational age, weight, and length, were compared between the treatment groups (iron + folate, or iron + folate + zinc) using t-tests or chi-square tests, as appropriate. We also compared maternal and child characteristics at birth among participants and nonparticipants of the follow-up study, and those with complete or missing data on cardiometabolic risk factors at follow-up. No statistically significant differences were observed ($P > 0.05$). To assess the association between measures of body size and composition and cardiometabolic factors, we used correlation analysis. For normally distributed variables (measures of anthropometry and blood pressure) we used Pearson’s correlation coefficients, and for non-normally distributed variables (lipid profile and insulin resistance measures) we used Spearman’s correlation coefficients. Because there is no accepted definition of metabolic syndrome for children under 10 years of age, we used modified criteria of the National Cholesterol Education Program (NCEP) ATP III guidelines for adults to identify children who are at risk of metabolic syndrome, as described by Stewart et al. (9, 30). Children were considered to be at risk of metabolic syndrome if they met 3 or more of the following criteria: 1) abdominal obesity, defined as WC ≥ 90th percentile of the reference population (third National Health and Nutrition Examination Survey, NHANES III: 58.3 cm for girls and 57.6 cm for boys) (31); 2) high triglycerides, defined as TG ≥ 95th percentile of the reference population (American Academy of Pediatrics: 120 mg/dL [1.37 mmol/L] for girls and 85 mg/dL [0.97 mmol/L] for boys) (32); 3) low HDL-cholesterol, defined as HDL-cholesterol < 5th percentile of the reference population (American Academy of Pediatrics: 36 mg/dL [2.0 mmol/L] for girls and 38 mg/dL [2.1 mmol/L] for boys) (32); 4) high blood pressure, defined as SBP or DBP ≥ 90th percentile of the reference population (age-, sex-, and height-specific) (33); and, 5) high glucose, defined as fasting plasma glucose ≥ 5.6 mmol/L (American Diabetes Association) (34).

Anthropometric and blood pressure measures were normally distributed; therefore, we used the t-test to compare differences by treatment group; insulin resistance measures followed a
CHAPTER 5: MATERNAL ZINC SUPPLEMENTATION AND CARDIOMETABOLIC PROFILE AT 4.5 YEARS

log-normal distribution, hence we used the Wilcoxon ranksum test. A total of 17 children had triglycerides, total cholesterol, or HDL-cholesterol concentrations outside the detectable limit of the assay (triglycerides <0.51 mmol/L or >7.34 mmol/L, total cholesterol <2.59 mmol/L or >12.9 mmol/L, HDL-cholesterol <0.39 mmol/L or >2.59 mmol/L); we used tobit regression models for censored data to compare differences by supplement type (35). Because measures of lipid profile do not follow a normal distribution, they were transformed to normal using the ladder of powers developed by Tukey to meet normality assumptions for tobit regression (35, 36).

To compare differences in the proportion of children at risk of metabolic syndrome or any of its individual components, according to treatment type, we developed logistic regression models for each of the outcomes of interest (abdominal obesity, high triglycerides, low HDL-cholesterol, high blood pressure, insulin resistance, and at risk of metabolic syndrome). All models were adjusted for age and sex of the child. Interaction terms between treatment type and age or sex were tested, found not to be significant \( (p > 0.10) \), and, therefore, were excluded from the final models. With our final sample size \( (n = 159) \), and assuming 80% power and a 2-tail significance level of 0.05, the smallest difference we could detect between mean values of continuous outcomes was 0.4 SD; a difference of 0.41 is considered the recommended minimum effect size. Under the same assumptions, we could detect a reduction of 90% \( (OR = 0.1) \) in the odds of metabolic syndrome or any of its individual components associated with zinc supplementation; this difference is considered to correspond to a very large effect size (37).

The design and methods of the pregnancy and the follow-up study were approved by the Institutional Review Boards of the Instituto de Investigación Nutricional, Lima, Peru, and The Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland.

RESULTS

Figure 5.1 shows the enrollment and losses to follow-up of study participants by supplement type. Of the 242 women enrolled in the supplementation trial, 20 were lost to follow-
up, and 27 were excluded from the original analyses, due to significant obstetrical or medical complications. Therefore, 195 (80.6%) were included in the formal analysis (94 in the zinc group and 101 in the control group). The 195 participants included in the formal analysis, and 10 of the 27 excluded participants (who completed the protocol and whose babies survived the neonatal period and were free of congenital malformations) were identified for follow up. In all, 184 children (90%) were located and evaluated. Of these, 20 had no frozen plasma samples to conduct biochemical measurements, and 5 had missing information on blood pressure. For some cases, plasma samples were missing due to refusal of the blood draw at follow-up or due to insufficient volume to conduct these biochemical analyses. The present report is, therefore, restricted to 159 children with complete biochemical and blood pressure data, 86 (85.1%) from the control group and 73 (77.7%) from the treatment group. Missing data between treatment groups were non-differential ($p=0.67$). Table 5.1 shows selected characteristics of study participants at enrollment. Participants were similar in maternal and child characteristics at baseline.

Shown in table 5.2 are the measures of central tendency and dispersion of the cardiometabolic risk factors. There were no statistically significant differences in variables related to anthropometry and body composition, lipid profile, or insulin resistance according to treatment group ($p > 0.05$). The sex-specific correlations between selected anthropometric measures of body size and composition and measures of lipid profile, blood pressure, and insulin resistance are presented in table S5.1, supplemental table). There were no statistically significant associations between anthropometric measures and measures of lipid profile or blood pressure ($p > 0.05$). Among males, there was a trend of an inverse association between anthropometric measures and measures of insulin resistance, which reached statistical significance only for weight and WC with insulin and HOMA-IR. On the other hand, among females, there was a trend for a positive association between anthropometric measures and measures of insulin resistance, which reached significance for weight, BMI, FMI, the sum of 4 SF, WC, and FFMI, with insulin and HOMA-IR.
Selected cardiometabolic risk factors and risk of metabolic syndrome, in study children, by prenatal supplement type, are shown in table 5.3. A total of 29 (18.2%) children had waist circumference $\geq 90^{\text{th}}$ percentile of the reference population, 31 (19.5%) children had triglycerides $\geq 95^{\text{th}}$ percentile of the reference population, 119 (74.8%) had HDL-cholesterol concentration $< 5$th percentile of the reference population, 38 (23.9%) had SBP or DBP $> 90^{\text{th}}$ percentile of reference population, 8 (5.0%) had plasma glucose $\geq 5.6$ mmol/L, and 16 (10.1%) children met the definition of at risk for metabolic syndrome. A total of 20 (12.6%) children had no cardiometabolic risk factors present, 71 (44.7%) had 1, 52 (32.7%) had 2, 14 (8.8%) had 3, 2 (1.3%) had 4, and no children had all cardiometabolic risk factors present. Of the 38 children who were classified as having high blood pressure, 35 (92%) had high DBP. Of the 16 children who met the definition of being at risk for metabolic syndrome, 15 (94%) had low HDL-cholesterol, 12 (75%) had high blood pressure, 10 (63%) had high triglycerides, 7 (44%) had high waist circumference, and 6 (38%) had high glucose concentration. There were no statistically significant differences in the risk of developing metabolic syndrome or any of its individual components, according to prenatal supplement type.

DISCUSSION
We examined the effect of prenatal zinc supplementation on the cardiometabolic profile of Peruvian children at 4.5 years of age. In this study, zinc supplementation did not affect measures of anthropometry, lipid profile, blood pressure, and insulin resistance of participants. No differences according to supplement type were observed when analyzing the measures as continuous variables, or using a definition of ‘at risk of’ metabolic syndrome or any of its individual components (abdominal obesity, high triglycerides, low HDL-cholesterol, high blood pressure, and high glucose).

We know of only one other study which has evaluated the long-term effects of prenatal zinc supplementation on cardiometabolic outcomes in childhood. Stewart et al. examined the
effect of prenatal micronutrient supplementation on the risk of metabolic syndrome in children 6-8 years of age in rural Nepal (9). Specifically, they examined the effect of prenatal supplementation with 1) vitamin A (control), 2) folic acid (400 µg), 3) folic acid with iron (60 mg), 4) folic acid with iron and zinc (30 mg), and, 5) a multiple micronutrient supplement containing folic acid, iron, zinc, and additional minerals and vitamins. In agreement with our study, prenatal supplementation with folic acid, iron, and zinc did not affect measures of blood pressure, lipid profile, or insulin resistance in children as compared to a control group. However, there was a significant reduction in microalbuminuria – a marker of kidney dysfunction and risk factor for cardiovascular disease – in the groups receiving folic acid (OR; 95% CI: 0.56; 0.33, 0.93), or folic acid, iron, and zinc (OR; 95% CI: 0.53; 0.32, 0.89), as compared to the control group. Even if the reduction in microalbuminuria was somewhat greater in the group receiving supplemental zinc, the effect observed cannot be attributed to zinc directly because the authors did not explicitly contrast the group receiving iron + folic acid with that receiving iron + folic acid + zinc, which is the contrast in our study.

There are two other studies examining the effect of prenatal multiple micronutrient supplementation (containing 15 mg of zinc) on individual cardiometabolic outcomes, particularly blood pressure, with contradictory results (38, 39). A study conducted in rural Bangladesh found that prenatal supplementation with multiple micronutrients was associated with higher diastolic blood pressure at 4.5 years (0.87 mmHg; 95% CI: 0.18, 1.56) when compared to those receiving folic acid (400 µg) and iron (30 mg or 60 mg) (36). No differences were found, however, between the prenatal treatment groups in systolic blood pressure, kidney volume, or glomerular filtration rate. A second study, also conducted in Nepal, found that prenatal supplementation with multiple micronutrients was associated with lower systolic blood pressure at 2.5 years (2.5 mmHg; 95% CI: 0.5, 4.6), when compared to those receiving folic acid (400 µg) with iron (60 mg) (37). In both studies, the dose of zinc used was lower than the one in our study and, because zinc was one
of the multiple micronutrients included in the supplement, the changes in blood pressure cannot
be attributed solely to zinc.

The role of zinc status in the development of cardiometabolic diseases has been studied
more frequently for the postnatal period, but the evidence is not conclusive. Results from
observational and intervention studies suggest that zinc deficiency may play a role in the
development of insulin resistance, in abnormalities in lipid metabolism, and in cardiovascular
risk. Observational studies in humans in adults from developing countries have shown that lower
serum zinc concentrations and lower consumption of dietary zinc are associated with an increased
prevalence of hypertension, hypertriglyceridemia, coronary artery disease, and diabetes (40, 41).
Results from interventional studies also suggest a beneficial effect of zinc supplementation in
response to metabolic and lipid metabolism, but mainly among adults with conditions known to
influence zinc metabolism (42-44). We are aware of three different meta-analyses of randomized
controlled trials examining the effect of supplemental zinc on cardiometabolic outcomes among
adults. One found a modest but significant overall reduction in fasting glucose concentrations
after zinc supplementation, with a greater effect among patients with type 2 diabetes, metabolic
syndrome, and obesity, as compared to the effect observed in healthy individuals (42). A second
meta-analysis conducted among patients with type 2 diabetes found that zinc supplementation
was associated with a reduction in fasting blood glucose, 2 hour post-prandial blood glucose,
HbA1c, total cholesterol, and LDL-cholesterol (43). A third meta-analysis found no overall effect
of zinc supplementation on markers of lipid metabolism (44). However, when stratifying the
analysis according to health status, a decrease in HDL-cholesterol among healthy subjects, but an
increase in HDL-cholesterol among subjects with type 2 diabetes or those undergoing
hemodialysis was observed among zinc-supplemented individuals, as compared to those
receiving placebo.

There are several strengths of our study that should be mentioned. First, the sample was
drawn from a randomized, double-blind, controlled trial. Second, this is a long-term follow-up
study evaluating the effect of prenatal supplementation on children 4.5 years of age, in which
durable effects of prenatal zinc supplementation on autonomic function during childhood were
demonstrated. Here we expanded our evaluation to consider whether differences in related
parameters of cardiometabolic risk would be detectable. Third, we evaluated cardiometabolic
outcomes combined (being at risk of metabolic syndrome), and each of its individual components
(anthropometry, lipid profile, blood pressure, and insulin resistance), allowing us to test the
effects of zinc on different cardiometabolic domains.

Our study does have limitations. Because there is no standard definition of metabolic
syndrome in children under 10 years of age, we used modified criteria available for adults with
modified cut-offs appropriate for children, to identify children who are at risk of metabolic
syndrome, therefore making comparisons with other studies, challenging. Another limitation is
related to the number of missing values for the cardiometabolic outcomes, due to the non-
availability of frozen plasma samples to conduct biochemical analyses ($n = 20$). Even if the
missingness was not differential by treatment group, it decreased our sample size and, therefore,
our power to detect differences between the two groups. As mentioned earlier, with our final
sample size ($n = 159$) we could detect a reduction in the risk of metabolic syndrome or any of its
individual components of 90% or greater, which is a very large effect size. However, we also
analyzed the outcomes of interest in a continuous scale, which provided enough power to detect
differences of 0.4 SD or greater. These differences are considered to correspond to small effect
sizes; therefore, relevant differences in cardiometabolic outcomes between the two groups would
have been detected. Second, we conducted measurements of lipids, glucose, and insulin on
plasma samples which had been frozen for several years, and concentrations can decrease
progressively with time (45-47). However, because the concentrations of metabolites assessed are
within normal values for children of their age and sex, it is likely that only minimal degradation
occurred (48). Moreover, there is no reason to think that degradation occurred differentially by
treatment group. As a result of the method used to measure the concentration of lipids in plasma,
we were unable to detect values below or above particular limits; however, because censored data is more informative than missing data, we were able to impute those values and still include them in the analysis.

In this study, prenatal supplementation with zinc had no discernible effects on cardiometabolic parameters or risk of disease at 4.5 years of age. These children may be too young for any changes to be observed in the cardiometabolic measures used; additional follow-up at later ages may reveal detectable differences. By not using markers that can identify changes in endothelial dysfunction or insulin resistance at earlier stages, such as intima media thickness and distensibility, or glucose tolerance and HbA1c, it is possible that we missed early differences by supplement type (49, 50). It has been suggested that rapid weight gain in infancy needs to be present for prenatal nutritional insults to have an effect on the risk of components of metabolic syndrome later in life; fast weight gain in infancy is not seen in this population (51-54). It is also possible that a stronger clustering of cardiometabolic factors is needed for a long-term effect of prenatal zinc to be observed. The degree of clustering of cardiometabolic factors varies with age, with stronger clustering and associations occurring in adulthood as compared to childhood (55).

Our findings are in line with this suggestion; 14 (8.8%) children had 3 cardiometabolic risk factors present, 2 (1.2%) had 4, and none had all 5 factors present. Central adiposity and insulin resistance are considered instrumental in the development of the metabolic syndrome (56). In our study, abdominal obesity and glucose intolerance were observed in few participants – out of the 16 children classified as at risk of metabolic syndrome, only 7 (44%) had abdominal obesity and 6 (38%) had high glucose – therefore it is possible that these children are too young to present alterations in these components. In addition, in our study population lipid metabolism components had a high contribution when classifying children as being at risk of metabolic syndrome –, 15 (94%) had low HDL-cholesterol and 10 (63%) had high triglycerides. HDL-cholesterol concentrations have also been reported to be low in children from developing countries, and are particularly low as compared to those in U.S. children (9). These differences
could be attributed to specific components of the local diet; however, ethnic differences cannot be ruled out and, therefore, the clinical relevance of these low HDL-cholesterol concentrations in these populations is unknown. It is also possible that the zinc effects are only observed when classifying individuals by “normal” or “at risk” status, and we may not have had adequate power to detect potentially important differences; we found no evidence of differences according to supplement type, when comparing cardiometabolic risk factors on a continuous scale, yet we observed a tendency of a protective effect of zinc on abdominal obesity, low HDL, and high glucose, when comparing these risk factors in a dichotomous scale which did not reach statistical significance. Unlike the observed effects on autonomic function, there is no established developmental role of prenatal zinc status on cardiometabolic profile.

In conclusion, our study found no difference in measures of child cardiometabolic risk at 4.5 years depending on whether mothers received supplemental zinc during pregnancy. Data currently available do not support the hypothesis that maternal zinc supplementation reduces the risk of offspring cardiometabolic disease, other than our previously reported findings on autonomic function. Because of the uniqueness of the study design and the findings on autonomic function, it would be important to evaluate the effect of prenatal zinc supplementation at older ages, and include additional markers of cardiometabolic diseases that may identify earlier changes in dyslipidemia, endothelial dysfunction, and insulin resistance.
### Table 5.1 Selected profile characteristics during pregnancy and at delivery by prenatal supplement type for 159 Peruvian children

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Maternal supplement type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron + folate</td>
</tr>
<tr>
<td>Maternal†</td>
<td>86</td>
</tr>
<tr>
<td>Age, y</td>
<td>23.4±4.9</td>
</tr>
<tr>
<td>Height, cm</td>
<td>152.4±5.1</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.6±3.4</td>
</tr>
<tr>
<td>Primiparity, %</td>
<td>57.0</td>
</tr>
<tr>
<td>Maternal education, %</td>
<td></td>
</tr>
<tr>
<td>Primary or less</td>
<td>4.9</td>
</tr>
<tr>
<td>Secondary incomplete</td>
<td>28.0</td>
</tr>
<tr>
<td>Secondary complete</td>
<td>50.0</td>
</tr>
<tr>
<td>Beyond secondary</td>
<td>17.1</td>
</tr>
<tr>
<td>Birth</td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth, wk</td>
<td>39.3±1.2</td>
</tr>
<tr>
<td>Weight at birth, g</td>
<td>3277±386</td>
</tr>
<tr>
<td>Length at birth, cm</td>
<td>49.7±1.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD or percent; † evaluated at a mean age of 13 weeks gestation. No statistically significant differences by supplement type ($P_s > 0.05$)
### Table 5.2 Cardiometabolic risk factors in 159 Peruvian children 4.5 y by prenatal supplement type

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Maternal supplement type</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iron + Folic acid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Iron + Folic acid + Zinc</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>86</td>
<td>73</td>
</tr>
<tr>
<td>Anthropometry and body composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>17.4±2.4</td>
<td>17.2±2.2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>102.4±3.4</td>
<td>102.9±4.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>16.6±1.64</td>
<td>16.3±1.3</td>
</tr>
<tr>
<td>BMI-for-age, Z-score</td>
<td>0.82±1.0</td>
<td>0.65±0.9</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>54.7±4.2</td>
<td>55.1±4.2</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>6.4±1.7</td>
<td>6.2±1.3</td>
</tr>
<tr>
<td>Fat mass index, kg/m²</td>
<td>6.1±1.4</td>
<td>5.8±1.0</td>
</tr>
<tr>
<td>Lipid profile*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma triglycerides, mmol/L</td>
<td>0.88 (0.71, 1.10)</td>
<td>0.86 (0.65, 1.06)</td>
</tr>
<tr>
<td>Plasma total cholesterol, mmol/L</td>
<td>3.43 (3.08, 3.79)</td>
<td>3.54 (3.28, 4.01)</td>
</tr>
<tr>
<td>Plasma HDL-cholesterol, mmol/L</td>
<td>0.75 (0.54, 0.96)</td>
<td>0.74 (0.59, 0.98)</td>
</tr>
<tr>
<td>Plasma LDL-cholesterol, mmol/L</td>
<td>2.28 (1.84, 2.66)</td>
<td>2.48 (1.87, 2.86)</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>84.2±7.8</td>
<td>85.1±10.2</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>59.9±8.1</td>
<td>57.7±9.6</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>68.0±7.3</td>
<td>66.8±8.6</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mmol/L</td>
<td>4.44 (4.11, 4.94)</td>
<td>4.44 (4.11, 4.92)</td>
</tr>
<tr>
<td>Plasma insulin, µIU/ml</td>
<td>9.00 (5.09, 16.00)</td>
<td>10.02 (5.30, 15.01)</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.73 (0.91, 3.00)</td>
<td>1.98 (0.95, 2.97)</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (IQR)

p-values are calculated using t-test or Wilcoxon ranksum test

BMI = Body Mass Index, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure

* p-values were calculated using tobit regression for left censored variables for triglycerides (n=11), total cholesterol (n=10), and HDL-cholesterol (n=6); data were missing for LDL-cholesterol (n=30)
Table 5.3 Prevalence of cardiometabolic factors and risk of metabolic syndrome in 159 Peruvian children 4.5y by prenatal supplement type

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Maternal supplement type</th>
<th>Iron + Folic acid</th>
<th>Iron + Folic acid + Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>86</td>
<td>73</td>
</tr>
<tr>
<td>Abdominal obesity (WC ≥ 90th percentile)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td>16 (18.6)</td>
<td>13 (17.8)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>0.95 (0.42, 2.13)</td>
<td></td>
</tr>
<tr>
<td>High triglycerides (≥ 95th percentile)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td>16 (18.6)</td>
<td>15 (20.6)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>1.13 (0.52, 2.48)</td>
<td></td>
</tr>
<tr>
<td>Low HDL cholesterol (&lt; 5th percentile)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td>65 (75.6)</td>
<td>54 (74.0)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>0.92 (0.45, 1.88)</td>
<td></td>
</tr>
<tr>
<td>High blood pressure (SBP or DBP ≥ 90th percentile)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td>20 (23.3)</td>
<td>18 (24.7)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>1.08 (0.52, 2.24)</td>
<td></td>
</tr>
<tr>
<td>High glucose (≥ 5.6 mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n (%)</td>
<td></td>
<td>5 (5.8)</td>
<td>3 (4.1)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>0.69 (0.16, 3.01)</td>
<td></td>
</tr>
<tr>
<td>At risk for metabolic syndrome (child met 3 of the above criteria)</td>
<td></td>
<td>10 (11.6)</td>
<td>6 (8.2)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td>0.68 (0.23, 1.97)</td>
<td></td>
</tr>
</tbody>
</table>

All models are adjusted by age and sex
Cutoffs for cardiometabolic risk factors were as follows: WC ≥ 90th percentile (NHANES III: 58.3 cm for girls and 57.6 cm for boys) (29), triglycerides ≥ 95th percentile (ADA: 1.37 mmol/L for girls and 0.97 mmol/L for boys) (30), HDL-cholesterol < 5th percentile (ADA: 2.0 mmol/L for girls and 2.1 mmol/L for boys) (30).
Figure 5.1 Participants enrolled in the study and lost to follow-up by prenatal supplement type.
REFERENCES


Table S5.1. Correlation between anthropometric characteristics and cardiometabolic markers in 159 Peruvian children 4.5 years of age, by sex

<table>
<thead>
<tr>
<th>Anthropometric measures</th>
<th>Lipid profile</th>
<th>Blood pressure</th>
<th>Insulin resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triglycerides</td>
<td>TC</td>
<td>HDL-c</td>
</tr>
<tr>
<td>Males (n = 82)</td>
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</tr>
<tr>
<td>Weight</td>
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<td>0.08</td>
</tr>
<tr>
<td>Height</td>
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<td>-0.01</td>
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<tr>
<td>BMI</td>
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<td>0.09</td>
</tr>
<tr>
<td>FMI</td>
<td>-0.07</td>
<td>-0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>Sum of 4 SF</td>
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<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>WC</td>
<td>-0.13</td>
<td>-0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>SS/Tri SF ratio</td>
<td>0.07</td>
<td>-0.13</td>
<td>-0.06</td>
</tr>
<tr>
<td>FFMI</td>
<td>-0.01</td>
<td>-0.11</td>
<td>0.04</td>
</tr>
<tr>
<td>Females (n = 77)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>0.15</td>
<td>-0.07</td>
<td>-0.04</td>
</tr>
<tr>
<td>Height</td>
<td>0.09</td>
<td>-0.08</td>
<td>-0.19</td>
</tr>
<tr>
<td>BMI</td>
<td>0.14</td>
<td>-0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>FMI</td>
<td>0.15</td>
<td>-0.08</td>
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<td>0.05</td>
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<td>SS/Tri SF ratio</td>
<td>0.20</td>
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<tr>
<td>FFMI</td>
<td>0.14</td>
<td>-0.04</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Pearson’s correlation coefficients for blood pressure measures; Spearman’s correlation coefficients for lipid profile and insulin resistance measures

* p-value < 0.05, ** p-value < 0.01

BMI = Body Mass Index, FMI = Fat Mass Index, SF = skinfolds, WC = waist circumference, SS = subscapular, Tri = triceps, FFMI = Fat-free Mass Index, TC = total cholesterol, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial blood pressure, HOMA-IR = homeostasis model assessment for insulin resistance

Left-censored data were imputed for triglycerides (n=11), TC (n=10), HDL-c (n=6); data were missing for LDL-c (males=22; females=8).
CHAPTER 6: FETAL ORIGINS OF CHILD AUTONOMIC CONTROL IN PERUVIAN CHILDREN

ABSTRACT

Introduction: Imbalances in autonomic nervous activity are associated with negative health outcomes in adults. Differences in autonomic control are observed in the fetal period and remain stable into the postnatal period.

Aims: This study examines associations between several cardiac measures of autonomic control from mid-gestation into childhood.

Study design: Heart rate measures were collected at 20, 24, 28, 32, 36, and 38 week gestation and again at 4.5 years of age.

Subjects: One hundred and sixty five Peruvian children whose mothers were part of a prenatal zinc supplementation trial.

Outcome measures: Child heart period (HP) and four measures of cardiac variability including standard deviation of HP (HP-SD), range of HP, mean square of successive differences (MSSD), and a measure of vagal tone (V) at 4.5 years.

Results: Fetal heart rate (HR) was inversely associated with child HP, all measures of cardiac variability, and a measure of vagal tone (V) at 4.5 years. Two measures of cardiac variability or patterning in the fetal period, HR range, and the number of episodic accelerations of heart rate were also significantly associated with childhood measures of heart period and variability.

Conclusions: These results provide evidence of fetal origins of postnatal autonomic control.
INTRODUCTION

Cardiovascular and metabolic diseases are important contributors to morbidity and mortality worldwide. In 2002, cardiovascular diseases (CVD) accounted for approximately 25% of deaths and 7% of Disability Adjusted Life Years (DALYs), and Type 2 diabetes mellitus (DM) accounted for an additional 2% of deaths and 1% of DALYs worldwide (1). Further increases in CVD and DM disease burden are projected in the near future, predominantly in the developing world (2). It is now widely accepted that the risk of developing cardiometabolic disease starts before birth. There is evidence that differences in the functioning of homeostatic systems mediating stress can be predisposing individuals to cardiometabolic disease. The hypothalamic-pituitary-adrenal (HPA) axis has been the most widely studied (3).

Less studied is the autonomic nervous system (ANS), which coordinates automated organ functions critical during homeostasis as well as in response to environmental stimuli. These functions are accomplished through two broad branches – the parasympathetic (PNS) and the sympathetic (SNS), which operate independently, but most often interact in a coordinated fashion in which one system predominates over the other. The PNS promotes anabolic functions related to day-to-day living, such as food digestion and absorption, whereas the SNS promotes metabolic activities and prepares the individual to cope with certain environmental stimuli. Patterns of heart rate (HR) and its variability (HRV) have been considered a window to the human ANS because they reflect variation in the degree of autonomic control of the heart within individuals. Although several factors can affect the HR of an individual, the ANS is the main determinant of its baseline value, and variations in HR inform regarding adaptability to environmental influences (4, 5). Higher HR and lower HRV are interpreted as evidence of greater sympathetic control of cardiac function within the context of this dynamic relation, and an increasing number of studies suggest that the predominance of sympathetic over parasympathetic activity is associated with obesity, high blood pressure, dyslipidemia, and DM, and with all-cause mortality including CVD (6).
Although it is recognized that the development of the ANS originates during the fetal period, relatively few studies have documented the development and stability of individual differences in autonomic control from the prenatal to postnatal period. We are aware of only four published studies evaluating the consistency of cardiac measures of autonomic function from fetal life into childhood (7-10). In a first study including 21 U.S. children, a positive correlation between fetal HR in late pregnancy (on average 32 days before birth) and infant HR at 6 different ages up to 1 year of age was reported ($r = 0.25$ to $0.73$) (7). In this same study, also a positive correlation between fetal HRV and infant HRV was reported, although this association was less consistent than for HR. In a second study, based on a secondary analysis of a nationally-representative dataset of 11,000 British children, it was found that low fetal HR during labor (<120 bpm) was associated with a lower HR at age 10 years (8). In this study, the stability of HRV from the prenatal into the postnatal period was not evaluated. Two other studies conducted in 52 (9) and 79 (10) U.S. children have also reported a moderate positive correlation between fetal HR and HRV measured at multiple gestational ages in the second half of pregnancy, and again in infancy, up to 2 years of age.

Although these studies strongly support the prenatal origins of individual differences in heart rate and its variation, with the exception of the study evaluating fetal HR during labor and then at age 10 years in 11,000 children – which did not evaluate the stability of HRV, they are limited in the number of participants included and the postnatal age at follow-up. Moreover, these studies were conducted in developed country populations, and it is important to understand if these associations generalize to populations from developing countries exposed to greater environmental disadvantages. The level and trajectories of HR patterns during gestation, and neurobehavioral development during infancy have previously been reported to differ between populations from different geographical, ethnic, and cultural backgrounds (11-13). Therefore, the main goal of this study was to further understand the fetal origins of autonomic control by
assessing the associations between several cardiac measures of autonomic control studied from 20 to 38 weeks gestation to 4.5 years of age in a sample of Peruvian children.

**METHODS**

**Study design and participants**

Between 1998 and 2000, a double-blind, randomized clinical trial was conducted in 242 pregnant women in a periurban area of Lima, Peru, to assess the effect of prenatal zinc supplementation on fetal neural development and growth. When children were approximately 4.5 years of age, they participated in a follow-up study to evaluate the long-term effects of supplementation on health, nutritional, developmental, and autonomic outcomes. Results relating to zinc supplementation are published elsewhere (14-16). In addition, the longitudinal data were used to evaluate whether individual differences in prenatal cardiac measures continue to the postnatal period, independent of supplementation, which is the focus of this report.

**Prenatal period**

*Enrollment.* Details of recruitment, enrollment, and supplementation are provided in earlier reports (14,15). Briefly, women who were receiving care at the Hospital Materno Infantil San José (Villa el Salvador, Lima, Peru), who were classified with a low-risk pregnancy, carrying a singleton fetus, and who had lived on the Coast of Peru for at least 6 months before pregnancy were eligible for the study. Participants were enrolled between 10-16 weeks gestation, and randomized within parity (primipara/multipara) and week of gestation (10-13/14-16 wk) strata to receive 60 mg iron (ferrous sulfate) and 250 µg folic acid, with or without 25 mg of zinc (zinc sulfate) daily throughout pregnancy.
**Prenatal Protocol.** Electronic fetal monitoring (EFM) was conducted at 20, 24, 28, 32, 36, and 38 week gestation. Women were monitored for 50-minutes while resting comfortably in a semirecumbent, left-lateral position using standard cardiotocography (Toitu, MT 320, Wayne, Pa). This duration was selected to exceed the 30-40 minute recording time recommended to ensure intrafetal consistency (17, 18). Testing took place between 9 a.m. and 6 p.m., and women were instructed to eat 1 ½ hours prior to testing but not thereafter. No systematic differences in FHR have been found when testing during diurnal hours (19).

**Derivation of fetal cardiac measures.** FHR data were continuously digitized, via streaming software and analyzed off-line using customized software (GESTATE: James Long Company, Caroga, NY; J.A.D). The data underwent error rejection procedures based on moving averages of acceptable values as needed. Variable extraction included measures of rate and variability computed in 1-minute epochs and averaged over the 50-minute recording. FHR was quantified in beats per minute (bpm), and the following measures of heart rate variability were computed: 1) standard deviation of FHR (FHR-SD), 2) range of FHR, and 3) number of FHR accelerations, defined as any increase above the baseline FHR of 10 bpm or more lasting for at least 15 seconds.

**Postnatal Period**

**Recruitment at 4.5 years.** Of the 242 women enrolled in the supplementation trial, 20 were lost to follow-up, and 27 were excluded from the original analyses due to significant obstetrical or medical complications. Therefore, 195 (80.6%) were included in the formal analysis (94 in the zinc group and 101 in the control group) (14). For the follow-up study, the 195 participants included in the formal analysis, and 10 of the 27 excluded participants (who completed the protocol and whose babies survived the neonatal period and were free of congenital
malformations) were identified for follow up. Of the 205 eligible participants, 184 children (90%) were located and evaluated, 86 whose mothers received zinc and 98 whose mothers did not (16).

**Child protocol.** The child protocol included interviews with the caregiver and review of clinical records to collect information on socioeconomic conditions of the family, child feeding practices, and health, nutritional, and developmental history. In addition, a health exam, a nutritional evaluation, and neurobehavioral developmental testing were performed. Pertinent to this report is the collection of HR measures. Cardiac monitoring was conducted by electrocardiography (ECG), once children were familiar with testing procedures. A belt with two embedded pediatric electrodes was positioned on the child’s chest under a shirt. ECG data were collected with the children at rest for approximately 5 minutes. To reduce movement artifact while collecting measures at rest, children remained in a seated position and were exposed to interesting visual stimuli to maintain quiescent. R-waves were collected, amplified, and timed by a standard commercially available apparatus (Mini-Logger 2000, Mini Mitter, Bend, OR).

**Derivation of child cardiac measures.** ECG data were transferred to a computer, manually edited for artifact, and processed using MXedit software (Delta Biometrics, Bethesda MD). To derive cardiac measures, each tracing was divided in 30-second epochs, each epoch was analyzed separately, and arithmetic means were computed for each cardiac measure. Baseline heart period (HP) is the mean interval (ms) between successive R-R waves; this metric is essentially the inverse of heart rate. The following measures of HP variability were computed: 1) standard deviation of HP (HP-SD); 2) range of HP per 30-minute epoch; 3) mean square of successive differences (MSSD), a time-dependent method of analyzing variation in successive HP; and 4) vagal tone ($V$), the natural logarithm of the extracted variance calculated using methods developed by Porges, which is a measure of respiratory sinus arrhythmia (20).
Missing ECG data were typically due to signal artifact. ECG data were available for 165 (80%) of the 184 children at follow-up, 79 from the zinc group and 87 from the control group. Of these, 3 in the zinc group and 4 in the control group were not included in the original analysis (14).

**Data analyses**

Data analyses were performed using Stata 12.0 (Stata Corporation, College Station, Texas). Univariate distributions of the variables of interest were explored, to identify outliers and skewed distributions. Because the number of accelerations is not normally distributed, we also estimated the models using the squared-transformed equivalent; the results were similar and robust, and, therefore, for easier interpretability, we present the untransformed variables. The type of transformation was selected using the ladder of powers developed by Tukey (21). Given the complexity of fetal data and based on the observed changes in FHR patterns across pregnancy, we used several approaches for data reduction to summarize the existing data and to develop the most parsimonious models. For FHR and FHR-SD, we created mean of pregnancy summary measures (arithmetic mean of all available measures between 20 and 38 weeks gestation – hereafter referred as “mean FHR” and “mean FHR-SD”). FHR decreased throughout pregnancy, and all available measures were significantly correlated (0.35-0.78). FHR-SD increased across pregnancy, and all measures were moderately correlated (0.18-0.56). For both measures, the highest correlations were observed between a current value and the preceding one, suggesting that the measures probably relate through an autoregressive process, and that a mean value is justified. FHR accelerations and range of FHR increased closer to the end of pregnancy; hence, we focused our analysis on the latest available measure (38 weeks gestation). Bivariate analyses were conducted to evaluate associations between fetal cardiac measures and those at 4.5 years.
Multivariate linear regression models were fitted using the available cardiac measures at 4.5 years as outcomes (HP, HP-SD, range of HP, MSSD, and $I$), and the available fetal cardiac measures (mean FHR, mean FHR-SD, range of FHR at 38 weeks, and number of FHR accelerations at 38 weeks) as covariates. For each outcome, nested models were developed using a stepwise approach, introducing one FHR measure at a time and comparing each model with the previous one, using the Likelihood Ratio Test (LRT) to determine the best fitting models. Adjusted R-squared values are reported as a reference. Previously, we reported in these same participants that prenatal zinc supplementation had an effect on fetal and child HR measures; therefore, models were adjusted for supplement type, as well (14, 16). Other factors tested during the analysis include sex, body surface area at 4.5 years ($\text{BSA} = 0.024265 \times \text{height in cm}^{0.3964} \times \text{weight in kg}^{0.5378}$), size at birth, gestational age at birth, and being born small for gestational age (<10th percentile of birth weight for gestational age) (22). To assess if the associations between fetal and child cardiac measures were modified by prenatal supplement type or sex, interaction terms between fetal cardiac measures and supplement type or sex were created and tested in the multivariate analysis; none was found to be statistically significant ($p > 0.05$). A total of 20 participants had no data available for the 38 week visit, due to early delivery; therefore, the final sample in the multiple regression models is 145. Because most participants excluded from the original analysis were born before the 38th week, only 1 of them ended up being included in the final models. Collinearity was assessed using the Variance Inflation Factor (VIF>10). All of our models had a VIF close to 1 suggesting no collinearity was present. Statistical significance was defined as $p < 0.05$.

RESULTS

Characteristics of study sample
CHAPTER 6: FETAL ORIGINS OF CHILD AUTONOMIC CONTROL

Shown in table 3.1 are selected characteristics of the study sample. Preliminary analyses showed no differences in these characteristics by supplement type; therefore, combined data are presented (14, 16).

Fetal and child measures of autonomic control

Shown in table 6.2 are the fetal measures of autonomic control for the 165 Peruvian fetuses included in this report. Throughout gestation, there was a decline in FHR from 148.8 bpm at 20 weeks to 143.2 bpm at 38 weeks. There was an increase in HRV across pregnancy. The HR-SD increased from 3.0 at 20 weeks to 4.0 at 38 weeks, whereas the average number of FHR accelerations was 0.5-1.8 from 20-28 weeks and 3.7-4.2 from 32-28 weeks, and the range of FHR changed from 16.3-20.4 during 20-28 weeks, to 22.8-24.0 from 32 weeks onward. Shown in table 6.3 are the cardiac measures of autonomic control at follow up in these 165 children. There are statistical differences in these variables by supplement type, and these have been previously published (14, 16).

Associations between fetal and child cardiac measures of autonomic control

Figures S6.1-S6.5 (supplemental) present the bivariate associations between fetal and child cardiac measures of autonomic control and tables 6.4-6.6 present results from the final multiple regression analysis evaluating associations between fetal and child cardiac measures of autonomic control. Faster mean FHR was inversely associated with child HP (i.e., faster child heart rate), all measures of HRV, and parasympathetic control at 4.5 years. Higher range of FHR at the end of pregnancy was associated with greater child HP (i.e., slower HR), all measures of HRV, and V. The number of FHR accelerations at end of pregnancy was not associated with child HR measures in the bivariate analysis, but in the multivariate models it was inversely associated with HP, all measures of HRV, and V at 4.5 years. Child sex was significantly
associated with HP in the bivariate analysis (on average, girls had a HP of 22.5 ms lower than boys, corresponding to a difference of approximately 4 bpm, \( p=0.008 \)); however, in the multiple regression model statistical significance attenuated and sex was excluded from the final model. FHR-SD was not associated with any of the cardiac measures of autonomic control at 4.5 years in the bivariate or multivariate analyses. Because there have been reports of associations between intrauterine growth restriction, prematurity, and differences in autonomic control, we assessed whether weight at birth, gestational age at birth, or being born small for gestational age were associated with cardiac measures at 4.5 years (23-26). None was significantly associated with cardiac measures at 4.5 years in bivariate or multivariate analyses. In addition, because body size and composition have been associated with differences in autonomic control, we assessed if child BSA and BMI were associated with any of the cardiac measures at 4.5 years (27). Neither was found to be associated in either the bivariate or multivariate analyses. The models presented accounted for 19% (HP), 20% (HP-SD and MSSD), 18% (range of HP), and 12% (V) of the explained variances in the measures at 4.5 years.

**Sensitivity analysis**

As mentioned, a total of 20 individuals were born before the 38-week visit and were excluded from the final models. A comparison was conducted of selected prenatal and postnatal characteristics between those excluded and included in the final models. As expected, children born before the 38-week visit were 313 g lighter (\( p=0.005 \)) and 1.5 cm shorter (\( p=0.005 \)) at birth; they were born 2.7 weeks earlier (\( p<0.001 \)) and were more likely to be preterm (22% vs 0%, \( p<0.001 \)). Cardiac measures at 4.5 years, however, did not differ between the two groups.

**DISCUSSION**
Results from this study confirm persistent associations between multiple cardiac measures of autonomic control during the fetal period and comparable cardiac measures of autonomic control in childhood. To our knowledge, this is the first study reporting this association from the prenatal period through almost the first 5 years of childhood using multiple fetal and child measures of autonomic control. Our results are consistent with previous findings showing stability of heart rate and variability in children from the United States, from the prenatal to the postnatal period, and further expand these findings to a sample of Peruvian children from different geographic, ethnic, cultural, and sociodemographic background (7-10).

We found that mean FHR was inversely associated with all cardiac measures of autonomic control at 4.5 years. On average, fetuses with faster heart rates continue to display faster heart rates (i.e., lower heart period) as children 4.5 years after birth, as well as less variability in heart rate and lower $V$. These results are generally consistent with three prior studies that assessed associations between prenatal and postnatal HR measures over shorter follow-up periods (14-16). One of them also reported an inverse association between fetal HR and infant HR-SD at 1 year of age (9).

Overall, the associations between fetal and childhood measures of HRV were detectable but less consistent than those for HR. The range of FHR at the end of pregnancy was positively associated with all cardiac measures of autonomic control at 4.5 years of age; fetuses with greater range of FHR (interpreted as greater HRV) had slower HR (greater HP), higher HRV (as measured by HP-SD, range of HP, and MSSD), and greater parasympathetic control (as measured by $V$) at 4.5 years. Contrary to expectations, we found no significant associations between FHR-SD (averaged fetal measure or at each of the six gestational age-specific time points) and the cardiac measures of autonomic control at 4.5 years, in either the bivariate or multivariate analyses. No associations between the number of FHR accelerations at the end of pregnancy and the cardiac measures of autonomic control at 4.5 years were detected in the bivariate analysis;
however, significant inverse associations emerged in the multiple regression analysis after adjusting for prenatal supplement type, mean FHR, and range of FHR. Fetuses with more FHR accelerations at the end of pregnancy had lower HP (faster HR), lower HRV (as measured by HP-SD MSSD, and range of FHR), and lower parasympathetic control (as measured by V) at 4.5 years. Three previous studies have shown a stability of HRV from the prenatal into the postnatal period up to 1 year of age (7, 9, 10), and one of them also reported an inverse association between fetal HRV and HR at 1 year (9). Our findings related to range of FHR at 38 weeks gestation are consistent and complement those previously published, and together provide support of the stability of fetal HRV into childhood and the inverse association between fetal HRV and postnatal HR (10, 11).

Our findings related to the number of FHR accelerations (which can also be considered a measure of fetal HRV) challenge interpretation, but the consistency of the direction of associations with all cardiac measures at 4.5 years makes the results worthy of further investigation. FHR acceleration patterns are commonly used to assess fetal well-being in the antenatal period in high-risk pregnancy. In general, the absence of FHR accelerations is considered a sign of fetal hypoxia; however, the short- and long-term clinical significance of individual differences in the number of FHR accelerations in normal pregnancies has not been established. Moreover, the level and trajectories of FHR accelerations throughout pregnancy differ between populations, suggesting population-specific factors affecting neurobehavioral development. As compared to a sample of U.S. fetuses, the Peruvian fetuses in the current study had fewer FHR accelerations at each of the 5 gestational ages sampled, and this difference became greater as pregnancy progressed (11). In addition, the number of FHR accelerations increased in all fetuses up to 32 weeks gestation, but after this point the number of FHR accelerations continued to increase in U.S. children, whereas it decreased in Peruvian children. On average, at 38 weeks gestation, U.S. fetuses had 7 FHR accelerations and Peruvian fetuses
had 3 (11). Given the differences in FHR acceleration patterns across diverse populations, replication of our FHR acceleration findings in other populations is needed.

Previously, we have shown that prenatal zinc supplementation exerts an independent effect on fetal measures of autonomic control, and these changes are detectable during childhood. Here, we evaluated whether the relation between measures during the two periods differed depending on prenatal supplement type, and found no differences. The unadjusted and adjusted regression coefficients for prenatal supplement type also did not vary significantly after introducing other covariates in the models (data not shown). Thus, we find no greater divergence beyond the period of supplementation, which suggests no lingering effect beyond the period of supplementation (16).

There are several strengths to this study that increase assurance of our findings. The consistency of the associations between several measures of autonomic control, during pregnancy and childhood, suggests that the associations are not spurious, and the longitudinal prospective design of the study increases certainty of the temporal causal inference of the associations identified. As part of our protocol, EFM and child ECG monitoring were conducted under standard conditions controlling, as much as possible, external factors that could potentially alter the cardiac measures. In addition, EFM was conducted at six gestational age-specific time points during pregnancy, which allowed us to assess fetal neural development throughout gestation, and identify influences of fetal autonomic control at age-specific time points during pregnancy, on the postnatal measures. Although the original design of this study was a randomized clinical trial and not an observational study, we were able to adjust for the intervention during the analyses and still had adequate power to identify statistically significant associations in multivariate models. It would have been expected that loss to follow-up could be an issue 4.5 years after the pregnancy study was completed; however, around 90% of the eligible participants were located and enrolled in the follow-up, with ~80% contributing data to the analyses presented here. Our sample size is
much larger than that of previous studies with similar prenatal and postnatal protocols \((ns = 21, 32,\text{ and } 79)\), and constitutes the largest study ever published documenting the fetal origins of cardiac measures of autonomic control \((7, 9, 10)\).

Some design weaknesses should be considered. The most important is the lack of information available on environmental factors acting in the childhood period that could have affected ANS maturation after birth. Certain factors, such as temperature, stress, and nutrition, among others, have been shown to modulate ANS development in the postnatal period \((29)\). Attention to these factors was not the focus of this study, and we did not have continuous follow up of the children postnatally. We were able, however, to assess for some potential confounding factors and important interaction effects.

We found no significant associations between size at birth (birth weight or small for gestational age) or gestational age, and any of the cardiac measures at 4.5 years. This is in direct contrast to studies reporting associations between small size at birth and preterm delivery and measures of autonomic control \((23-26)\). Because we restricted our study to low-risk pregnancies and our analyses to participants with HR data at 38 weeks, our final models excluded all children born preterm, and the proportion of babies small for gestational age is small. Thus, our results suggest that variation in birth size among term infants does not appear to explain differences in autonomic function.

Our findings, together with those previously reported, strongly indicate stability of autonomic control from the prenatal to the postnatal period. The persistence of the stability of these differences later in life merits further study in other populations, as do the clinical significance of differences of autonomic control during the childhood period and its impact on long-term health outcomes. The association between adult autonomic control and the risk of cardio-metabolic diseases has been established; therefore, understanding its early origins could present an opportunity for early prevention.
### Table 6.1. Selected characteristics of 165 Peruvian children

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girl, %</td>
<td>49.1</td>
</tr>
<tr>
<td>Weight at birth, g</td>
<td>3279 (409)</td>
</tr>
<tr>
<td>Length at birth, cm</td>
<td>49.8 (2.0)</td>
</tr>
<tr>
<td>Gestational age at birth, week</td>
<td>39.2 (1.3)</td>
</tr>
<tr>
<td>Prematurity, %</td>
<td>2.5</td>
</tr>
<tr>
<td>Small for gestational age, %</td>
<td>13.6</td>
</tr>
<tr>
<td>Body Mass Index at 4.5 y, kg/m²</td>
<td>16.4 (1.5)</td>
</tr>
</tbody>
</table>

*Note. Values are mean (standard deviation) or percent.*
Table 6.2. Cardiac measures of autonomic control in 165 Peruvian fetuses at 20-38 weeks gestation *

<table>
<thead>
<tr>
<th>Cardiac measures</th>
<th>Gestational age in weeks (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 (163)</td>
</tr>
<tr>
<td><strong>Heart Rate</strong></td>
<td></td>
</tr>
<tr>
<td>FHR, bpm</td>
<td>148.8 (5.6)</td>
</tr>
<tr>
<td><strong>Heart Rate Variability</strong></td>
<td></td>
</tr>
<tr>
<td>FHR-SD, bpm</td>
<td>3.0 (0.6)</td>
</tr>
<tr>
<td>Range of FHR, bpm</td>
<td>16.3 (5.8)</td>
</tr>
<tr>
<td>Number of FHR accelerations</td>
<td>0.5 (0.8)</td>
</tr>
</tbody>
</table>

*Data published previously by supplement type in a larger sample (n = 195) (21)

Note. M (SD). FHR = fetal heart rate, FHR-SD = standard deviation of FHR, bpm = beats per min.
Table 6.3. Cardiac measures of autonomic control in 165 Peruvian children at 4.5 years of age *

<table>
<thead>
<tr>
<th>Cardiac measures</th>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Heart Rate</strong></td>
<td></td>
</tr>
<tr>
<td>HP, <em>ms</em></td>
<td>564.7 (55.6)</td>
</tr>
<tr>
<td><strong>Heart Rate Variability</strong></td>
<td></td>
</tr>
<tr>
<td>Child HP-SD, <em>ms</em></td>
<td>6.8 (0.8)</td>
</tr>
<tr>
<td>Range of HP, <em>ms</em></td>
<td>137.9 (54.5)</td>
</tr>
<tr>
<td>MSSD, <em>ms</em></td>
<td>22.1 (12.3)</td>
</tr>
<tr>
<td><strong>Parasympathetic control</strong></td>
<td></td>
</tr>
<tr>
<td>( V, \ln (\text{ms})^2 )</td>
<td>5.5 (1.1)</td>
</tr>
</tbody>
</table>

* Data published previously by supplement type in a larger sample \( (n = 195) \) (23)

Note. \( M (SD) \). HP = heart period, HP-SD = standard deviation of HP, MSSD = mean square of successive differences, \( V \) = Vagal tone, *ms* = milliseconds.
Table 6.4. Multiple linear regression analysis for fetal variables predicting child heart period at 4.5 years of age (n = 145) *

<table>
<thead>
<tr>
<th>Fetal variables</th>
<th>β (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean FHR</td>
<td>-3.28 (-5.13, -1.44)</td>
<td>0.001</td>
</tr>
<tr>
<td>Range of FHR - 38 weeks</td>
<td>1.80 (0.75, 2.85)</td>
<td>0.001</td>
</tr>
<tr>
<td>Number of FHR accelerations – 38 weeks</td>
<td>-4.51 (-7.45, -1.56)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

* Model adjusted for prenatal supplement type

Note. FHR = fetal heart rate; HP = heart period.
**Table 6.5. Multiple linear regression analysis for fetal variables predicting child heart rate variability at 4.5 years (n = 145) **

<table>
<thead>
<tr>
<th>Fetal variables</th>
<th>HP-SD</th>
<th></th>
<th>MSSD</th>
<th></th>
<th>Range of HP</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β (95% CI)</td>
<td>p-value</td>
<td>β (95% CI)</td>
<td>p-value</td>
<td>β (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Mean FHR</td>
<td>-0.05 (-0.08, -0.03)</td>
<td>&lt;0.001</td>
<td>-0.74 (-1.11, -0.37)</td>
<td>&lt;0.001</td>
<td>-3.42 (-5.08, -1.77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Range of FHR – end of pregnancy</td>
<td>0.03 (0.02, 0.05)</td>
<td>&lt;0.001</td>
<td>0.40 (0.19, 0.61)</td>
<td>&lt;0.001</td>
<td>1.88 (0.94, 2.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of FHR accelerations – end of pregnancy</td>
<td>-0.05 (-0.10, -0.01)</td>
<td>0.010</td>
<td>-0.96 (-1.56, -0.37)</td>
<td>0.002</td>
<td>-2.76 (-5.40, 0.11)</td>
<td>0.041</td>
</tr>
</tbody>
</table>

*Note. FHR = fetal heart rate; HP = heart period; HP-SD = standard deviation of HP; MSSD = mean square of successive differences.

* Models adjusted for prenatal supplement type
Table 6.6. Multiple linear regression analysis for fetal variables predicting vagal tone at 4.5 y ($n = 145$) *

<table>
<thead>
<tr>
<th>Fetal variables</th>
<th>$\beta$ (95% CI)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean FHR</td>
<td>-0.06 (-0.09, -0.02)</td>
<td>0.004</td>
</tr>
<tr>
<td>Range of FHR – end of pregnancy</td>
<td>0.03 (0.01, 0.06)</td>
<td>0.002</td>
</tr>
<tr>
<td>Number of FHR accelerations - end of pregnancy</td>
<td>-0.08 (-0.14, -0.02)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

* Model adjusted for prenatal supplement type

Note. FHR = fetal heart rate; $\nu$ = vagal tone.
REFERENCES


**CHAPTER 6: FETAL ORIGINS OF CHILD AUTONOMIC CONTROL**

**A**

![Plot A](image)

\[ r = -0.26 \]

**B**

![Plot B](image)

\[ r = -0.03 \]
Figure S6.1 Relationship between fetal cardiac measures of autonomic control (Fetal Heart Rate, FHR (A), SD of the FHR, FHR-SD (B), range of FHR (C), and number of FHR accelerations (D), and child heart period (HP) at 4.5 years. \( r = \) Pearson’s correlation coefficient. Hollow circles represent data for children with 38-week visit, solid circles represent data for children without a 38-week visit (for range of FHR and number of FHR, value at 36 weeks is presented).
A

B

r = -0.27

r = 0.06
Figure S6.2 Relationship between fetal cardiac measures of autonomic control (Fetal Heart Rate, FHR (A), SD of the FHR, FHR-SD (B), range of FHR (C), and number of FHR accelerations (D), and SD of child heart period (HP-SD) at 4.5 years. $r =$ Pearson’s correlation coefficient. Hollow circles represent data for children with 38-week visit, solid circles represent data for children without a 38-week visit (for range of FHR and number of FHR, value at 36 weeks is presented).
Figure S6.3 Relationship between fetal cardiac measures of autonomic control (Fetal Heart Rate, FHR (A), SD of the FHR, FHR-SD (B), range of FHR (C), and number of FHR accelerations (D), and range of child heart period (HP) at 4.5 years. $r =$ Pearson’s correlation coefficient. Hollow circles represent data for children with 38-week visit, solid circles represent data for children without a 38-week visit (for range of FHR and number of FHR, value at 36 weeks is presented).
Figure S6.4 Relationship between fetal cardiac measures of autonomic control (Fetal Heart Rate, FHR (A), SD of the FHR, FHR-SD (B), range of FHR (C), and number of FHR accelerations (D), and child mean square of successive differences (MSSD) at 4.5 years. r = Pearson’s correlation coefficient. Hollow circles represent data for children with 38-week visit, solid circles represent data for children without a 38-week visit (for range of FHR and number of FHR, value at 36 weeks is presented)
Figure S6.5 Relationship between fetal cardiac measures of autonomic control (Fetal Heart Rate, FHR (A), SD of the FHR, FHR-SD (B), range of FHR (C), and number of FHR accelerations (D), and child vagal tone ($V$) at 4.5 years. $r$ = Pearson’s correlation coefficient. Hollow circles represent data for children with 38-week visit, solid circles represent data for children without a 38-week visit (for range of FHR and number of FHR, value at 36 weeks is presented)
CHAPTER 7: CONCLUSIONS

Cardiovascular and metabolic diseases are considered global public health problems. Ischaemic heart disease, cerebrovascular disease, and type 2 diabetes are the top 1st, 2nd, and 8th causes of death worldwide (1). It has been projected that global disease and the financial burden of cardiovascular and metabolic diseases will continue to increase in importance, predominantly in developing countries.

Maternal nutrition, with respect to both macronutrient and micronutrient deficiencies, is also a public health problem, especially in developing countries (2). Besides the negative consequences on maternal, infant and child survival and well-being, an adverse nutritional environment during the prenatal period can cause structural and physiological changes in the developing organs with long-term functional consequences, predisposing individuals to an increased risk of certain diseases later in life (3, 4).

In this study it is examined whether fetal growth and the development of the autonomic nervous system influence the risk of long-term health outcomes, with emphasis on cardiometabolic disease. To address the study aims, data were used from pregnant women who were participating in a maternal zinc supplementation trial in Lima, Peru, between 1998 and 2000 and a follow-up of their offspring, in 2003 when they were approximately 4.5 years of age. A wealth of information was available from the pregnancy study on the nutritional and health status of the mother during pregnancy, as well as on the growth and neurobehavioral development of the fetus, during the second half of pregnancy. Information available at follow-up included the past and current health, nutritional, and developmental status of the child, as well as their cardiorespiratory status, as measured by electrocardiography. In 2011, additional measurements in cryopreserved plasma samples of the children were conducted at follow-up. In this study, it was evaluated whether intrauterine growth, prenatal zinc, and the development of the fetal
autonomic nervous system are associated with certain anthropometric, biochemical, and cardiac measures that are believed to be precursors of cardiometabolic disease in adulthood.

**Summary of major findings**

Overall, the findings of the present study support the hypothesis that intrauterine growth and development have an association with anthropometric and cardiometabolic parameters in early childhood (4.5 years of age), that are known to be precursors of cardiometabolic disease in adulthood; however, zinc supplementation received by the pregnant mother did not appear to influence the presence of cardiometabolic conditions in early childhood.

**Intrauterine growth**

Childhood undernutrition has important implications for health and human capital, especially in low- and middle-income countries (LMICs) (5, 6). For obvious methodological reasons, anthropometry is commonly used to evaluate the nutritional status of children in epidemiologic studies; therefore, associations between anthropometric measures of body size and composition during childhood, and health, educational, and economic outcomes have been reported. In the long-term, childhood stunting has been associated with reduced stature and fat free mass (3, 7), lower achieved schooling (6, 8), decreased economic productivity (8), adverse maternal outcomes (females) (9), and increased risk of cardiometabolic diseases (10). Increased weight and body fat during childhood have been associated with higher adult fat mass and also with an increased risk of developing cardiometabolic diseases (11, 12).

There is now compelling evidence that the fetal nutritional environment can influence a variety of long-term health outcomes including body size and composition. Size at birth has been positively associated with later height, weight, and fat-free mass (and to lesser extent with fat mass), and negatively associated with abdominal adiposity (13). These findings could be interpreted as larger fetuses becoming larger children overall; however, due to the decreased
accumulation of adipose tissue in the abdominal region – which is considered a metabolic risk factor – they may have a lower cardiometabolic risk as compared to those who suffered from intrauterine growth restriction (14).

There is consensus about birth weight being limited in its ability to characterize the prenatal nutritional environment because it only provides information on the size of the fetus at the end of the prenatal period. In this study, we used serial fetal biometry, evaluated by ultrasonography at 20, 24, 28, 32, and 36 weeks of gestation, to assess whether fetal size and growth (change of size in time) in the second half of gestation are associated with various anthropometric measures of body size and composition at 4.5 years of age in a sample of Peruvian children. By modelling intrauterine growth using information at 5 set time points during gestation, we can increase our ability to identify distinct growth trajectories or specific periods in fetal life during which nutritional exposures can have a lasting impact in later size and body composition.

Consistent associations were observed between fetal size and growth, and later body size and composition. Both fetal size and fetal growth were positively associated with anthropometric measures of child size, adiposity, and lean mass (females only). In addition, an inverse association was found between fetal size and growth and a measure of adiposity in the trunk relative to that in the extremities. Although there were some differences according to sex and fetal dimension, these associations were relatively consistent when using any of the three individual fetal dimensions (head circumference, abdominal circumference, or fetal femur length) or a composite measure (estimated fetal weight).

Probably the most consistent relationship reported in the literature is the positive association observed between fetal length (FL or crown-rump length, CRL) or fetal linear growth (deviations in FL or CRL in pregnancy) and child length/height (15-18). Fetal FL correlates with length at birth \( (r = 0.7) \) (19), and length during the first year of life is correlated with final attained height (20, 21). In this study, some differences were observed in the association of fetal
FL with height in childhood, which was dependent on the sex of the child. In males, FL size was positively associated with height in childhood, beginning at week 20 of gestation, with stronger associations observed with progression of pregnancy. However, in the model evaluating the impact of FL growth on height in childhood, only the size of the FL at 20 weeks – and not the FL growth variables – was associated with child height. These findings could be interpreted as fetal FL size throughout pregnancy being associated with child height to the extent that it is determined by prior measures, and possibly due to a stronger genetic influence in height. In females, we also observed a positive association between fetal FL size and child height, but this reached significance only at 36 weeks of gestation. On the contrary, in the model evaluating the impact of FL growth on height in childhood, conditional FL at both 24 and 36 weeks was independently associated with child height. Because previous reports have not stratified analyses according to sex, we are not certain that these dissimilarities are also present in other populations – which might suggest biological differences – or are population-specific.

Greater fetal growth was associated with greater overall fat mass, fatness relative to height, lean mass, and adiposity in the abdominal area, and lower trunk fat relative to the extremities in childhood. The association between fetal growth and adiposity is complex (13). Initial studies have consistently reported a positive association between birth weight and BMI later in life; however, more recent studies indicate that birth weight is a stronger predictor of lean mass as compared to fat mass. Further, birth weight tends to be inversely associated with abdominal adiposity. In addition, the association between fetal growth and later adiposity is likely dependent on the timing of exposure to variations in the prenatal nutritional environment. Studies in survivors of the Dutch famine indicate that fetuses exposed to an adverse nutritional environment in early pregnancy have an increased risk of later obesity, whereas those fetuses exposed to famine in later pregnancy were less likely to become obese (22).

Studies linking intrauterine growth as measured by fetal biometry, have reported an inverse association with total adiposity and/or central to peripheral adiposity in early childhood
up to 6 years of age (23-26). It was found that fetal size and growth were positively associated with later total adiposity, but inversely related to a measure of adiposity in the trunk relative to that in the extremities. One of the critical principles of the developmental origins paradigm is that the programming effects become more noticeable in cases where there is a mismatch between an adverse nutritional environment in early life and an abundant nutritional environment later in life. It is possible that, in our population, certain factors contributing to growth differences during the prenatal period, such as nutritional deficiencies, continue to be present after birth, with very limited compensatory growth occurring, and, therefore, the programming effects on body composition only become apparent when using a measure of relative central fatness rather than measures of overall fatness.

Associations with fetal size tend to become apparent beginning at 28 weeks of gestation, whereas those with fetal growth become more pronounced starting at week 32. These findings suggest that the period between 28 and 32 weeks of gestation may be critical for fetal growth and development and, therefore, of increased susceptibility to programming effects. Previous reports in this same sample of fetuses (and a comparison population from the U.S.) indicate that the period between 28 and 32 weeks of gestation is also critical for neurobehavioral development (27).

Maternal zinc supplementation and cardiometabolic outcomes

It has been proposed that maternal micronutrient status during pregnancy results in differences in cardiometabolic functional outcomes throughout life (28). There is some evidence from studies in animals and humans indicating that zinc may have a role. In animals, zinc deficiency during pregnancy results in a decreased number and size of nephrons, glomerular filtration rate, lean body mass and insulin response to glucose, and increased systolic blood pressure and body fat (29, 30). In humans, maternal zinc supplementation during pregnancy has been associated with improved fetal and child autonomic function and a reduction in the risk of
microalbuminuria and peripheral adiposity in childhood; all suggestive of an improved cardiometabolic profile (31-34).

Maternal zinc supplementation during pregnancy did not affect measures of anthropometry, lipid profile, blood pressure and insulin resistance of their offspring, when they were evaluated at 4.5 years of age. No differences according to supplement type were observed when analyzing the measures as continuous variables, or using cutoffs to classify children as ‘at risk of’ metabolic syndrome or any of its individual components (abdominal obesity, high triglycerides, low HDL-cholesterol, high blood pressure, and high glucose).

One other previously published study has assessed the long-term effect of prenatal micronutrient supplementation on the cardiometabolic risk in childhood (9). In agreement with our study, prenatal supplementation with folic acid, iron, and zinc did not affect measures of abdominal obesity, blood pressure, lipid profile, or insulin resistance in children, as compared to a control group. But a significant reduction in microalbuminuria in the groups receiving folic acid (OR; 95% CI: 0.56; 0.33, 0.93), or folic acid, iron, and zinc (OR; 95% CI: 0.53; 0.32, 0.89), as compared to the control group (vitamin A only). Even if the reduction in microalbuminuria was somewhat greater in the group receiving supplements containing zinc, the effect observed cannot be attributed to zinc directly because the authors did not explicitly contrast the group receiving iron + folic acid with that receiving iron + folic acid + zinc.

Findings from the present study, together with those previously reported in the literature, do not favor the hypothesis that maternal supplementation with zinc during pregnancy decreases the risk of cardiometabolic disease in their children, with the exception of our prior findings on autonomic control (32).

**Cardiac measures of autonomic control**

Imbalances in the autonomic nervous function in adulthood are associated with negative health outcomes, including cardiometabolic conditions (35). The fetal period and infancy are
critical periods of ontogeny of the autonomic nervous system. Although there is some evidence indicating some degree of plasticity of neural systems throughout life, the majority of its development occurs early in life, particularly during the prenatal period during which the majority of neuron production occurs (36). Consequently, factors modifying neural development during the fetal period will likely have long-lasting functional consequences. Animal studies of dietary restriction during pregnancy provide supportive evidence of this (37, 38). Further, in humans, maternal zinc supplementation during gestation resulted in changes in the pattern of cardiac measures consistent with improved parasympathetic activity in the offspring; these differences remained into childhood (31, 32, 39).

Several studies conducted in U.S. children have indicated that the autonomic function remains moderately stable from the prenatal period into childhood (40-42). To date, no studies have replicated this association in diverse populations. It is essential to examine whether these observations can be reproduced in populations from developing countries that are known to be exposed to adverse nutritional environments in utero (43). This is particularly important because some dissimilarities in fetal neurobehavioral development have been reported between populations from different geographical, ethnic and cultural backgrounds (27). Further, the association between adult autonomic control and the risk of cardio-metabolic diseases has been established; therefore, understanding its early origins is of particular relevance in developing-country settings, where the developmental origins of health and disease paradigm seems to be of greater significance.

In this study, associations were examined among several cardiac measures of fetal autonomic control, evaluated between 20 and 36 weeks of gestation, and comparable measures at 4.5 years of age. Mean fetal heart rate was inversely associated with all cardiac measures of autonomic control at 4.5 years. On average, fetuses with faster heart rates continue to display faster heart rates (i.e., lower heart period) as children, as well as less variability in heart rate and lower parasympathetic control. The associations between fetal and childhood measures of heart
rate variability were detectable but less consistent than those for heart rate. Fetuses with a wider range of fetal heart rate (interpreted as greater heart rate variability) had slower heart rate (i.e. greater heart period) as children, as well as greater heart rate variability and higher parasympathetic control.

Results from the present study confirm previous findings showing stability of heart rate and variability from the prenatal period through infancy, and expand these associations into the early childhood period (40-42). Further, we were able to replicate these associations in a sample of Peruvian children who are known to be ethnically different, come from different sociocultural environments, and who have fetal heart rate and variability trajectories discrepant with those among U.S. children (27). The available evidence supports the hypothesis that the autonomic control has its origin in utero.

**Strengths and Limitations**

There are several strengths in our study that increase the validity of its results. First, this study was conducted in a population from a developing country. Most studies into the fetal programming of long-term disease have been conducted in developed countries. No previously published studies assessing the association between prenatal and postnatal cardiac measures of autonomic control, or the relationship between fetal biometric measures of fetal growth and body size and composition in childhood have been conducted in developing countries. It is important to evaluate how the prenatal environment influences later health outcomes in populations undergoing nutrition and epidemiologic transitions, who are known to be exposed to greater nutritional disadvantages during early life, and in whom increasing economic affluence is creating nutritionally abundant environments, predominantly in urban areas. This study replicated findings, previously reported in children from developed countries, showing associations between prenatal and postnatal cardiac measures of autonomic control and measures of intrauterine growth.
and later body size and composition. Results from the present study increase the generalizability of these observations to populations in more disadvantaged settings.

Second, fetal measures of growth and neural development were evaluated in a longitudinal, prospective manner, and a follow-up was conducted when children were 4.5 years of age. Although causality cannot be proven, the study design used established temporality which – together with the consistency of the associations observed regardless of the growth or neurobehavioral measure used – provides evidence for a causal relationship between prenatal exposures and postnatal outcomes evaluated.

Third, as part of the study protocol, all evaluations in the prenatal and postnatal period (including electronic fetal monitoring, assessment of fetal dimensions using ultrasonography, and child anthropometry and electrocardiogram monitoring) were conducted by trained and standardized operators, which increase the validity of the measurements.

Fourth, evaluation of fetal cardiac measures and biometry were conducted at regular 4-week intervals, beginning at 20 weeks of gestation, which allowed comparability of measurements across study participants and the assessment of fetal growth and neural development throughout the second half of gestation. Further, it allowed the identification of specific periods of time during gestation that are critical in the programming of long-term outcomes; previous studies relating intrauterine measurements of fetal growth with long-term outcomes typically include 1 or 2 ultrasound evaluations, which is insufficient to identify these critical periods (44).

Fifth, for the analyses related to fetal growth and child size and body composition, we used both individual dimensions of fetal size and a composite measure (estimated fetal weight) to characterize fetal growth, and we used various measures of child size and body composition to characterize child growth in a comprehensive way. This allowed to explore whether specific dimensions of fetal size were associated with particular body composition outcomes.
There are some methodological strengths specific to the analyses corresponding to the effect of prenatal zinc on cardiometabolic outcomes, the most important being that we used a double-blinded randomized controlled trial design. This type of design is considered the gold-standard in epidemiology research. Further, with zinc supplementation we were able to experimentally manipulate the nutritional environment of the mother and, therefore, of the fetus, as was evidenced by an increase in serum and urinary zinc concentrations in the mother and in the umbilical cord at birth (45).

Finally, it would have been expected that loss to follow-up could be an issue 4.5 years after the pregnancy study was completed; however, around 90% of the eligible participants were located and enrolled in the follow up. For the analyses related to the cardiac measures of autonomic control, the sample size was much larger than that of previous studies with similar prenatal and postnatal protocols (\( ns = 21, 32, \) and 79), and constitutes the largest study ever published documenting the fetal origins of cardiac measures of autonomic control (40, 41, 46).

Despite the strengths of this study, there are some limitations to it. The fetal protocol included measurements starting at 20 weeks of gestation, meaning that the results can only be extrapolated to the second half of pregnancy; there is evidence suggesting that programming of long-term outcomes is likely dependent upon the timing when variations in the fetal nutritional environment occur (22, 23, 25, 26, 44). Because participants in the pregnancy study were not contacted until they were 4.5 years of age, information is missing on environmental factors that could have influenced their postnatal growth and neurobehavioral development which were not controlled for. Attained size and body composition in childhood result from the combination of genetic potential, dietary quality during pregnancy, infancy and early childhood, and exposure to infections in early life, whereas certain factors, such as temperature, stress, and nutrition, among others, have been shown to modulate ANS development in the postnatal period (2, 47). Attention to these factors was not the focus of this study, and there was not continuous follow-up of the children postnatally.
Although the sample size was larger than most studies evaluating the prenatal origins of cardiac measures of autonomic control, it was smaller than some of the previous studies assessing the relationship between intrauterine growth and child body composition (23, 25, 26). In addition, in the specific case of the analyses related to the effect of prenatal zinc on cardiometabolic outcomes in childhood, the sample size was further decreased due to the non-availability of frozen plasma samples with which to conduct biochemical analyses, decreasing the statistical power to detect differences between the two groups.

Also specific to the analyses pertaining to the effect of maternal zinc supplementation on child cardiometabolic profile, because biochemical analyses were conducted on cryopreserved plasma samples, it is possible that concentrations of metabolites have decreases with time (43-45). However, because these concentrations were within normal values for children their age and sex, it is likely that only minimal degradation occurred (46). More importantly, there is no reason to think that degradation occurred differentially by treatment group.

Lastly, although 4.5 years can be considered a long-term follow-up for certain health outcomes, it is possible that these children may still be too young to detect certain physiologic changes in their cardiac and metabolic systems that could be obvious later in childhood or adolescence.

**Implications and further research**

It is now widely accepted that the nutrition environment in early life can program the risk of long-term disease, including cardiovascular and metabolic diseases, yet there is much more that needs to be learned regarding the mechanistic processes. To date, most studies in this area rely on birth weight – an imperfect measure of the prenatal nutritional environment – to characterize the fetal nutritional environment. It is imperative to use more refined measures of fetal growth, as well as prospective measures of maternal nutrition during pregnancy, to better define the prenatal nutritional environment, and it is also important to include measurements of
the prenatal environment at earlier stages in pregnancy. The relative paucity of data from fetal growth during the first trimester of pregnancy, makes it difficult to discriminate the effects of exposures during distinct stages of gestation in terms of long-term programming.

It is also essential to better understand the effect postnatal environmental factors have on modifying the relationships between prenatal exposures and long-term health outcomes, particularly the role of specific dietary patterns during childhood. In addition, studies are needed with longer follow-up periods after birth. Evaluating outcomes at young ages may impair our ability to detect differences in cardiometabolic functions of individuals that could be apparent later in life.

Although results from observational studies suggest that maternal diet is associated with certain health outcomes in the offspring, at present, there is inadequate evidence supporting that maternal dietary interventions are effective in improving the cardiometabolic profile of their offspring. Well-designed, highly-powered, randomized clinical trials of maternal nutritional interventions and their effect on the medium- and long-term morbidity and mortality outcomes in their offspring will be essential to move in this direction. Furthermore, there is an urgent need for conducting studies in this area, among populations from developing countries undergoing the nutrition and epidemiologic transition, which are likely to be at high risk of the programming effects related to prenatal undernutrition. Low- and middle-income countries are struggling to find solutions at the levels of policy, health care delivery, and health education in relation to the double burden of disease they are facing.
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CURRICULUM VITAE

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EDUCATION

2007- **Ph.D candidate, International Health, Program in Human Nutrition**
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2007  **M.H.S. in Clinical Epidemiology**
Cayetano Heredia Peruvian University, School of Medicine, Lima, Peru.

2003  **M.D.**
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PROFESSIONAL EXPERIENCE

2014- **Affiliate Research Faculty.** Dietetics Program, Division of Health Sciences, Idaho State University. Pocatello, ID, USA.

2010-2014 **Assistant Professor.** Public Health Program, Division of Health Sciences, Idaho State University. Pocatello, ID, USA.

2013-2014 **Program Director.** Public Health Program, Division of Health Sciences, Idaho State University. Pocatello, ID, USA.

2009 - 2010 **Graduate Research Assistant.** Center for Human Nutrition, Johns Hopkins Bloomberg School of Public Health. Baltimore, MD, USA.
Study: Child Health Epidemiology Research Group, Folic Acid Section (PI: Laura Caulfield, PhD)
Principal responsibilities: Conduct of systematic reviews and data management.

2008 - 2009  **Teaching Assistant.** Center for Human Nutrition, Johns Hopkins Bloomberg School of Public Health. Baltimore, MD, USA.
Course: Principles of Human Nutrition: Summer 2008, Summer 2009, and Fall 2009 (Instructor: Benjamin Caballero, MD, PhD)

2007 - 2008  **Graduate Research Assistant.** Center for Human Nutrition, Johns Hopkins Bloomberg School of Public Health. Baltimore, MD, USA.
Study: Growing Leaps and Bounds (PI: Benjamin Caballero, MD, PhD)
Principal Responsibilities: development of food models, data forms, data collection for the 24 hr dietary intake recall for children 3-24 months of age, development of food frequency questionnaire.


2005-2006  **General Practice Physician.** “Suiza Lab” Clinical Laboratories, Lima – Peru.

2003-2004  **General Practice Physician.** Naval Medical Center, Department of Emergency Medicine, Lima – Peru.

2002-2003  **Medical Intern.** Daniel Alcides Carrion Hospital. Lima, Peru.

PROFESSIONAL ACTIVITIES

Memberships
2008-  **Member,** International Society for Developmental Origins of Health and Disease
2008-2013  **Member,** American Public Health Association
2008-  **Member,** American Society for Nutrition
2008-  **Member,** International Epidemiological Association
2003-  **Member,** Peruvian College of Physicians

Journal Reviewer
2009-  **Reviewer,** Revista Peruana de Medicina Experimental y Salud Pública (Spanish)
2011-  **Reviewer,** Obesity
2013-  **Reviewer,** Journal of Obesity and Weight Loss Therapy
2013-  **Reviewer,** Frontiers in Epidemiology

HONORS AND AWARDS

2009  **Nevin Schrimshaw International Nutrition Foundation Fellowship.** Peruvian representative at the workshop “Latin American Nutrition Leadership Program”. Santiago, Chile
2009  American Heart Association Pre-doctoral Fellowship and Weinberg Foundation research award. American Heart Association Mid-Atlantic Affiliate, Maryland

2009  Harry Prebluda Fellowship in Nutritional Biochemistry, Center for Human Nutrition, Johns Hopkins School of Public Health, Maryland

2008  Proctor and Gamble Scholarship, International Health, Johns Hopkins School of Public Health, Maryland

2008  Harry D. Kruse Fellowship in Nutrition, Center for Human Nutrition, Johns Hopkins School of Public Health, Maryland

2007  Adele Diaz Scholarship for Research in Nutrition, Center for Human Nutrition, Johns Hopkins Bloomberg School of Public Health, Maryland

PUBLICATIONS

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Peer reviewed publications


TEACHING

*Masters in Public Health (MPH) advisees*


**Mateja Savoie, RD MPH.** Thesis title: Nutrition-related behavior change in adult participants of a supplemental nutrition assistance program-education (2013).

**Michael Wucinich, MPH.** Thesis title: Rural anesthesiologist compensation: revisiting the rural pass-through (2013)

**Nicholas Scarborough, MPH.** Thesis title: Hospitalizations and mortality due to Staphylococcus aureus and MRSA, United States, 2009-2010 (2013)


**Joshua Reeder, MPH.** Thesis title: Multiple case study of Fuel Up to Play 60 (2014).

*Final Oral participation (Masters in Public Health)*


**Tamara Salazar, MPH.** Thesis title: Exploring support and attitudes that may influence smoke-free campus policy change (2012).

**Deepthi Moparthi, MD MPH.** Thesis title: Impact of types of sex education on teen birth rates by state, age, race, and ethnicity (2012).


Kristin Moore, MPH. Thesis title: Effectiveness of comprehensive community health screening events in Ada County, Idaho: are they connecting individuals with personal health services? (2014).

Oluwafemi Abimbade, MD MPH. Thesis title: Key stakeholder perceptions regarding budget cuts in Idaho mental health services (2014).

Final Oral participation (Masters in Science)

Nikki Mills, MPAS. Physician Assistant Project (2013)


Joanna Nichols, MPAS. Physician Assistant Project (2014)

Danichi Yoshida, MPAS. Physician Assistant Project (2014)

CLASSROOM INSTRUCTION

Primary Instructor

Applications in Epidemiology (2011-2014). This 3-credit course provides a broad overview of epidemiological principles and methods used in public health. It emphasizes conceptual foundations of and critical review of epidemiologic study designs.

Biostatistics (2010-213). This 3-credit course provides a broad overview of biostatistical methods and concepts used in public health. It emphasizes conceptual understanding of the calculation and interpretation of statistical estimation and inferential statistics.
US and Global Health Systems (2010). This 3-credit course explores the historical and contemporary factors that shape health systems in the US and internationally.

Technological Applications in Public Health (2011-2014). This 3-credit course introduces software programs for data management and analysis in public health practice.

Research and Writing in Health (2012-2013). This 3-credit course is designed to facilitate the development of a thesis proposal and the completion of the MPH thesis.

RESEARCH GRANT PARTICIPATION

State Oral Health Evaluation
Sponsoring Agent: Idaho Department of Health and Welfare
Role: C-Principal Investigator, 10% effort
Date: Dec 2013-Aug 2014
Project description: Collaborative project with the Evaluation and Surveillance Specialist at the Idaho Department of Health and Welfare (IDHW) to build the evaluation capacity of the State Oral Health program, as required by the IDHW’s new 5-year grant from the CDC.

Gateway to Health
Sponsoring Agent: Portneuf Health Care Foundation, Pocatello-Idaho, USA
Role: Co-Principal Investigator
Date: August 2012-November 2012
Project description: Project to develop a Community Needs Assessment of Southeastern Idaho.

Decreasing jail recidivism in Bannock County, Southeast Idaho
Sponsoring Agent: Portneuf Health Care Foundation, Pocatello-Idaho, USA
Role: Principal Investigator, 10% effort
Date: June 2012-June 2013
Project description: Community study to determine the incidence and prevalence of jail recidivism in Bannock County Adult Correctional System, to explore its determinants and recommend best practices.

Long-term effects of individual differences of fetal autonomic control and growth on cardio-metabolic markers
Sponsoring Agent: Division of Health Sciences, Idaho State University, Pocatello-Idaho, USA
Role: Principal Investigator, 10% effort
Date: Dec 2010-Jun 2011
Project description: Retrospective cohort of ~200 Peruvian children (from fetal period) to determine association between individual differences in fetal autonomic control and growth on cardio-metabolic markers at 4.5 years of age.

Diarrhea caused by coliforms and other agents in infants in Lima, Peru
Sponsoring Agent: Cayetano Heredia Peruvian University, Lima - Peru
Children’s Health Institute, Lima – Peru
Role: Co-investigator, 10% effort (PIs: Theresa Ochoa, MD; Claudio Lanata, MD, MPH)
Date: Jan 2008- Oct 2009
Project description: Funding extension for the study “Diarrhea caused by coliforms and other agents in infants in Lima, Peru”. Prospective community cohort of ~1000 Peruvian children to determine incidence of diarrhea, clinical presentation, and risk factors associated with diarrhea; and prevalence of etiologic agents in children with diarrhea and healthy controls.

Determinants of Childhood Obesity in Lima, Peru
Sponsoring Agent: International Life Sciences Institute – Latin America
Role: Co-Principal Investigator (Co-PI: Reyna Liria, RD, MPH); 30% effort Nov 2006-Aug 2007, 5% effort Sep 2007-Nov 2007
Date: Nov 2006 – Nov 2007
Project: Cross-sectional study to determine the prevalence of obesity in children 3rd-6th grade in Lima, Peru. Case-control study to determine risk factors for obesity in the same population.

The Nutrition Transition in Peru
Sponsoring Agent: ORC Macro International
Role: Co-investigator, 20% effort (PI: Claudio Lanata, MD, MPH)
Date: Aug 2006-Aug 2007
Project: Secondary data analysis of Demographic Health Studies from Peru to describe the nutrition profile trends in children less than 5 years of age and women in reproductive age, and multivariate analysis to determine factors associated with obesity in the same population.

ACADEMIC SERVICE

Dietetics Program
Member, Faculty Search Committee in the Dietetics Program (2015).

MPH Program
Chair, Department Chair Search Committee in the Department of Community and Public Health (2014).
Chair, Faculty Search Committee in the Public Health Program (2014).

Division of Health Sciences
Member, Interprofessional Affairs Council (2012-2014).
Member, Division of Health Sciences Promotion and Marketing Committee (2011-2012)
Member, Research Day Committee (2011)

Idaho State University
Member, Advisory Board of Early Learning Center (2010-2011).

PRESENTATIONS


ADDITIONAL INFORMATION

My research interests are in the area of the nutrition transition, obesity, and chronic diseases in developing countries.

My specific interests include:

1. The Nutrition Transition
2. Determinants and health consequences of obesity
3. Early determinants of chronic diseases

Keywords
Nutrition
Obesity
Nutrition Transition
Chronic Diseases
Developing Countries
Developmental Origins of Health and Disease

LANGUAGES
Fluent in English and Spanish at the Professional Level.