THE RELATIONSHIP BETWEEN MATERNAL BODY COMPOSITION AND DIET WITH FETAL DEVELOPMENT IN LOW-INCOME WOMEN IN BRAZIL

By

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ABSTRACT OF THE DISSERTATION

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The assessment of body composition requires the use of techniques that can accurately detect changes in total body water (TBW), and the accuracy of bioelectrical impedance analysis (BIA) during pregnancy has been questioned. However, BIA was found to estimate TBW reliably during pregnancy. Previous studies of body composition during pregnancy suggest that the fat free mass (FFM) component of gestational weight gain (GWG) might be more important towards the end of gestation for fetal development while others suggest that the general GWG during mid-pregnancy is more important. Such associations are unclear regarding the components of GWG during the second trimester of gestation, particularly fat mass (FM). Therefore, the objective of this study was to compare the use of segmental BIA with whole body BIA and assess the relationship between maternal FM and fetal growth and birth weight. This study further sought to analyze the fatty acid composition of breast milk from a subsample of women with premature infants and their cognitive and linguistic development. Based on the experiments of this research, there was a high level of disagreement between segmental and whole body BIA, where FFM was

underestimated and FM overestimated by segmental BIA. With the use of whole body BIA, FM was significantly related to fetal growth and birth weight. In the subsample of women with preterm babies, the concentration of essential and long-chain polyunsaturated fatty acids was optimal, and we did not find any developmental delays in these children at 1yr. In conclusion, further studies need to verify our findings in this and other populations. As the Brazilian diet transitions into a Westernized one and the prevalence of obesity increases, the study of maternal diet, body composition, and breast milk quality is more important now than ever.

ABBREVIATION LIST

AA	Arachidonic acid
ALA	Alpha-linolenic acid
BIA	Bioelectrical impedance analysis
BIS	Bioimpedance spectroscopy
BF	Body fat
BWT	Birth weight
BWTadj	Birth weight adjusted for gestational age
CHD	Coronary heart disease
DEXA	Dual energy X-ray absorptiometry
DHA	Docosahexaenoic acid
EFA	Essential fatty acid
EFW	Estimated fetal weight
EPA	Eicosapentaenoic acid
FFA	Free fatty acids
FFM	Fat free mass
FFQ	Food frequency questionnaire
FG	Femur growth
FL	Fetal length
FM	Fat mass
GA	Gestational age
GDM	Gestational Diabetes Mellitus
GLC	Gas liquid chromatography
GWG	Gestational weight gain
H	Height

HC	Head circumference
HPL	Human placental lactogen
IUGR	Intrauterine growth restriction
L	Length
LA	Linoleic acid
LCPUFA	Long-chain polyunsaturated fatty acid
R	Resistance
SAS	Statistical Analysis Software
SES	Socioeconomic status
SPSS	Statistical Package for the Social Sciences
TBW	Total body water
T2D	Type II diabetes
UERJ	State University of Río de Janeiro
Xc	Reactance
Z	Impedance

DEDICATION

I dedicate this dissertation to my parents, Jorge and Jannette, to my sisters, Alana and Adriana, to my grandparents, abuelita Aurín and abuelita Freddy, abuelito Lúper and abuelito Carlos, my cousin Mari Luz, and to my husband, Pedro for bringing so much joy and light to my life, for the inherited and shared values, for listening, for being my best friends. I love you.

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1 INTRODUCTION

1.1 Statement of the problem

In developing countries, such as Brazil, malnutrition due to unequal social and economic challenges, is highly prevalent and a major public health concern (1, 2). Since pregnant and lactating women have higher nutritional needs than normal, it is not uncommon to find that fetal development and subsequent birth weight are poor in developing countries. More recently, obesity is replacing undernutrition in all strata of society, thus maternal gestational weight gain (GWG) is higher than recommended and babies are born heavier. Furthermore, birth weight is a determining factor for childhood growth and adult health, and this could place future generations at risk for poor health. However, studies examining maternal body composition and fetal growth in low-income women are scarce and have reached different conclusions.

It is important to study the relationship of maternal diet and body composition with fetal development and birth weight with a clear understanding of the metabolic milieu involved in pregnancy, the objective of this study. This study will help determine the relationship between maternal body composition and fetal growth during the second trimester of pregnancy and neonatal health in Rio de Janeiro, Brazil. Furthermore, we will study breast milk fatty acid concentration from the first week of pregnancy and its relationship with premature infant cognitive, linguistic, and motor development. The main hypothesis is that a higher fat mass (FM) or fat free mass (FFM, body tissue that is not fat, such as muscle, organs, and bone, and water) at mid-pregnancy will be associated with

greater fetal growth and higher birth weight, independent of age, energy intake, and physical activity (**Figure 1**). Our secondary hypothesis is that higher [EFA] and [LCPUFA] in breast milk; important components of the central nervous system, retina, and organ tissues, will be associated with better cognitive, linguistic, and motor development at 1 year in infants born preterm (**Figure 2**).

Figure 1. Main hypothesis

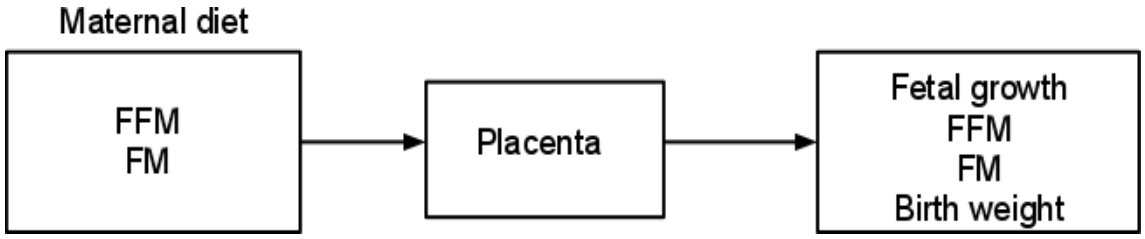
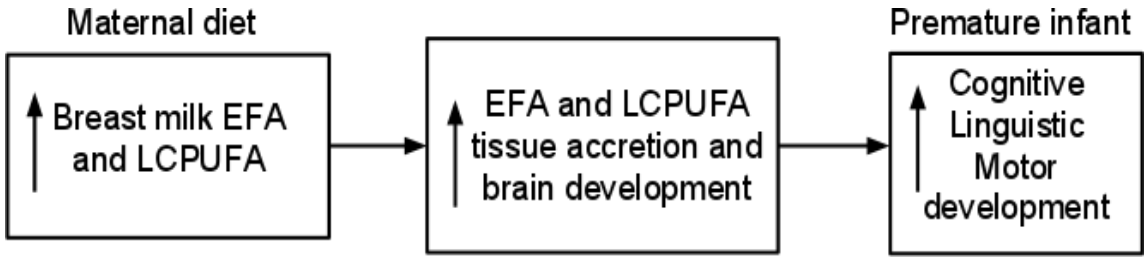


Figure 2. Secondary hypothesis



2 LITERATURE REVIEW

2.1 Overview of Nutrition and Health in Developing Countries

The improvement of nutritional health and status is an important aspect of public health that promotes the social and economic success of a nation. In both children and adults living in developing countries, those of lower socioeconomic status are more likely to have poor access to healthy food, suffer from undernutrition, and suffer premature mortality due to higher rates of all diseases and conditions (3, 4). While the prevalence of growth retardation (stunting), a key marker of long-term nutritional health, fell from 48% in 1980 to 33% in developing countries in 2000 (5), malnutrition still remains a major public health problem, as a third of children under 5 living in developing countries are stunted (3, 4). Furthermore, there are 820 million people affected by hunger in developing countries (6). A poor nutritional status is a major determinant of disease and is also associated with impaired productivity, factors that influence the health and economic development of future generations (7). Thus, the role of public health, particularly nutrition, is fundamental to improve education, child mortality, maternal health and disease, and social and economic development (8).

While undernutrition is still a major public health problem, it is being replaced by overweight and obesity in developing countries (9, 10). Obesity is defined as an abnormal or excessive fat accumulation that may impair health (9). Currently, 65% of the world's population lives in countries where obesity is more lethal than undernutrition (9). What was mainly a burden of developed countries,

is becoming more prevalent in developing countries, especially in urban areas as a consequence of reduced physical activity and energy imbalance, where the diet is shifting to an energy dense, micronutrient poor one (9). It is estimated that 35 million children are overweight in developing countries while there are 8 million in developed countries. Obesity is associated with an increased risk of coronary heart disease, type 2 diabetes, cancer, hypertension, dyslipidemia, stroke, liver and gallbladder disease, sleep apnea and respiratory problems, osteoarthritis, and gynecological problems (11).

Populations living under more affluent conditions have the means to obtain healthier foods while the poor do not (12). Therefore, it is imperative to study how these changes in food consumption affect the cycle of maternal-child obesity. Preventive measures for undernutrition and obesity are necessary because of the later risk for disease beginning in the womb. Both under and overnutrition need to be treated simultaneously to prevent and alleviate the loss of productivity in a country and improve health.

2.1.1 Socioeconomic Challenges in Brazil

Social inequality and wide income disparities are still major problems in Brazil, and the level of poverty is significantly above the norm for a transitional country (13). For example, earnings of women are on average 29% lower than men even when working women receive on average an additional year of education, and this is not different for blacks compared to whites (14). Part of the cause for these continued social problems rests in the repeated economic challenges in Brazil that have aggravated poverty. In the 1960's and 70's,

poverty was reduced by economic growth (higher employment and wages), whereas in the 1980's a recession in the private sector prompted a decline in income for all social groups, but affected the lowest income groups the most (13). More recently, between 1995 and 2004, the prevalence of poverty decreased by 8 percent and was attributed to an increase in the gross domestic product (GDP) and improvements in income distribution (15). Still, these statistics do not necessarily mean that the poor have felt these economic successes. Thus, studying the relationship between maternal diet and fetal growth has great potential to contribute to the long-term health of Brazilian children, and can influence social and economic development in Brazil (14), potentially leading to decreased poverty and a more egalitarian society.

2.1.2 The Nutrition Transition and Double Burden in Brazil

One of the most significant public health challenges Brazil has faced is the "nutrition transition", changes in dietary and activity patterns determined by the interaction between economic, demographic, environmental, and cultural changes (10, 16, 17). In Brazil, the diet has shifted from a decreased consumption of cereals, legumes, fruits and vegetables to an energy-dense, micronutrient-poor, one with higher consumption of fats in general, fats of animal origin, and processed foods that are rich in sugar and sodium (18). Undernutrition in Brazil has decreased (19-21), but it is still a public health problem, where pregnant women and children from lower socioeconomic groups are the most affected (22, 23). Strategic actions still are needed to continue to alleviate a problem that involves poor nutrition bilaterally (lacking micronutrients

and energy or energy dense but micronutrient-poor) to prevent poor growth without promoting obesity. Data from the present study may prove to be important in developing these strategies.

Obesity is replacing undernutrition in the more developed regions of Brazil and may increase the risk for chronic disease (1, 10, 24). While nationally representative surveys in Brazil report that undernutrition in children has decreased (20), this is not true for all socio-economic status (SES) groups. For example, low-income women are more susceptible to both undernutrition and risk of becoming overweight than higher-income women (12, 24). Regardless of the cause of the double burden, it reflects a cycle of disease and a significant burden in health care services of $4.5 billion per year (25). Therefore, it is essential to focus on maternal nutritional status, beginning *in utero* and throughout life, battling social inequality to prevent the cycle of socioeconomic inequality, poor growth and development, and chronic disease.

2.2 Growth and human health

The most important indicator of health and well being of children and society in general is growth status (5, 26). Stunting (height-for-age less than -2 SD of the WHO Child Growth Standards median) and wasting (weight-for-height less than -2 SD of the WHO Child Growth Standards median), for example, are used to determine the extent of undernutrition, moderate, chronic, or acute, respectively (27). Stunting may begin *in utero* by poor maternal diet and has been associated with increased central fat and chronic disease in adulthood (28-30) and varies by region in Brazil depending on socioeconomic status (21, 31).

Major factors that contribute to stunting include lack of access to food, inadequate basic sanitation, low education levels, short period of breast-feeding, and deficient health services (19, 26). On the other hand, obesity rates in low-income populations are increasing at an alarming rate due to the availability of energy dense and micronutrient-poor foods (32). Children that are born to obese mothers tend to have a faster growth rate begining *in utero* until adolescence (33, 34). This growth rate is also related to an increased risk for obesity and chronic disease in adulthood (35). Research to monitor growth patterns according to socio-economic changes in developing countries must be a priority to prevent poor or heightened growth during pregnancy and the imminent development of chronic disease, the very focus of this study.

2.3 Pregnancy Physiology Overview

Pregnancy is a state of intense physiological and physical adaptations (36, 37) and a key period of the life cycle that determines the future health of the offspring. In a normal pregnancy, the maternal cardiovascular system adapts by decreasing the total peripheral resistance after the sixth week of gestation causing a circulatory underfilling, which activates the renin-angiotensin-aldosterone system and causes a plasma volume expansion (36, 37). The plasma volume expansion increases to approximately 50% by the third trimester of pregnancy (36, 37). Consequently, there is an increased blood flow to the fetus, which is positively correlated with birth weight (38). Heart rate and cardiac output also increase by 10-15 bpm and peak during the second trimester (39). Blood pressure decreases at mid-pregnancy due to the increased vasculature of

the uterus, the uteroplacental circulation, and the reduced vascular resistance of the skin and kidneys (40).

The renal system adapts by increasing the size of the kidneys and renal plasma flow up to 65% until mid-pregnancy and falls thereafter to normal levels (36). The glomerular filtration rate also increases up to 55% until the end of pregnancy, so there is an increased filter load of metabolites. There is an increased sodium and potasium retention (950 and 350 mmol, respectively) and an increased glucose, amino acid, soluble vitamin, and calcium excretion, the latter being countered by increased intestinal absorption at 24 weeks of pregnancy (36).

The gastrointestinal system is also highly adapted in pregnancy. The intestinal tract has decreased motility and salt and water absorption increases. The liver synthesizes more fibrinogen, albumin, and plasma globulin due to estrogen, thus more hormone-binding globulins. The liver also decreases amino acid extraction (36). These modifications favor optimal nutrient partitioning, necessary for maternal and fetal growing needs.

2.3.1 Fuel Utilization in Pregnancy

Pregnancy can be described as glucosuric and hyperlipidemic (41, 42). Early in pregnancy, insulin, hepatic glucose production, and basal glucose remain at normal levels (43). After 6-12 weeks of gestation fasting plasma glucose falls by 0.11 mmol/l and remains under pre-pregnancy levels until mid-pregnancy, and it is accompanied by increased muscle insulin sensitivity (44). This sensitivity triggers a higher rate of glycogen synthesis and storage, fat

deposition and cell amino acid uptake (for gluconeogenesis) in early pregnancy. As pregnancy progresses, insulin production increases and may be 3- to 3.5-fold higher in the late third trimester. This insulin production favors lipogenesis and fat storage, influenced by lower cortisol, human placental lactogen (HPL), estrogens, and progestins (45, 46). Simultaneously however, insulin resistance develops (47, 48), where the insulin response in late pregnancy decreases by 50-75% (49), which causes large changes in the postprandial concentration of macronutrients. Therefore, maternal insulin resistance in late pregnancy improves the mobilization of glucose, lipoproteins, and amino acids to the fetus after a meal (45).

Maternal fat storage is stimulated from early- to mid-pregnancy and mobilization is favored in late pregnancy by numerous alterations in lipid metabolism. Specifically, changes in early pregnancy are under hormonal control by estrogen, progesterone and insulin, which inhibit lipolysis and promote fat deposition (47). After midpregnancy, the same mechanism that induces hepatic glucose production by increased human placental lactogen (HPL) acts against insulin and induces lipolysis and release of FFA (50, 51). Therefore, the mother can use FFA as a fuel source and glucose is preserved for fetal development. Triacylglycerols, fatty acids, cholesterol, lipoproteins, and phospholipids increase steadily throughout pregnancy as a consequence of higher estrogen levels and insulin resistance (52). Cholesterol, as part of lipoproteins, increases after 8 weeks until term and is used for steroid synthesis by the placenta (45). Especially, HDL and VLDL levels increase, the latter by up to 50% by the 36th

week of pregnancy (53). Furthermore, VLDL triglyceride at term is directly related to birth weight and placental weight (36). A better understanding of energy metabolism during pregnancy is key to study the association of maternal diet and body composition with fetal growth.

2.3.2 Energy requirements during pregnancy

Based on a model of an average weight gain of 13.8 kg during pregnancy, Butte and King (54) summarized that:

> "The energy requirement of a pregnant woman is the level of energy intake from food that will balance her energy expenditure when the woman has a body size and composition and level of physical activity consistent with good health, and that will allow for the maintenance of economically necessary and socially desirable physical activity. In pregnant women the energy requirement includes the energy needs associated with the deposition of tissue consistent with optimal pregnancy outcome."

Pregnancy is highly anabolic, so dietary energy requirements increase and a positive energy balance is favored for the accumulation of fat and protein for the synthesis of new maternal and fetal tissue (55, 56). The higher energy needs during pregnancy are also due to the increased maternal basal metabolic rate (BMR), the energy spent at rest (54, 57). The elevated BMR is in part explained by the growing tissues and other physiological changes that occur during pregnancy, increased cardiac output, maternal circulation, respiration, and renal function (58). Initially, energy requirements during pregnancy were based on the gestational weight gain (GWG) (58), however not all factors that influence the high variation in individual women during pregnancy are completely understood, and the variability in maternal expenditure is controversial. Variable maternal pre-pregnancy nutritional status, activity energy expenditure (AEE), and

GWG (54, 59) need to be considered for individual energy needs assessments and recommendations.

One study proposed that BMR is significantly correlated with GWG, and maternal body fat (56). Others have suggested that decreases in free triiodothyronine, and free tetraiodothyronine, hormones that control metabolic rate, could lead to higher BMR (60). FFM and FM both have been correlated with higher BMR but not consistently (2, 57, 61). Interestingly, Prentice (2) showed that pregnant women in developing countries increase BMR to a lower extent compared to women in developed countries. Furthermore, Prentice (62) found that in developing and developed countries, increases in BMR during pregnancy were highly correlated with pre-pregnancy percent fat. Studies now show that pre-pregnancy nutritional status has a high influence on pregnancy BMR increase (2, 57).

In developing countries, as well as in some cases in developed countries, some women have BMR depressions, which are not completely understood physiologically, and further studies are necessary to explain this. Furthermore, there is a tendency for pregnant women to underreport their energy intake (63, 64). These factors combined warrant a deep understanding of the physiological changes that occur in pregnancy, the importance of the pre-pregnancy nutritional status, and the influences of the composition of weight gain during pregnancy to make recommendations of dietary and weight gain needs during pregnancy to favor optimal fetal growth.

2.3.3 Specific nutrient needs during pregnancy overview

Other than meeting individual energy needs, pregnant women should reach the recommended intakes of essential fatty acids (EFA), long chain polyunsaturated fatty acids (LCPUFA) (65), micronutrients (66, 67) and antioxidants (68). The inclusion of whole foods such as meat, fish, and eggs in the diet of pregnant women is an excellent way to include highly bioavailable protein, fatty acids, and micronutrients essential for the development of the fetus and for the improvement of the quality of breast milk (69).

LCPUFAs such as n-3 and n-6 fatty acids serve as a functional part of membrane phospholipids, precursors for eicosanoids, ligands for membrane receptors and transcription factors that regulate gene expression (69). Specifically, docosahexaenoic acid (DHA) and arachidonic acid (AA) are LCPUFAs accumulated in the fetus that can only be obtained through placental transfer and interconversion from alpha-linolenic acid (ALA) or linoleic acid (LA), respectively (70). DHA is preferentially transferred via the placenta, resulting in higher DHA levels in the neonate brain and retina (71). Therefore, a higher concentration of maternal dietary ALA and DHA improve infant and child visual and neural maturation (72, 73).

Iron, zinc, calcium, vitamins B12 and A, and riboflavin are among the most important micronutrients for the mother and fetus during pregnancy (69). Iron is found in many proteins such as enzymes or transport proteins like hemoglobin and myoglobin (74). Zinc is involved in gene expression, cell division and differentiation, and DNA and RNA synthesis (75), processes extremely relevant

to maternal and fetal health and survival. Vitamin B12, a methyl group transferor, is involved in protein synthesis, fat and carbohydrate metabolism, among other important biochemical reactions (76). Vitamin A is required for embryonic eye development (77) and for the expression of many developmental genes (78). Studies of micronutrient deficiency are abundant (79-82) and emphasize the need for dietary supplementation for women living in underdeveloped and developing countries where the diet may be low energy-dense, adequate or energy-dense, however micronutrient poor.

2.4 Maternal Diet and Fetal Growth

The maternal diet during pregnancy is key to improving fetal development and optimizing infants' physical and intellectual potential to increase their future opportunities in life. Adequacy in terms of calories and nutrients prevents intrauterine growth restriction (IUGR) and stunting, as well as promotes cognitive function, a well-defined factor that has long-term effects on the economic potential of the individual, and ultimately the nation. During pregnancy, EFA, LCPUFAs (65), micronutrients (66, 67), and antioxidants (68) are also essential to prevent poor fetal growth. Essential fatty acids (ALA and LA) are fats that cannot be synthesized by the body and must be obtained in the diet and are necessary for normal fetal and infant neurodevelopmental function, visual acuity, improved cognition and motor development (83, 84). Preventive measures, such as beneficial changes in maternal diet, are certain to have a greater impact on fetal growth and long-term nutritional status, compared to treatment of poor birth outcomes.

The benefits of improved infant feeding after birth may be attenuated by the effects of intrauterine programming or by intergenerational effects of maternal stunting (85). There are three windows of fetal growth where maternal nutrition can potentially influence fetal development. Winick (86) described those phases as cellular hyperplasia, hyperplasia and hypertrophy, and predominantly hypertrophy. Kunz and King (87) also suggested that maternal nutrition and metabolism affect the health of the baby by three mechanisms. First, early exposures may affect the developing embryo directly or indirectly via placental development. Second, during organogenesis, the environmental influences could alter cell types in number and function in organs like the heart, pancreas, or kidneys, which may predispose to disease. Third, developed organs could be affected in terms of the regulatory set points by which they function. These mechanisms have been supported by many animal studies on maternal diet and offspring health by protein energy restriction during key periods of growth of the fetus, total calorie energy restriction, micronutrient deficiency, or supplementation studies (PUFAs, vitamins and minerals) (88-90). Preventative measures by beneficial alterations in maternal diet and weight gain will have a much better impact on fetal growth, rather than treatment of poor birth outcomes.

2.5 Maternal diet and breast milk composition

The diet of pregnant or lactating women influences the breast milk nutrient content. As a woman stores fat during pregnancy, it is essential for her to consume the best quality of fats possible so that during critical periods of fetal growth and lactation, these will be available to the baby. In this sense, the

concentration of EFAs and LCPUFAs and some vitamins may as important as total energy intake (91, 92). DHA can be consumed from fish, for example, and the body can synthesize i(93). DHA is a very important LCPUFA for fetal and infant neurodevelopmental function and visual acuity, and improved cognition and motor development (83, 84). Low levels of DHA at birth and during breast-feeding are associated with poorer neural and visual maturation in infancy and childhood (94).

LCPUFAs accumulate predominantly during the third trimester of pregnancy in the brain but continue to be essential during breastfeeding, when such fatty acids accumulate in the developing central nervous system (CNS), the retinal cells, and various organs (95). Particularly, DHA and arachidonic acid (ARA) accumulate rapidly in gray matter up to the second year of life (96, 97). DHA sustains synaptogenesis and myelination, defined as the formation of the lipid myelin bilayer that surrounds the axons, thus becoming essential for normal cognitive and behavioral functions (98, 99). Several studies support the benefits of maternal DHA intake and breastfeeding or of infant formula DHA supplementation with infant developmental outcomes (72, 100-102). Such effects may be long-lasting on the infant (103). Therefore, pregnant and lactating women are recommended to reach a daily intake of 200-300 mg DHA (65, 104), but there might be a dietary deficiency of DHA and breast milk concentrations of essential fatty acids (EFA), especially among women in low-income parts of the world (105, 106).

Torres (106) reviewed the intake of EFA and LCPUFA in Brazil, and found that pregnant women are below the recommended daily intake for pregnant and lactating women. Furthermore, studies of the sources of fat intake in Brazilian lactating women showed that the diet is comprised of approximately >80% n-6 LCPUFA, showing a decline in fish consumption and an increased intake of meat and vegetable oils (107, 108), thus a high n-6: n-3 ratio. An ideal ratio of n-6 to n-3 would be 1:4, a balance that favors normal growth and homeostasis (109). To our knowledge there are no studies on such dietary changes and the possible effects, especially of maternal fat consumption, on the infant/child population in Brazil. Therefore, further studies are needed to better understand the possible impacts of the maternal diet on breast milk composition and infant development taking into account the transitional diet in Brazil.

2.5.1 Premature infant development and nutrient intake

Studies of premature infant breastfeeding and formulafeeding have shown that breastfeeding provides significant benefits for the infant (110-113). Premature infants who received maternal milk had increased host defense and reduced infection, and better cognitive and motor development in later life (96, 114). Compared to infants who received formula, premature infants fed maternal milk had a lower risk for the metabolic syndrome, especially those fed fortified maternal milk (111, 115, 116). These protective effects of breast milk for premature infants point to the importance of the quality of breast milk.

Compared to infants born at term, premature infants are at an increased risk of suffering from motor impairment, attention and learning disabilities (117-

120). The second and third trimesters of gestation are periods of vulnerability to nutritional insults. In particular, there is an accelerated rate of LCPUFA accretion in the central nervous system (CNS) and organ membranes (95). Therefore, potential disabilities may be associated with disturbances in nutrient transfer prenatally and postnatally to essential fatty acid-poor milk during breastfeeding. DHA and AA, for example, both accumulate rapidly in grey matter during the third trimester of gestation and up to the second year of life (96, 97). DHA is crucial for synaptogenesis and myelination (the formation of the lipid myelin bilayer that surrounds axons, which is essential for the development of behavioral, cognitive, and affective functions) (98). Thus, an optimal maternal EFA and LCPUFA status are essential prenatally and during breastfeeding for proper brain maturation, development, and visual acuity of premature infants (95, 121, 122).

Some studies show that women with premature infants have higher breast milk LCPUFA during the first week postpartum compared to breast milk of women with full-term births (123-125) conferring a protective effect for the infant. For example, mothers of pre-term infants had twice the concentration of AA (0.82 wt%) and DHA (0.33 wt%) compared to full-term infants (AA, 0.44 wt% and DHA 0.15, wt%, respectively) in one study (124) and 1.5 more AA and twice the DHA than full-term infants in another study (125). A recent review concluded that breastfeeding was associated with higher infant IQ and sight skills (126). Together, these findings further heighten the need to monitor maternal diet, establish different DRIs for lactating women with preterm rather than term infants, and evaluate the need for maternal and/or infant supplementation Therefore, the

objective of this study was to assess the fatty acid composition of breast milk in low-income women and to determine the relationship between the FA composition and cognitive, linguistic, and motor development in breastfeda premature infants.

2.6 Bioimpedance Analysis

Bioimpedance analysis (BIA) is a relatively simple, low cost, safe, and reproducible technique that can be used to assess body composition in health and disease (127-131). BIA is based on the resistance (R) and reactance (Xc) measurements of body tissues against a small alternating electric current (typically 800 μA, 50 kHz for single frequency BIA) (132). This current will flow easily through tissues with high water/electrolyte content (fat free mass) and result in low R-values while tissues such as fat present high R (133). Another component of the BIA measurement is capacitance (C, the number of cells and level of integrity of the cellular membranes), which increases with increasing Xc, the energy stored in the body due to intact cell membranes. However, BIA assumes that the body is a cylindrical conductor of a specified length (L) or height (Ht) and an area (A) or volume (V) (134), where $R = \rho L/A$ or $R = \rho L/V$ and ρ is the resistivity of the tissues or the opposition to the flow of the electric current. Furthermore, the BIA technique uses prediction equations that have been developed in euvolemic populations (134), and may not be appropriate for populations with an altered body geometry and body fluids, such as during pregnancy (135-137).

2.6.1 Bioimpedance Analysis During Pregnancy

The maternal nutritional status during pregnancy is very relevant to fetal growth, as discussed above, and for the health of the mother for the prevention of future disease (138-142). BIA is an inexpensive body composition assessment technique that has been safely used during pregnancy to assess changes in total body water (TBW), hydration status, FM, and FFM (143-145). However, Buchholz (135) reviewed the use of BIA and bioimpedance spectroscopy (BIS) in clinical populations and concluded that the sensitivity of BIA to changes in the trunk is very low and argued that BIA does not accurately predict TBW, especially in populations with altered trunk geometry, such as during pregnancy. This fact may invalidate prediction equations produced in healthy, non-pregnant samples and applied to pregnant women. However, to date there are no prediction equations developed from samples of pregnant women. Furthermore, no study has looked to evaluate the accuracy of BIA compared to segmental BIA during pregnancy for the measurement of body fat and FFM controlling for the changing trunk. Segmental measurements of body composition by BIA may result in more accurate assessments during pregnancy.

Segmental BIA of the arms, legs, and trunk may account for the variability in body build among populations (146) and may help create reference ranges for body composition during pregnancy. While whole body BIA considers that the body is a single conducting cylinder, segmental BIA considers that the arms, legs, and trunk are five interconnected conducting cylinders (147). Although the trunk has a more complex non-uniform structure due to the organs and the

growing fetus, uterus, placenta, and amniotic fluid during pregnancy, it was previously reported that segmental BIA could estimate body TBW more accurately when extracellular fluid was redistributed in the trunk and limbs (148). Thus, a segmental approach of body composition assessment during pregnancy may provide more accurate measurements of FM, FFM, and TBW.

2.7 Maternal body composition changes during pregnancy and fetal growth

Maternal FM, FFM, and TBW each independently affect fetal development. While the relationship between GWG and fetal growth and birth weight is known (149-152), studies of maternal body composition and fetal growth or birth weight are contrasting. During the first trimester, maternal body composition does not seem to have a significant contribution to fetal growth. Villar (153) reported that maternal FM during the start of the second trimester of pregnancy is positively associated with birth weight, while Mardones-Santander (154) reported the same relationship during the third trimester of pregnancy, and Lederman (155) found a weak negative association between FM and birth weight. Others have found no association of maternal FM and birth weight (136, 156, 157). Maternal total body water (TBW) (155) near term and higher maternal FFM during the third trimester are both positively associated with birth weight (156, 158). Furthermore, Thame (157) reported that, in adolescent and mature pregnant women, the gain in FFM towards the end of gestation was more important in determining birth weight than overall GWG in both groups of women. Because most of the variance in GWG during the second trimester is accounted for by fat (136, 153), maternal FM during the second trimester may be a key

factor for fetal growth and the influence of the components of GWG warrants further research. Based on these studies, it appears that maternal FM and FFM individually (different from maternal weight) during mid-pregnancy may have differential effects on fetal growth and birth weight.

Pregnancy is a state of constant nutrient partitioning; a balance between a highly anabolic state for fat and protein deposition and catabolism for nutrient availability for the fetus (45, 46, 50, 51). Previous studies did not find strong associations between maternal diet and birth weight or placental weight (159, 160). Furthermore, Duggleby (161) reported that increased length at birth was related to maternal protein turnover but not maternal protein intake. This suggests that maternal dietary macronutrients are deposited as FM or FFM in growing tissues and then supplied to the fetus. Because women of a similar pre-pregancy weight and GWG might have a very different body composition, it is of outmost importance to understand the associations between maternal body composition, nutrient turnover, and fetal growth, since the uterine environment might be the most important factor to determine a baby's future health. Based on the existing literature, it is clear that maternal GWG and dietary intake alone may not be appropriate predictors of fetal growth and BWT. Therefore, the aim of this study was to assess the relationship between maternal body composition during the second trimester of gestation and fetal growth in low-income women from Brazil.

2.8 *In utero* and post-natal nutrition of the offspring

Maternal diet and body composition influence fetal health and future health, such that, low birth weight is related to obesity and chronic disease in adulthood. The “thrifty phenotype hypothesis” explains how poor fetal and infant growth is related to a higher risk of developing impaired glucose tolerance and the metabolic syndrome (162). Thus, a poor condition in early life programs a postnatal phenotype that will be beneficial for survival under similar poor nutrition conditions as *in utero*, however, unfavorable if nutrition is more abundant (163). To date there is sufficient epidemiological evidence worldwide and evidence from animal studies to backup the concept of fetal programming (164).

As populations undergo rapid socio-economic transitions in the developing and the developed worlds (89), the need to find ways to reduce the mismatch between an undernourished fetal development and a postnatal energy rich diet and to prevent maternal obesity is greater now than ever. Therefore, it is necessary to understand some basic concepts and theories that are well established regarding fetal programming for postnatal survival and how they relate to the programming of disease later in life. In 1989 Hales and Barker suggested the “thrifty phenotype hypothesis”, trying to explain how poor fetal and infant growth was related to a higher risk of developing impaired glucose tolerance and the metabolic syndrome (162). Their hypothesis states that a poor condition in early life programs a postnatal phenotype that will be beneficial for survival under similar poor nutrition conditions as in utero, however, unfavorable if nutrition is more abundant (163). This was supported by the Dutch Hunger

Winter study where the effect of the “thrifty phenotype hypothesis” was most marked in those that were in utero in their third trimester during the hunger. Another hypothesis called the “thrifty genotype hypothesis” had also been proposed since 1962 based on studies of Pima Indians in America (165). This hypothesis explained the high prevalence of obesity and diabetes in these populations, and it was suggested that genetic changes might accumulate in order to become beneficial for survival under famine conditions, but again, become harmful if the society becomes more affluent, implying ameliorated nutrition (165). To date there is sufficient epidemiological evidence worldwide and evidence from animal studies to backup fetal programming. It is now known that under- or over-nutrition in the fetal environment and infectious or chronic disease states, and hypoxia of the mother may expose the fetus to abnormal hormonal, growth factor, and cytokine or adipokine cues (164). Thus, metabolic, immune system, vascular, hemodynamics, renal, growth, and mitochondrial parameters are altered resulting in poor glucose homeostasis, insulin resistance, type-2 diabetes, hypertension, cardiovascular disease, obesity, and heart disease (164).

2.8.1 Studies of fetal programming: Undernutrition and overnutrition

Fetal programming refers to the process in which an environmental stimulus during a critical window of time, early in life, permanently affects subsequent structure, function or developmental schedule of the organism (163). Specifically, nutritional programming takes place during the maternal care period, and the adaptations tend to be long-term and irreversible (166). As a suboptimal

nutritional environment in the uterus may be reflective of maternal undernutrition, placental insufficiency, or even overnutrition, the extent of programming can be influenced by the length and timing of the insults and by the level to which they occur (138, 167, 168) Furthermore, there may be intergenerational effects, where previous undernourishment or disease status of the grandmother may be passed on to the mother and have an effect on her offspring (164). Fetal programming is then a concept that relates poor early growth (due to maternal disease or unfavoravel nutritional state) to disease in adulthood.

Barker has studied extensively the relationship between maternal malnutrition and offspring with consistent findings on the risk of developing CHD, hypertension, T2D, and cancer (138, 140, 141, 169, 170). Based on the Hesinki study, Barker suggested that the programming of coronary heart disease (CHD), type II diabetes (T2D), and hypertension might originate through developmental plasticity and compensatory growth (139). For example, babies that had a low birth weight and gained weight rapidly between ages 3-11 y or that had a high BMI in adulthood had double the risk of developing CHD and T2D (139). Furthemore, they also found that CHD was related to impaired placental growth and the early onset of insulin resistance in childhood (138), where beta-cell dysfunction, impaired glucose tolerance, and diabetes are associated with IUGR (171).

Studies of Pima Indians have shown that there is a U-shaped relationship between birth weight and onset of disease later in life (172). Maternal undernutrition or overnutrition and diabetes can lead to smaller or larger infants,

where neonates from obese mothers have larger fat stores and are at an increased risk of early onset of chronic disease (87, 173). Phillips proposed that thin women or women that have a low weight gain during pregnancy have offspring with a higher blood pressure, and found instead that mothers with increased truncal fat had babies with higher blood pressure, possibly as an effect of increased cortisol (168). Recently, Dabelea proposed potential pathways that link maternal overnutrition to increased fetal and infant adiposity and the early onset of disease (35). They suggest that with fetal overnutrition there is a disregulation of the adipoinsular axis that might lead to altered energy, appetite and adipocyte metabolism resulting in increased infant adiposity and the early onset of T2D, gestational diabetes, CHD, and the metabolic syndrome (35). In conclusion, there is sufficient evidence that an extreme maternal nutritional status can strongly influence fetal development and future health. However, such associations in women with apparent normal weight are not well understood, specifically regarding the effect of varying maternal body composition on fetal development. Future studies should focus on the effects of the maternal nutritional status (body composition and diet) in well-nourished women.

Based on the existing literature, it is clear that the socioeconomic and nutritional transitions in developing countries have a great impact on the health of its populations, especially pregnant women. Pregnant women go through intense physiological adaptations to favor nutrient partitioning and fetal growth. All adaptations have specific time windows prone to fetal growth insults that can arise from maternal over- or under-nutrition. Since maternal dietary intake

determines maternal body composition, a clear understanding of how maternal body composition is related to fetal growth is necessary, the purpose of this dissertation.

3 RATIONALE

3.1 Significance of the Research

It is necessary to study fetal growth using novel techniques and precise measures of maternal health to better understand how maternal diet may influence long-term health of children. There is a clear gap up knowledge on the relationship between maternal body composition at different stages of pregnancy and fetal growth/birth weight, especially in low-income women from developing countries. Furthermore, studies of breast milk fatty acid composition among Brazilian women and how they may relate to infant development are scarce. Therefore, this project will better characterize the relationship between maternal diet and body composition and birth weight and further describe the relationship between maternal breast milk fatty acid composition and infant development.

3.2 Importance of Research

The results of the present study will allow improving the understanding of factors that determine fetal growth and development. Fetal growth will be associated with maternal body composition and diet, providing new evidence of food-based approaches to combating undernutrition in the early stages of life, a key objective of public health. Furthermore, maternal docosahexaenoic acid (DHA) breast milk composition has not been thoroughly studied in this population, providing evidence supporting the dietary reference intakes (DRIs) in

the US as well as Brazil and other developing countries.

The results will be shared with Brazilian and the international community by published articles and presentations, as well as the lay media. Results will also be made directly available to the women who volunteer. Thus, the public health and scientific impact is significant by promoting maternal-fetal health as the study reaches low-income pregnant women that are most likely food insecure and may be suffering from nutritional deficiencies or excessive energy intake.

4 HYPOTHESES AND SPECIFIC AIMS OF THE RESEARCH

4.1 Hypotheses

The central hypothesis of this thesis is that a higher percentage FM and percentage FFM (body tissue that is not fat, such as muscle, organs, and bone, and water) at mid-pregnancy will be associated with greater fetal growth and higher birth weight, independent of socioeconomic status (SES), education level, and age. Furthermore, infants born preterm that received breast milk with higher [EFA] and [LCPUFA] will perform better at cognitive, linguistic, and motor development tests at 1 year of age. We hypothesize the following:

1. Low-income pregnant women will show lower or higher rates of fetal growth according to their FM during the second trimester of gestation.
2. BIA will be a reliable method to assess body composition during pregnancy.
3. Low-income pregnant women will not have adequate breast milk DHA.
4. Higher concentration of breast milk LCPUFA will be positively related to infant development at 12 months of age.

4.2 Specific aims

1. Determine the relationship between maternal percentage lean and fat tissue with fetal growth.
2. Determine the accuracy of BIA to estimate body composition during pregnancy.

3. Determine the variability of DHA in breast milk among low-income pregnant women and its relationship with infant development.

5 METHODOLOGY AND EXPERIMENTAL DESIGN

5.1 Subject recruitment

Two hundred low-income Brazilian women (15-40 yrs old) were recruited in their fourth month of pregnancy. The study sample consisted of women of mixed ethnicity, where genetic influences include mainly black and native and white. The time from recruitment through the final measurement for each woman was 3-4 months. Dietary intake and anthropometric measures were assessed during the first hospital visit and at the eighth month of pregnancy. Fetal growth was determined through ultrasound measurements made during the first visit and seventh month of gestation. At birth, infant body weight and the placental weight were measured with a digital scale to the nearest 0.1 g. Newborn length was measured and ponderal index [birth weight(grams)/length^3(centimeters^3)] was calculated as an index of fatness. Gestational age was estimated using the date of the last menstruation.

5.2 General Protocol

The recruitment timeline is outlined in Table 5-1. A rolling admissions system was used for recruitment such that in the first month we conducted baseline measures on “Group 1” and continued recruiting subsequent groups and then began conducting ultrasound measures on Group1 as we recruited. Thus, approximately 12 months were required to collect all data, including birth weight.

All women were approached in a random fashion and asked to participate. To be able to participate, the woman had to meet the following inclusion criteria:

singleton pregnancy, between the ages of 15-40, non-smoker, non-diabetic (or any known chronic disease), no know HIV infection, and no known malformation. Every woman who met the inclusion criteria was given a demographic questionnaire and a food frequency questionnaire (FFQ). She was subsequently invited to return for body composition analyses. Upon her return according to her gestational age (GA), informed consent forms were provided and signed at the time of recruitment at the research room at the maternity ward. All parts of the document were read and explained in detail to the subject by the project coordinator. The provided information included the purpose of the study and the participant's role, procedures to be used, discomforts and possible risks of participating, an explanation on compensation of expenses and possible damages, expected benefits, voluntary participation and right to withdraw from the study, confidentiality, and the availability of results once the study was completed.

A subsample of 25 healthy preterm infants born adequate for gestational age (AGA) was recruited between March 2005 and November 2007. Twenty-five mothers gave a breast milk sample. Written informed consent was obtained from the children's parents or guardians before their participation in the study.

The protocol used in this sutdy was approved by the Rutgers University and the Instituto Fernandes Figueira (IFF) Institutional Review Board and Research Ethics Committee.

5.3 Dietary assessment

Dietary intake was assessed using a food frequency questionnaire (FFQ) validated in the Brazilian adult population. Research assistants administered the FFQ at the time of recruitment (20 weeks GA) during the second trimester of gestation (33 weeks GA). Data were manually entered for analysis.

5.4 Body composition: Bioelectrical Impedance Analysis

A BIA 310e Bioimpedance Analyzer (Biodynamics Corp., Seattle, WA, USA) was used to measure body composition. All measurements were conducted with an alternating electric current of 800 μA, 50 kHz. Prior to the measurement, women were asked to refrain from exercise and coffee the day prior to the appointment and to come in properly hydrated (2-3 8 oz. glasses of water within 3 hours of the test). The subject remained in a relaxed standing position with arms slightly abducted from the body and legs slightly apart, and four sensor pads (two current electrodes and two voltage sense electrodes) were placed as described by Cornish (133). Briefly, two sensor pads were placed on the hand and foot of the dominant side for whole body bioimpedance. The sensor pads were subsequently rearranged such that the sensor pad on the corresponding wrist was transferred to the opposite foot to measure leg bioimpedance, and then replaced on the wrist after the measurement. Finally, the sensor pad on the corresponding ankle was transferred to the opposite wrist to measure arm bioimpedance. The current-injecting electrodes were kept on the same place at all times to avoid influencing the electrical current across the body.

R and Xc values were recorded and the analyzer automatically calculated all body composition values.

5.5 Anthropometric Measurements

Body weight (W) was measured using an electronic scale (W200-5 Welmy Corp., Santa Bárbara d´Oeste, SP, Brazil) with the women wearing light clothing (i.e. no shoes, jackets, or jewelry). Height (H) was measured to the nearest 0.1 cm with a Seca 206 wall-mounted stadiometer (Seca Corp., Hanover, MD, USA), BMI was calculated as weight (kilograms) divided by height (meters) squared. The same investigator performed all measurements.

5.6 Ultrasound

Fetal growth was assessed by serial ultrasound measurements on the second trimester at 20 and during the third trimester at 33 weeks of gestation. A 3.5 MHz linear array transducer was used to conduct all ultrasound measurements. Fetal length and estimated fetal weight (EFW) were estimated and used as indicators of fetal growth between the second trimester of gestation and birth, and femur growth rate was calculated from the second to the third trimester of gestation.

5.7 Mother's milk sample collection

Breast milk was manually expressed after breastfeeding in the morning between the 2nd and 7th day postpartum. A minimum of 1 ml of breast milk was collected from each subject in Eppendorf tubes that were labeled with the subject ID. All samples were stored in a freezer at 4°C for no more than 5 days before

being transported on ice to the laboratory where they were stored at -70°C until analysis.

5.7.1 Fatty acid analysis

The total lipid fatty acid content of the breast milk samples was analyzed using gas-liquid chromatography (GLC) as described previously (174). Lepage and Roy's (175) transesterification method was used to prepare the fatty acid methyl esters (FAME). FAME were measured in a PerkinElmer chromatography autosystem provided with a hydrogen flame ionization detector and a capillary column (60m x 0.30mm i.d.), packed with 10% SP 2330 (Supelco Inc, Bellefonte, PA, USA) as a stationary phase. Nitrogen was used as a carrier gas. Injection and detection temperatures were both at 220°C, and oven temperature was programmed to vary 5°C/min from 40 to 225 °C. Esters were identified by comparing retention times with known standards (Sigma Aldrich, St Louis, MO, USA) and quantification was done by calculating peak areas with an integrator. Results were expressed as relative area percentage (percentage of total FAME).

5.8 Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III)

For the subsample of healthy preterm babies, trained psychologists applied the Bayley-III on the same day of the medical appointment 9-12 months after birth in a separate room with the infant and the parent or guardian. After explaining the process of the test, the parent was interviewed and the physical state, the daily routine, and care and environment that surrounded the infant where assessed. Immediately after the interview and assessment, the test was

administered. The obtained raw scores were corrected based on the age of the infant according to the instructions, scales, and tables of the manual (176). For this study sample of premature infants, age was corrected for gestational age.

5.9 Statistical analysis

5.9.1 Bioimpedance analysis

Paired-sample t-tests were used to compare the mean percent body fat (BF%) and FFM measured by whole body BIA with the mean predicted BF% and FFM, respectively, using two sets of equations. Correlation plots and Bland-Altman assessments of agreement were used to compare whole body BIA BF% and FFM with the predicted values from segmental R and whole body Z. A range of agreement was defined as bias ± 1.96 SD. Data were analyzed as a group and stratified by short or normal stature (short stature ≤ 154.5 cm or normal stature >154.5 cm) (177). All statistical analyses were completed using SPSS 16. The significance level was set at $P < 0.05$.

5.9.2 Body composition

There were no significant differences in fetal or neonatal characteristics between boys and girls, thus they were analyzed together. All variables were checked for normality and serum glucose and physical activity were log transformed. Birth weight was adjusted for gestational age (GA) by regressing BWT against GA (BWTadj) and the residuals were used in subsequent analyses. Fetal growth (ΔFL), was calculated as birth length (cm) – fetal length at the second trimester, where fetal length = 6.18 + 0.59 x femur length in millimeters, according to Vintzileos (178). Mean values of fetal and birth

characteristics were analyzed in tertiles of maternal age. Multiple linear regression analyses were conducted for the following dependent variables: 1) BWTadj, 2) Placental weight, 3) Rate of change in estimated fetal weight per week (i.e. ΔEFW/ number of weeks), 4) Change in EFW (i.e. from week 20 until birth), and 5) Change in fetal length (ΔFL) (i.e. from week 20 until birth). Independent variables included %FM or %FFM controlling for gender, maternal glucose, energy intake, and physical activity level. Regression analyses were conducted for all women and by three predetermined groups of energy intake: low calorie intake <1500 kcal (LoE group), a normal calorie intake ≥1500 < 2500 kcal/d (normal or NL group), and a high calorie intake ≥2500 (HiE group). For the pre-determined groups of energy intake, the lower cut-off is based on the increased risk for suboptimal fetal development. The higher cutoff is based on the recommended increase in energy intake during the second trimester. A sensitivity analysis was done including and excluding outliers of energy intake (values that were below the 5^{th} or above the 95^{th} percentiles) and results were materially changed, therefore outliers were removed for analysis. All statistical analyses were performed using SPSS version 19 and significance was set at $p < 0.05$.

5.9.3 Breast milk fatty acid concentrations and infant development

FA values were skewed and were log transformed, so the analyses are derived from transformed data. Multiple linear regression analyses were conducted to assess the association between the FA content in colostrum and the Bayley-III outcome. Some infants underwent the test at either 9 or 12 months

or at both time points; therefore, time between birth and date of the test was adjusted for. The Bayley-III scores at 12 mo of age were used except for those infants for whom Bayley-III scores were available from 9 mo of age only (n=8). All values were controlled for sex, gestational age, and length of breast feeding and formula feeding. Additional simple linear regression analyses were conducted to solely assess the relationship between time of breastfeeding and the Bayley-III scores without controlling for any other variable. Exact age at day of the test was calculated as the time between term and date of the test and was used in the analyses that included expressive and receptive language and gross and fine motor development. Cognitive, general language and motor variables are adjusted for age at the moment of the test. A sensitivity analysis was done including and excluding outliers (values that fell under the 5^{th} (n=1) or above the 95^{th} percentiles (n=2) and results were materially unchanged, therefore all values were kept for analysis. The SAS program (Statistic Analysis System) version 8.0 (SAS Institute, Cary, NC, USA) was used for all analyses and statistical significance was set at $p < 0.05$.

5.10 Sample size calculation

Based on three studies of maternal supplementation and birth outcomes and two studies of EFA supplementation during pregnancy and breast milk fatty acid composition, we calculated (179) that approximately 200 women were necessary to have sufficient power to detect statistical differences in birth weight (**Table 5-2**).

5.11 Collaborating Sites

This study was a partnership of Rutgers, The State University of New Jersey, in the United States with the State University of Rio de Janeiro and the Fernandes Figueira Institute in Rio de Janeiro.

Table 5-1. Recruitment and ultrasound timeline (months)

1	**2**	**3**	**4**	**5**	**6**
B Gr 1	B Gr 2	B Gr3 US Gr 1	B Gr 4 US Gr 2	B Gr 5 US Gr 3	B Gr 6 US Gr 4

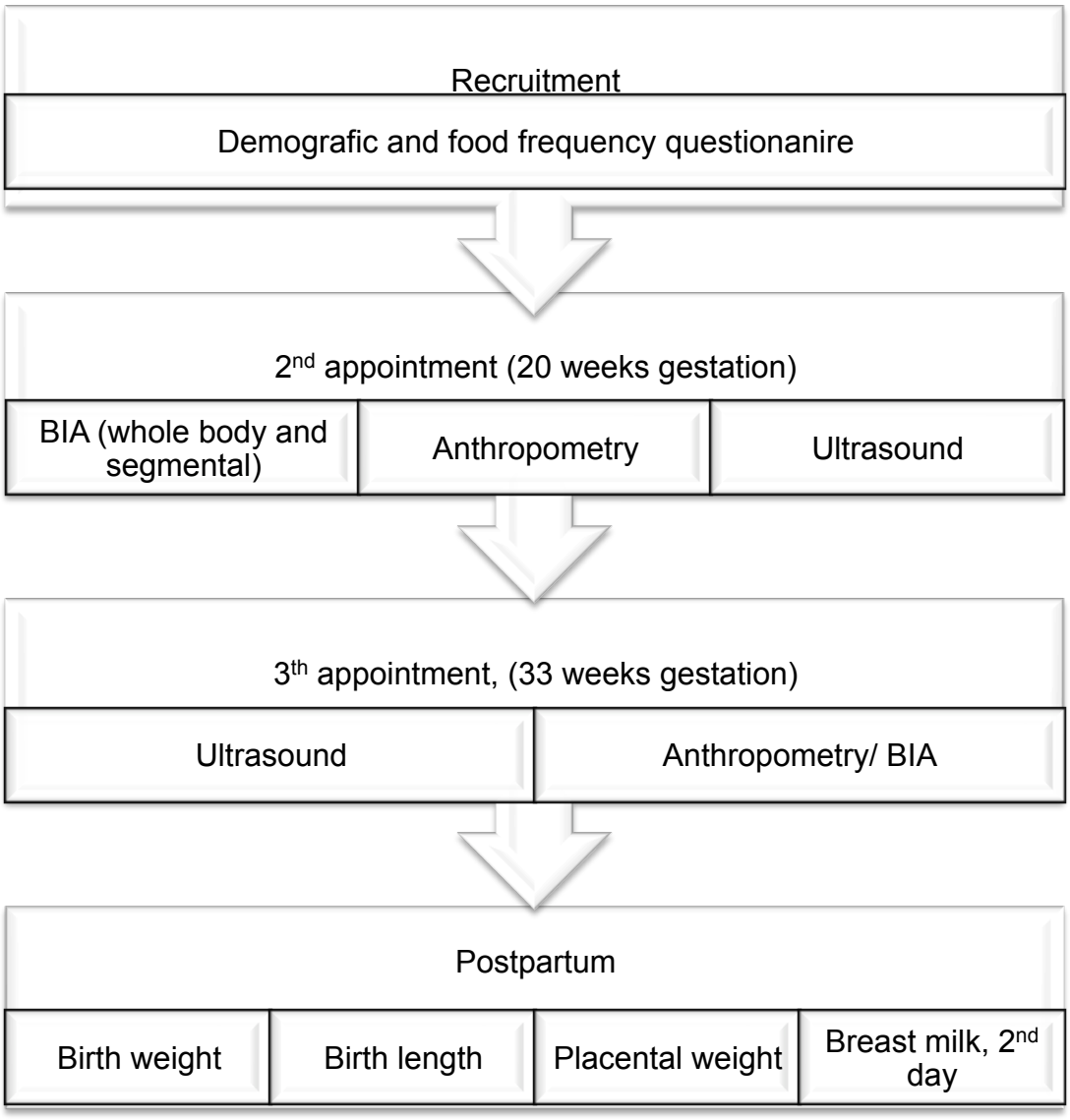
7	**8**	**9**	**10**	**11**	**12**
B Gr 7 US Gr 5	B Gr 8 US Gr 6	B Gr 8 US Gr 7	B Gr 10 US Gr 8		

B Gr : Baseline Group
US Gr : Ultrasound Group

Table 5-2. Power analysis

Reference	Mean Birth Weight Difference	SD	Sample Size/Group 80% Power	Sample Size/Group 90% Power
(180)	1400 g	600	20	31
(181)	500 g	250	6	8
(182)	100 g	200	63	85
(183)	100 g	300	142	190

Figure 3. Schematic of the study



6 MAIN EXPERIMENTS

SECTION A ESTIMATES OF BODY COMPOSITION DURING PREGNANCY USING BIOELECTRICAL IMPEDANCE ANALYSIS

Abstract

Background: Bioelectrical impedance analysis (BIA) is commonly used for body composition assessment, however prediction equations used with BIA equipment were developed in healthy populations from developed countries and the assumptions made regarding the body proportions may not apply to populations with altered body geometry, such as in pregnancy.

Objective: Compare BIA using body segments to whole body measurements in pregnant women.

Design: Subjects included 209 pregnant women aged 15-40 years and between 18 to 22 weeks gestation. Percent fat mass (%FM) and fat-free mass in kilograms (FFM) were estimated using whole body BIA and compared to the estimated parameters using two different sets of equations from body segment and whole body resistance (R) and impedance (Z).

Results: The estimates of %FM by the by the manufacturer were significantly lower (30% v. 34%) and significantly higher for FFM (47kg v. 43kg) compared to the equations by Baumgartner, ($P<0.001$). There was poor agreement between the body composition parameters estimated using prediction equations from segmental data and the parameters measured by whole body BIA.

Conclusion: Prediction equations based on segmental measurements and developed from non-pregnant women are a poor estimate of body composition

during pregnancy. Using whole body BIA may provide a better estimate of maternal body composition.

Introduction

Bioelectrical impedance analysis (BIA) is a common method to assess body composition and hydration status due to its safety, portability and affordability (127-131). BIA has a high correlation with more complex and expensive methods such as dual energy x-ray absorptiometry (DEXA) (184), underwater weighing (185), deuterium isotope dilution (D_2O) (186), and magnetic resonance imaging (MRI) (187, 188). However, high correlation does not mean good agreement, and it might conceal the bias between methods that a Bland-Altman plot can show (189). Furthermore, the use of BIA needs careful evaluation, as assumptions are often made that may violate the true geometry and homogeneity of the body (135) and lead to inaccurate or contradictory conclusions (190, 191).

The detailed scientific principles of BIA are discussed elsewhere (133). Briefly, BIA is based on the measurement of resistance (R, Ω) and reactance (Xc, Ω) when an alternating current (800 μA and 50 KHz in most commercial equipment) is conducted through the body. The BIA equipment calculates the respective body composition parameters through algorithms programmed by the manufacturer. One obstacle to the accuracy of the measurement is that the algorithms used by the manufacturer are often based on healthy euvolemic adults. Wrist-to-ankle (whole body) BIA follows the concept that the body consists of one single conducting cylinder (134) and may not be accurate for different populations, especially those with altered trunk geometry, such as patients with chronic renal failure, steatosis and chronic liver disease, dialysis,

and pregnant women, whose bodies go through profound body composition and physiological changes (135-137).

Throughout pregnancy the dimensions of the trunk change to accommodate the growth of the placenta and fetus, increased breast and womb tissue, amniotic fluid and retained water (192), and increased visceral adipose tissue (193). Specifically, during the first and second trimester blood volume, protein, and fat stores increase, and during the third trimester the fetus, placenta, and amniotic fluid predominate (194). It is a challenging task to measure and account for the effect of the densities of the fetus, placenta, and amniotic fluid by body composition methods such as BIA. For example, amniotic fluid volume can become altered (polyhydramnios or oligohydramnios) due to fetal or maternal disease (195) and these changes may not be detected without a site-specific assessment of body composition. Jensen et al. (196) used Culver and Viano's (197) method to construct concentric ellipses representing the different densities and mass changes of the trunk from 15 weeks of pregnancy until term. They found that during the second and third trimesters the mass of the lower trunk increased significantly compared to the other trunk segments. Wrist to ankle BIA does not account for such significant changes.

The trunk is not comprised of uniform tissues like the limbs, which have cylinder-like tissue compartments of fat, muscle and bone. In segmental BIA, five individual conductive cylinders can be measured (2 arms, 2 legs, and the trunk) (198), and may provide more accurate body composition estimates than wrist-to-ankle BIA (147). Therefore, to assume that the resistivity of the limbs is the same

in the trunk may be false due to the trunk's heterogeneous nature. Furthermore, since whole body BIA R is inversely proportional to the circumference of the cylinder, the arms and legs account for 97% of total R, so changes in the trunk are virtually undetectable (199). Thus, a segmental approach of body composition assessment during pregnancy may provide more accurate measurements of fat mass (FM), fat free mass (FFM), and total body water (TBW).

To address this issue, the objective of the present study was to compare body composition determined by BIA in pregnant women with predicted values using two sets of equations based on segmental R and whole body Z. The purpose was to evaluate the prediction of percent body fat (BF%) and FFM from segment length (L) and R or whole body Z with the hypothesis that given the changing trunk geometry and water content in pregnancy, segmental predictors rather than whole body measurements will provide a better assessment of body composition.

Subjects and Methods

Subjects

The study sample consisted of 209 low-income pregnant women from Rio de Janeiro, Brazil aged 15-40 y. All women were recruited during their second trimester (4-5 months) of gestation. Participants were included if they had a singleton pregnancy, were older than 15 or younger than 40 years old, did not smoke or use illegal drugs, and did not have a chronic disease or fetal malformation. The study protocol was approved by the Institutional Review Board

of Rutgers University and the Fernandes Figueira Institute, and all subjects signed an informed consent form at the time of recruitment.

Bioelectrical Impedance Analysis

A BIA 310e Bioimpedance Analyzer (Biodynamics Corp., Seattle, WA, USA) was used to measure body composition. All measurements were conducted with an alternating electric current of 800 μA, 50 kHz. Prior to the measurement, women were asked to refrain from exercise and coffee the day prior to the appointment and to come in properly hydrated (2-3 8 oz. glasses of water within 3 hours of the test). The subject remained in a relaxed standing position with arms slightly abducted from the body and legs slightly apart, and four sensor pads (two current electrodes and two voltage sense electrodes) were placed as described by Cornish (133). Briefly, two sensor pads were placed on the hand and foot of the dominant side for whole body bioimpedance. The sensor pads were subsequently rearranged such that the sensor pad on the corresponding wrist was transferred to the opposite foot to measure leg bioimpedance, and then replaced on the wrist after the measurement. Finally, the sensor pad on the corresponding ankle was transferred to the opposite wrist to measure arm bioimpedance. The current-injecting electrodes were kept on the same place at all times to avoid influencing the electrical current across the body. R and Xc values were recorded and the analyzer automatically calculated all body composition values.

Anthropometric Measurements

Body weight (W) was measured using an electronic scale (W200-5 Welmy

Corp., Santa Bárbara d´Oeste, SP, Brazil) with the women wearing light clothing (i.e. no shoes, jackets, or jewelry). Height (H) was measured to the nearest 0.1 cm with a Seca 206 wall-mounted stadiometer (Seca Corp., Hanover, MD, USA), BMI was calculated as weight (kilograms) divided by height (meters) squared. The same investigator performed all measurements. Height was used as a proxy for leg and arm length (L), where leg L was considered to be 0.4 of total height and arm L was estimated as (height – ¼ height)/ 2, considering that trunk width is approximately ¼ of total height (200). BF% and FFM from leg and arm R or whole body R (R_{WB}) or impedance (Z) from the Baumgartner et al. (185) and Bracco et al. (184) equations and body composition measured by whole body BIA were calculated with the following prediction equations:

Whole body BIA equation:

FFM = a * $HEIGHT^2$ + b * WEIGHT + c * AGE + d * R + e, where the weighing

constants for the terms are a, b, c, and d.

FM = WEIGHT – FFM

Baumgartner (185) equations from body segments:

$BF\%_L = -0.53 + 9.78 * (W * R_L/L_L^2)$

$BF\%_a = -8.20 + 8.13 * (W * R_a/L_a^2)$

$BF\%_B = -24.95 + 40.81 * (W * R_{WB}/H^2)$

$FFM_L = 20.88 + 1.09 * (L_L^2/R_L)$

$FFM_a = 18.04 + 1.87 * (L_a^2/R_a)$

$FFM_B = 8.60 + 0.74 * (H^2/R_{WB})$

Bracco (184) equations:

$FFM_{Bcc} = 18.2 + 0.46 * (H^2/Z_{WB})$

$FM_{Bcc} = W - FFM_{Bcc}$

$BF\%_{Bcc} = 1 + 100 * (FFM_{Bcc}/ W)$

Statistics

Paired-sample t-tests were used to compare the mean BF% and FFM measured by whole body BIA with the mean predicted BF% and FFM, respectively, using two sets of equations. Correlation plots and Bland-Altman assessments of agreement were used to compare whole body BIA BF% and FFM with the predicted values from segmental R and whole body Z. A range of agreement was defined as bias ± 1.96 SD. Data were analyzed as a group and stratified by short or normal stature (short stature ≤ 154.5 cm or normal stature >154.5 cm) (177). All statistical analyses were completed using SPSS 16. The significance level was set at $P < 0.05$.

Results

The physical characteristics of the study sample are presented in **Table A-1**. The mean age was 26.7 ± 7.7 years; mean weight and height were 67.2 ± 13.9 and 160.1 ± 6.7, respectively, and mean BMI was 26.2 ± 4.9 kg/m^2.

Percent body fat and FFM are given in **Table A-2**. Percent body fat measured by whole body BIA was significantly lower than predicted BF% (Baumgartner equations) from leg R by 9.7%, from arm R by 10%, and from R_{WB} by 5.1%. FFM measured by whole body BIA was significantly higher than predicted FFM from leg and arm R by 7.0 and 6.9 kg, respectively and significantly higher than predicted from R_{WB} by 4.1 kg. Percent body fat measured by whole body BIA was also significantly lower (by 7.6%) than predicted BF% using the equation by Bracco et al. from whole body Z. Whole body BIA FFM was significantly higher than FFM predicted by the Bracco equation by 10.5 kg. BF% and FFM from whole body BIA were significantly different ($P < 0.00$) from the predicted BF% and FFM using both the Baumgartner et al. and Bracco et al. equations.

Figures A-1 a and b through A-4 a and b show unstratified plots of identity (A) and Bland-Altman plots (B) and compare whole body BIA measurements with the predicted FFM using Baumgartner and Bracco's prediction equations. The limits of agreement and mean differences between methods for the unstratified Bland-Altman plots are presented in **Table A-3**. **Tables A-4 and 5** show the mean differences and limits of agreement for stratified analyses. There was higher agreement between women who were of short stature (≤ 154.5 cm) and

lower mean differences between methods for women of short height compared to women of normal height.

There was poor correlation between whole body BIA BF% and BF% based on Leg R and Arm R, respectively. For example, the limits of agreement between whole body BIA BF% and BF% based on Leg R ranged from 2.2 to -21.6, and the mean difference between the methods was -9.7. These two methods do not consistently provide similar measures because there is a level of disagreement that includes clinically important discrepancies of up to 40% BF between methods. Similarly, the disagreement of up to 15% between whole body BIA and Arm R BF% is clinically significant. There was also poor correlation (**Figures A 1a and 2a**) and significant disagreement (**Figures A 1b and 2b**) between whole body FFM and FFM based on Leg R and Arm R.

The correlation between whole body BIA BF% with the estimated BF% based on whole body R using Baumgartner's equation and whole body Z using Bracco's equation was higher than using arm and leg R, R^2= 0.83 and R^2=0.81, respectively. However, the disagreement between methods is as high as 20% BF. The correlation between whole body BIA FFM and estimated FFM from whole body R using the Baumgartner equation is R^2= 0.79 and from whole body Z by the Bracco equation is R^2= 0.77. However, the discrepancies go up to 20 kg for both equations (**Figures A 3a and 4b**).

Discussion

This study sought to evaluate the use of prediction equations based on segmental BIA during pregnancy compared to whole body BIA. Previous studies have used BIA during pregnancy to better define the relationship between maternal body composition and birth weight (145, 158) while others have used BIA to assess changes in hydration, edema, or for the clinical management of preeclampsia during pregnancy (143, 201, 202). Yet, only one study has investigated the accuracy of bioimpedance spectroscopy (BIS) for the measurement of body water during the reproductive cycle (203), and another study provided reference ranges of body composition during gestation (137). Buchholz (135) reviewed the use of BIA and BIS in clinical populations and concluded that the sensitivity of BIA to changes in the trunk is very low and argued that BIA does not accurately predict TBW, especially in populations with altered trunk geometry. This fact may invalidate prediction equations produced in healthy, non-pregnant samples and applied to pregnant women. However, to date there are no prediction equations for BIA developed from pregnant women. Furthermore, no study has looked to evaluate how the body composition assessment of whole body BIA compares to segmental BIA during pregnancy for the measurement of body fat and FFM, the purpose of this study.

Our findings showed significantly different mean BF% and FFM and poor agreement between methods. Specifically, segmental BIA predicted higher BF% and lower FFM with both sets of equations compared to whole body BIA. After stratification by short stature, whole body BIA and the two sets of equations

better predicted BF% and FFM. As would be expected, for short women (≤154.5cm) the mean differences between the estimations of FFM and BF% were lower compared to normal height women (>154.5cm).

Segmental BIA was successfully validated against more accurate methods of body composition assessment (187). Furthermore, Bracco compared segmental BIA to segmental DEXA, as a reference method, and developed body composition prediction equations for men and women (184). However, during pregnancy other factors need to be taken into account, such as the changing geometry of the trunk. For example, the prediction equations programmed into BIA equipment and used to estimate BF% and FFM include variables such as height, age, and weight to improve the accuracy of the prediction and theoretically adjust for the geometrical complexity of the body, however this implies that the prediction equations developed for one population may not be applied to another (204, 205). Another important factor to consider is that R is proportional to the L of the body or segment (cylinder) (134). Thus, the use of proxies for arm and leg L may have altered the estimation of BF% and FFM (206).

It is interesting to note that the mean sum of the measured arm and leg R was higher than whole body R. Because R is inversely proportional to FFM, the high R could have resulted in a higher prediction of BF% and lower FFM with the Baumgartner and Bracco equations. Moreover, the use of whole body R also predicted significantly high body composition parameters with the Baumgartner equations; however these values were on average only 5.11% higher and 4.10

kg lower for BF% and FFM, respectively. The mean differences were smaller than with the segmental predictors and more in accordance with the values measured by whole body BIA, suggesting that while BIA may not accurately detect changes in the trunk during pregnancy; it could still be a better option than using body segment parameters when the influence of the amniotic fluid on the trunk segment (through BIA) is unknown. Still, it is of outmost importance to develop prediction equations from whole body and segmental predictors during pregnancy that become available for optional use in commercial BIA devices.

A limitation in this study was some women presented edema in both legs and/or arms, but this does not seem to have had a significant effect on TBW, which fell into normal ranges or the group mean FFM, since it was still underpredicted by the prediction equations used. Second, measurements were conducted while standing, but this is unlikely to have caused any significant effect on the measurement, as the result is a slightly lower R reading (5-10 ohms) (207). Third, measurements were cross-sectional, and the use of longitudinal measurements, including pre and postpartum body composition would have provided clearer comparisons between segmental and whole body BIA. Fourth, the N for the segmental measures (N=88 for arm and N=86 for leg) was lower than for whole body BIA (N=209). The lower power possibly resulted in higher disagreement. A reference method to compare whole body BIA and segmental BIA estimates was not available, thus we were not able to validate segmental BIA during pregnancy. Therefore, this study is limited to the evaluation of agreement between two methods of body composition during pregnancy.

Finally, height was used as a proxy for arm and leg length, so it was not possible to correct for between individual variations in limb length for a given height.

Our findings indicate that there is poor agreement between the body composition parameters estimated using prediction equations from segmental data and the parameters measured by whole body BIA. Thus, prediction equations based on segmental measurements and developed from non-pregnant women are a poor estimate of body composition during pregnancy. Using whole body BIA may provide a better estimate of maternal body composition when more accurate techniques are not available. Future studies should develop algorithms and prediction equations from pregnant women based on segmental and whole body BIA parameters, including R and resistivity. We recommend the use of whole body BIA for pregnant women until a better method is available.

Table A-1 Physical characteristics of the study population

	N	Mean	Std. Deviation
Age	209	26.7	7.7
Height (cm)	209	160.1	6.7
Weight (kg)	209	67.2	13.9
BMI (kg/m^2)	209	26.2	4.9
Leg Length (cm)	209	64.0	2.7
Arm Length (cm)	209	60.0	2.5
Leg R (Ω)	86	258.4	43.6
Arm R (Ω)	88	330.1	50.1
Whole Body R (Ω)	209	564.1	82.5
Whole Body Xc (Ω)	199	61.3	10.8
Whole Body Z (Ω)	199	565.3	80.5

R, resistance in ohms (Ω); Xc, reactance in ohms (Ω); Z, impedance in ohms (Ω)

Table A- 2 Whole body BIA and segmental prediction equation BF%, FFM, and FM at 4-5 months (2nd trimester)*

	BIA**	Leg†	Arm†	Whole Body†
		Baumgartner et al. (185)		
BF%	30.2 ± 5.9	38.8 ± 6.3	39.2 ± 6.6	34.3 ± 8.2
FFM (kg)	47.1 ± 7.2	38.7 ± 3.2	38.9 ± 3.2	43.1 ± 6.3
FM (kg)	20.3 ± 7.9	26.1 ± 8.8	26.7 ± 11.0	24.2 ± 10.5
		Bracco et al. (184)		
BF%				39.6 ± 8.4
FFM (kg)				39.5 ± 3.8
FM (kg)				27.5 ± 10.8

***Means ± SD**

**From whole body bioimpedance analysis

†BF% and FFM from prediction equations significantly different from whole body BIA measurements ($P < 0.000$)

Table A-3 Mean differences, limits of agreement, and 95% confidence intervals

	Mean ± Std. Deviation*	95% Confidence Intervals		Limits of Agreement
		Lower	Upper	
$BF\%_{BIA}$ - $BF\%_{L}$	-9.7 ± 6.1	8.4	10.9	-2.22to 21.6
$BF\%_{BIA}$ - $BF\%_{a}$	-9.9 ± 4.5	9.0	10.9	-18.7 to -1.2
$BF\%_{BIA}$ - $BF\%_{B}$	-5.1 ± 3.8	-5.6	-4.6	-12.5 to 2.3
$BF\%_{BIA}$ - $BF\%_{Bcc}$	-10.5 ± 4.1	-11.1	-9.9	-18.6 to -2.4
FFM_{BIA} – FFM_{L}	6.9 ± 4.7	-7.9	-5.9	-2.2 to 16.2
FFM_{BIA} – FFM_{a}	6.9 ± 5.3	-8.2	-5.6	-3.6 to 17.4
FFM_{BIA} – FFM_{B}	4.1 ± 3.3	3.6	4.5	-2.4 to 10.6
FFM_{BIA} – FFM_{Bcc}	7.6 ± 4.1	7.0	8.1	-0.5 to 15.7

*$P < 0.000$

$BF\%_{L}$, percent body fat based on leg R; $BF\%_{BIA,}$ whole body BIA percent BF; $BF\%_{a}$, percent BF based on arm R; $BF\%_{B}$, Baumgartner estimation of percent BF from R_{WB}; $BF\%_{Bcc,}$ Bracco estimation of percent BF; FFM_{L}, fat free mass based on leg R; FFM_{BIA}, whole body BIA FFM; FFM_{a}, FFM based on arm R; FFM_{B}, Baumgartner estimation of FFM based on R_{WB}; FFM_{Bcc}, Bracco FFM.

Table A-4 Mean differences, limits of agreement, and 95% confidence intervals: Short height (≤ 154.5cm)

	Mean ± Std. Deviation*	95% Confidence Intervals		Limits of Agreement
		Lower	Upper	
$BF\%_{BIA}$ - $BF\%_{L}$	-10.0 ± 6.4	-13.6	-6.5	-22.6 to 2.5
$BF\%_{BIA}$ - $BF\%_{a}$	-8.2 ± 4.3	-10.6	-5.9	-16.7 to 0.2
$BF\%_{BIA}$ - $BF\%_{B}$	-5.1 ± 4.9	-6.7	-3.5	-14.9 to 4.6
$BF\%_{BIA}$ - $BF\%_{Bcc}$	-32.3 ± 12.1	-36.1	-28.4	-56.0 to -8.5
FFM_{BIA} – FFM_{L}	3.1 ± 3.6	1.1	5.1	-3.9 to 10.2
FFM_{BIA} – FFM_{a}	4.5 ± 2.5	3.2	5.8	-0.4 to 9.4
FFM_{BIA} – FFM_{B}	2.7 ± 3.1	1.7	3.6	-3.4 to 8.7
FFM_{BIA} – FFM_{Bcc}	4.6 ± 2.7	3.8	5.5	-0.7 to 10.0

*P < 0.000

$BF\%_{L}$, percent body fat based on leg R; $BF\%_{BIA,}$ whole body BIA percent BF; $BF\%_{a}$, percent BF based on arm R; $BF\%_{B}$, Baumgartner estimation of percent BF from R_{WB}; $BF\%_{Bcc,}$ Bracco estimation of percent BF; FFM_{L}, fat free mass based on leg R; FFM_{BIA}, whole body BIA FFM; FFM_{a}, FFM based on arm R; FFM_{B}, Baumgartner estimation of FFM based on R_{WB}; FFM_{Bcc}, Bracco FFM.

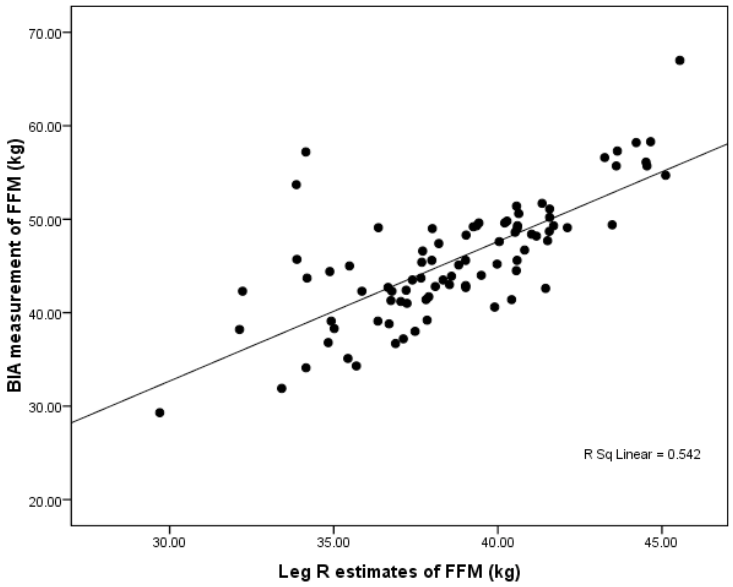
Table A-5 Mean differences, limits of agreement, and 95% confidence intervals: Normal height (>154.5cm)

	Mean ± Std. Deviation*	95% Confidence Intervals		Limits of Agreement
		Lower	Upper	
$BF\%_{BIA}$ - $BF\%_{L}$	-9.6 ± 6.1	-11.1	-8.2	-21.5 to 2.2
$BF\%_{BIA}$ - $BF\%_{a}$	-10.3 ± 4.4	-11.4	-9.3	-19.0 to -1.6
$BF\%_{BIA}$ - $BF\%_{B}$	-4.9 ± 3.8	-5.5	-4.3	-12.3 to 2.5
$BF\%_{BIA}$ - $BF\%_{Bcc}$	-32.2 ± 14.3	-34.4	-29.9	-60.3 to -4.1
FFM_{BIA} – FFM_{L}	7.8 ± 4.5	6.7	8.9	-1.1 to 16.7
FFM_{BIA} – FFM_{a}	7.7 ± 5.8	6.0	9.3	-3.7 to 19.1
FFM_{BIA} – FFM_{B}	4.3 ± 3.5	3.8	4.9	-2. 6 to 11.2
FFM_{BIA} – FFM_{Bcc}	8.3 ± 4.2	7.7	8.9	0.1 to 16.6

*P < 0.000

$BF\%_{L}$, percent body fat based on leg R; $BF\%_{BIA,}$ whole body BIA percent BF; $BF\%_{a}$, percent BF based on arm R; $BF\%_{B}$, Baumgartner estimation of percent BF from R_{WB}; $BF\%_{Bcc,}$ Bracco estimation of percent BF; FFM_{L}, fat free mass based on leg R; FFM_{BIA}, whole body BIA FFM; FFM_{a}, FFM based on arm R; FFM_{B}, Baumgartner estimation of FFM based on R_{WB}; FFM_{Bcc}, Bracco FFM.

a



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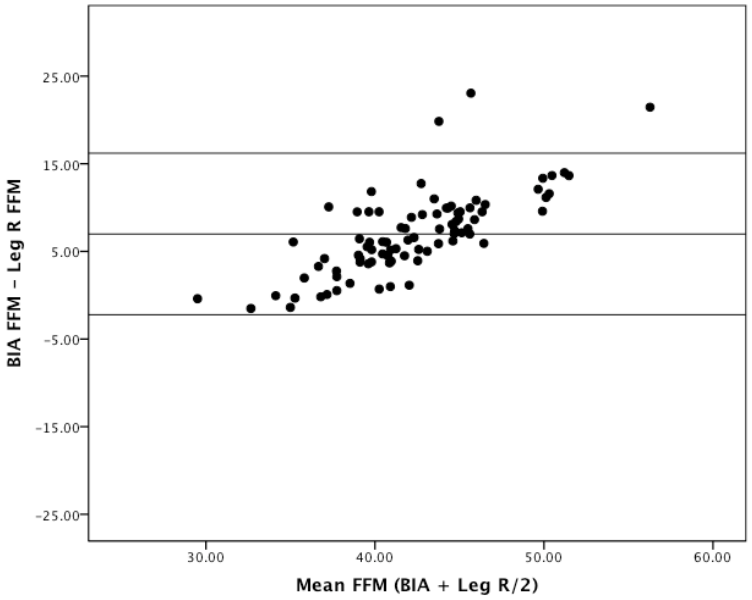
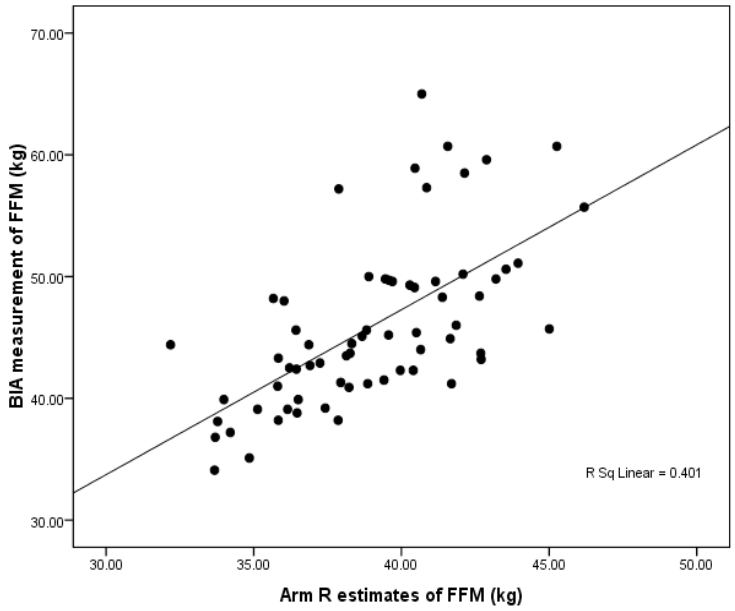


Figure A-1 a Correlation plot and b Bland-Altman plot showing the level of agreement between whole body BIA FFM and FFM predicted by Baumgartner's equation (185) from leg L and R.

a



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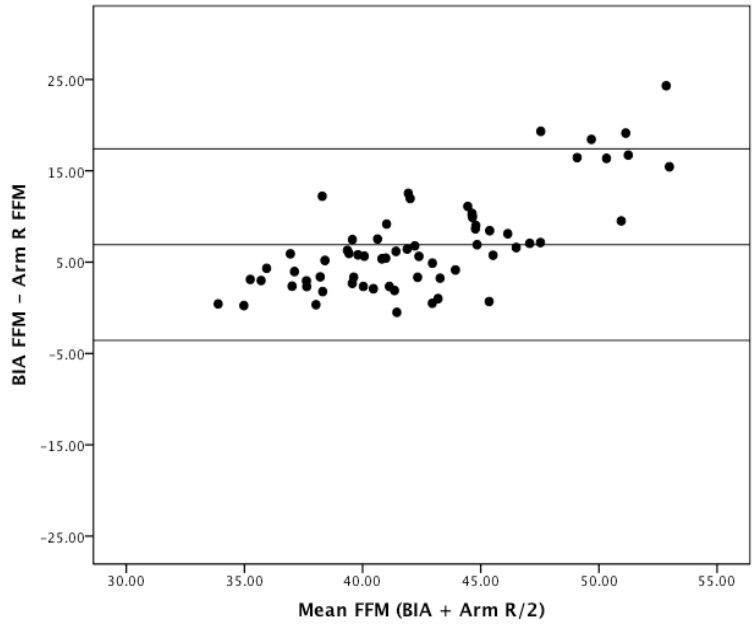
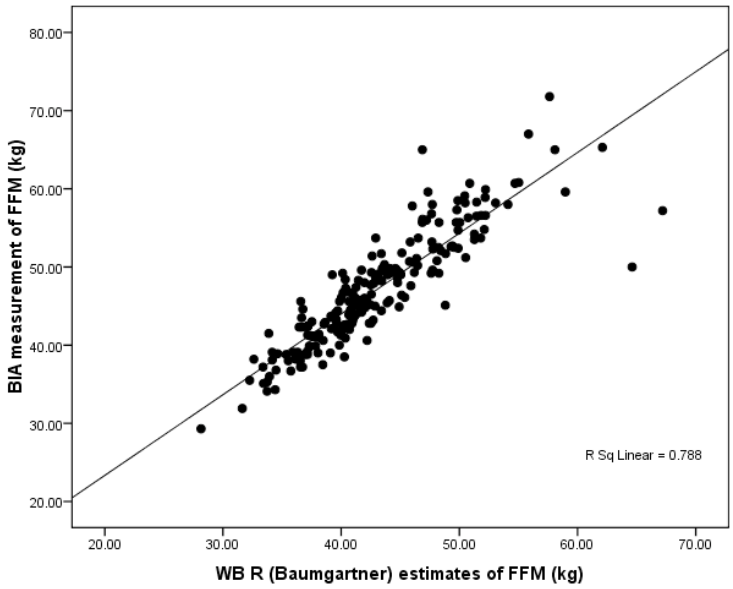


Figure A-2 a Correlation plot and **b** Bland-Altman plot showing the level of agreement between whole body BIA FFM and FFM predicted by Baumgartner's equation (185) from leg L and R.

a



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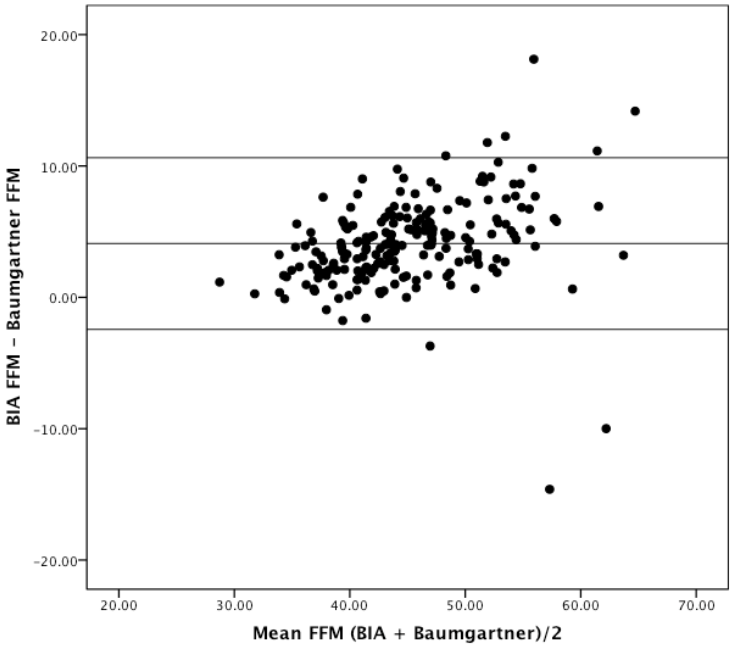
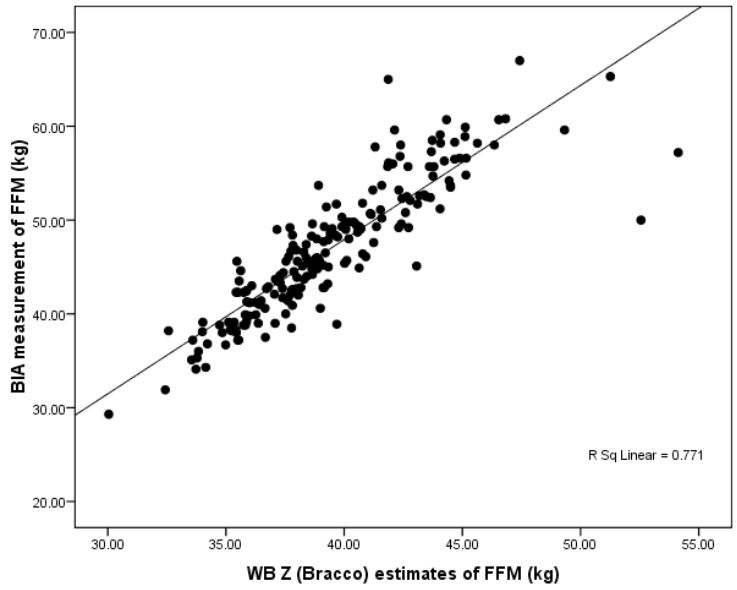


Figure A-3 a Correlation plot and **b** Bland-Altman plot showing the level of agreement between whole body BIA FFM and FFM predicted by Baumgartner's equation (185) from whole body H and R.

a



b

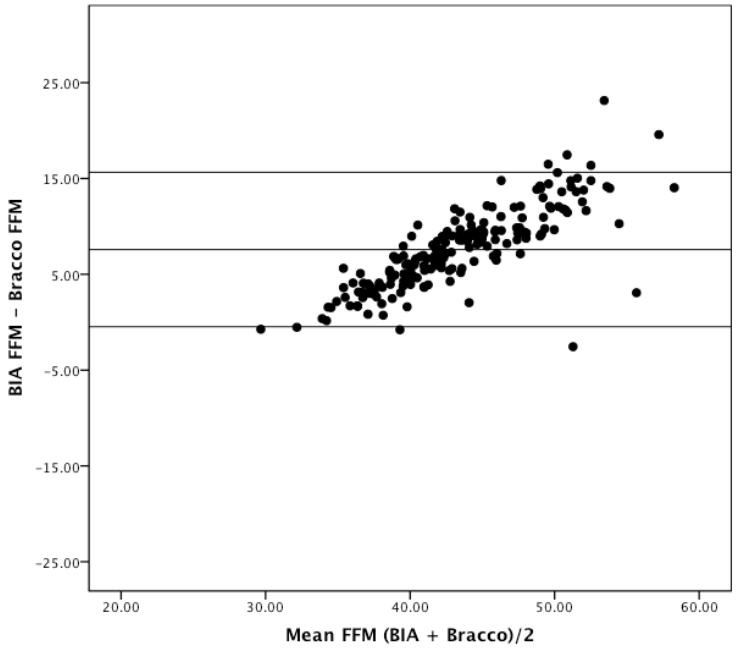


Figure A-4 a Correlation plot and **b** Bland-Altman plot showing the level of agreement between whole body BIA FFM and FFM predicted by Bracco's equation (184) from whole body W, H, and Z.

SECTION B FETAL GROWTH AND BIRTH WEIGHT ARE DETERMINED BY MATERNAL FAT MASS AT MID PREGNANCY IN LOW-INCOME BRAZILIAN WOMEN

Abstract

Background: The relationship between maternal body composition and fetal development is unclear.

Objective: To determine whether maternal body composition determines fetal growth in women with lower, normal, and higher energy intakes.

Design: In a sample of 124 women recruited from the Instituto Fernandes Figueira in Rio de Janeiro, Brazil, dietary intake was assessed using a food frequency questionnaire and body composition was estimated using bioelectrical impedance analysis. Fetal growth was assessed using serial ultrasound measure and infants underwent anthropometric measurements at birth. Multiple linear regression analyses were used to determine the association between fetal growth and birth weight with maternal percent fat-mass (%FM) and percent fat-free mass (%FFM), controlling for infant gender, maternal serum glucose, energy intake, and physical activity level

Results: Maternal characteristics were significantly different when divided by tertiles of age, but fetal and neonatal characteristics did not differ. For all women, %FM was positively associated with birth weight adjusted for gestational age (BWTadj), placental weight, change in estimated fetal weight (ΔEFW), and ΔEFW/week. After stratification by energy intake, the relationships persisted only for BWTadj, placental weight, and ΔEFW in women with a normal energy intake.

Conclusion: Maternal FM may be used as a reliable indicator of maternal nutritional status for recommendations on weight gain and fetal growth during pregnancy.

INTRODUCTION

The health of an infant is determined by complex interactions between maternal diet, body composition, and the placenta (138, 141, 171, 208, 209). A poor maternal diet can alter fetal development and increase the risk for chronic disease in adulthood, a concept known as fetal programming (138-140, 169-171, 210-212). However, in well-nourished women, the relationship between maternal diet, body composition and birth weight is still unclear (136, 153-158, 160, 161, 213, 214), particularly because women of similar weights might have a very different body composition. Possible determinants of fetal growth, such as maternal fat mass (FM) and fat-free mass (FFM) need to be investigated. Therefore, the focus of this study was to determine the relationship between maternal FM and FFM and fetal growth in a cohort of low-income Brazilian women.

Nutritional status during pregnancy, including maternal diet, the metabolic turnover of body fuels, and body composition (160), are important factors that influence fetal growth and birth weight and all should be considered . Maternal body composition can be determined by dietary intake and could be a more important predictor of birth outcome. For example, from early- to mid-pregnancy a woman becomes hyperphagic and fat storage is stimulated, whereas mobilization of fat is favored in late pregnancy by numerous alterations in lipid metabolism that promote accelerated fetal growth (45). Yet, the relationship between maternal FM throughout pregnancy and size at birth is yet to be understood (153, 154, 156), especially its importance relative to gestational

weight gain (GWG).

While the relationship between GWG and fetal growth and birth weight is known (149-152), studies of maternal body composition and fetal growth or birth weight are contrasting. Villar (153) reported that maternal FM during the start of the second trimester of pregnancy is positively associated with birth weight, while Mardones-Santander (154) reported the same relationship during the third trimester of pregnancy, and Lederman (155, 215) found a weak negative association between FM and birth weight. Others have found no association of maternal FM and birth weight (136, 156, 157). Maternal total body water (TBW) (155) near term and higher maternal FFM during the third trimester are both positively associated with birth weight (156, 158). Furthermore, Thame (157) reported that, in adolescent and mature pregnant women, the gain in FFM towards the end of gestation was more important in determining birth weight than overall GWG in both groups of women. Because most of the variance in GWG during the second trimester is accounted for by fat (136, 153), maternal FM during the second trimester may be a key factor for fetal growth and the influence of the components of GWG warrants further research. Based on these studies, it appears that maternal FM and FFM during mid-pregnancy may have differential effects on fetal growth and birth weight.

At the same time, maternal macronutrient intake and fetal growth are not consistently related, whereas maternal body composition may be more directly associated. Based on the existing literature, it is clear that maternal GWG and dietary intake alone may not be appropriate predictors of fetal growth and BWT.

Therefore, the aim of this study was to assess the relationship between maternal body composition during the second trimester of gestation and fetal growth in low-income women from Brazil.

METHODS

Subjects

The sample of this study consisted of 124 women from Rio de Janeiro, Brazil who were recruited at the Fernandes Figueira Institute. Healthy, non-smoking participants were recruited during their second trimester of singleton pregnancy between 15 and 40 years of age (one woman with 41 years of age was included). The study was approved by the Institutional Review Board of Rutgers University and the Fernandes Figueira Institue. Written informed consent was obtained at the time of recruitment.

Bioelectrical Impedance Analysis

Body composition was assessed using a BIA 310e Bioimpedance Analyzer (Biodynamics Corp., Seattle, WA, USA). All measurements were conducted with an alternating electric current of 800 μA, 50 kHz. Women were asked to refrain from exercise and coffee the day prior to the appointment and to arrive properly hydrated (2-3 8 oz. glasses of water within 3 hours of the test). Each subject remained in a relaxed standing position with arms slightly abducted from the body and legs slightly apart. Four sensor pads (two current electrodes and two voltage sense electrodes) were placed on the hand and foot of the dominant side for whole body bioimpedance. Resistance and reactance values

were recorded and the analyzer automatically calculated all body composition values.

Anthropometric Measurements

Maternal body weight was measured using an electronic scale (W200-5 Welmy Corp., Santa Bárbara d´Oeste, SP, Brazil) with the women wearing light clothing (e.g. no shoes, jackets, or jewelry). Height was measured to the nearest 0.1 cm with a Seca 206 wall-mounted stadiometer (Seca Corp., Hanover, MD, USA), BMI was calculated as weight (kilograms) divided by height [meters] squared. The same investigator performed all measurements.

Fetal growth (ΔFL), was calculated as birth length (cm) – fetal length at the second trimester, where fetal length = 6.18 + 0.59 x femur length in millimeters, according to Vintzileos (178). Infant body weight and the placental weight were measured with a digital scale to the nearest 0.1 g. Newborn length was measured and ponderal index [birth weight(grams)/length^3(centimeters^3)] was calculated as an index of fatness. Gestational age was estimated using the date of the last menstruation or ultrasound.

Physical Activity Measurement

A physical activity questionnaire based on the short form of the International Physical Activity Questionnaire (IPAQ) was used during the second trimester of pregnancy. The IPAQ measures the physical activity done at work, at home, for transportation, and leisure, and is calculated as moderate and vigorous activity minutes per day and multiplied by METs. The specific types of activity

that are walking, moderate-intensity activities and vigorous-intensity activities and the total score in MET-minutes/week is calculated as follows (216):

Total physical activity MET-minutes/week = sum of Total Work + Total Transport + Total Domestic and Garden + Total Leisure-Time MET-minutes/week scores.

Ultrasound

Fetal growth was assessed by serial ultrasound measurements on the second trimester at 20 and during the third trimester at 33 weeks of gestation. A 3.5 MHz linear array transducer was used to conduct all ultrasound measurements. Fetal length and estimated fetal weight (EFW) were estimated and used as indicators of fetal growth between the second trimester of gestation and birth, and femur growth rate was calculated from the second to the third trimester of gestation.

Dietary assessment

Dietary intake was assessed using a food frequency questionnaire (FFQ) validated in the Brazilian adult population. Research assistants administered the FFQ at the time of recruitment during the second trimester of gestation.

Statistical analyses

There were no significant differences in fetal or neonatal characteristics between boys and girls (N = 124), thus they were analyzed together. All variables were checked for normality and serum glucose and physical activity were log transformed. Birth weight was adjusted for gestational age (GA) by regressing BWT against GA (BWTadj) and the residuals were used in subsequent analyses. Mean values of maternal, fetal, and birth characteristics were analyzed by tertiles

of age. Analysis was performed using one-way ANOVA with post-hoc testing (Tukey's method).

Multiple linear regression analyses were conducted for the following dependent variables: 1) BWTadj, 2) Placental weight, 3) Rate of change in estimated fetal weight per week (i.e. ΔEFW/ number of weeks), 4) Change in EFW (i.e. from week 20 until birth), and 5) Change in fetal length (ΔFL) (i.e. from week 20 until birth). Independent variables included %FM or %FFM controlling for gender, maternal glucose, energy intake, and physical activity level. Regression analyses were conducted for all women and by three predetermined groups of energy intake: low calorie intake <1500 kcal (LoE group), a normal calorie intake ≥1500 < 2500 kcal/d (normal or NL group), and a high calorie intake ≥2500 (HiE group). For the pre-determined groups of energy intake, the lower cut-off is based on the increased risk for suboptimal fetal development. The higher cutoff is based on the recommended increase in energy intake during the second trimester. A sensitivity analysis was conducted including and excluding outliers of energy intake (values that were below the 5^{th} or above the 95^{th} percentiles) and results were materially changed, therefore outliers were removed for analysis. All statistical analyses were performed using SPSS version 19 and significance was set at $p < 0.05$.

RESULTS

Maternal and fetal-infant characteristics are presented in **Table 1A-B**. Mean values were analyzed in tertiles of age. Tertiles of age, weight, BMI, FM, FFM, %FM, %FFM were significantly different, while fetal and neonatal characteristics were not.

Multiple linear regression models for all women using %FM or %FFM as predictors controlling for gender, maternal glucose, energy intake and physical activity are shown on **Table 2**. %FM was a significant predictor of BWTadj **(Figure 1)**, placental weight, ΔEFW change, but not for FL in all women. In the analyses were stratification was done by our predetermined energy intake cutoffs, %FM positively predicted ΔFL. %FFM had a negative association with fetal and birth parameters (**Table 2**).

When the regression models were completed by the three predetermined groups of energy intake (LoE, NL, and HiE), %FM was positively associated with BWTadj for women in the normal energy intake (NL) (**Table 3**). Similarly, the regression models for placental weight showed that %FM had a significant association with placental weight in the NL group (**Table 4**). %FM was positively associated with ΔEFW for women in the NL and HiE groups (**Table 5**). %FM did not have a significant association with the change in EFW or fetal length from the second trimester until birth.

Table B-1: Maternal characteristics of low income Brazilian women (N=124)[1]

Variable	All Women (n=124)	≤ 22 y (n=48)	23-30 y (n=41)	>31 y (n=35)	P value for energy tertiles
Age (y)	25.9 ± 7.4	18.3 ± 2.4[a]	26.7 ± 2.2[b]	35.4 ± 3.0[c]	<0.001
Height (cm)	160.1 ± 7.3	159.3 ± 7.0	160.8 ± 7.1	160.3 ± 7.8	0.597
Weight (kg)	68.3± 15.4	61.1 ± 11.2[a]	68.9 ± 14.8[b]	77.4 ± 17.2[c]	<0.001
BMI (kg/m^2)	26.6 ± 5.3	24.0 ± 3.6[a]	26.5 ± 4.9[b]	30.0 ± 5.8[c]	<0.001
Lean mass (kg)	47.5 ± 7.9	44.2 ± 7.3[a]	47.9 ± 7.0[b]	51.8 ± 7.9[b]	<0.001
Fat mass (kg)	21 ± 8.9	17.3 ± 6.0[a]	21.3 ± 8.2[a]	25.8 ± 10.8[b]	<0.001
%FM	29.6 ± 6.2	27.7 ± 5.8[a]	29.5 ± 6.2[a]	32.1 ± 6.1[b]	0.006
%FFM	70.5 ± 6.8	72.3 ± 5.8[a]	70.5 ± 6.2[a]	67.9 ± 6.3[b]	0.008
Serum glucose transformed (mg/dl)	4.5 ± 0.2	4.4 ± 0.1	4.5 ± 0.2	4.5 ± 0.2	0.143
Income/ month (BRL)	1396 ± 1139	1398 ± 1514	1321 ± 803	1542 ± 1278	0.744
MET-minutes per week	1160 ± 1378	986.7 ±1129	1363 ± 1733	1190 ± 1278	0.523
MET-minutes per week (transformed)	2.8 ± 0.55	2.7 ± 0.56	2.9 ± 0.57	2.9 ± 0.51	0.262

[1]Analysis was performed using one-way ANOVA with post-hoc testing (Tukey's method). Values with different superscripts indicate significant differences.

Abbreviations: BMI : Body mass index,%FM : percent fat mass, %FFM : percent fat free mass, BRL : Brazilian real, BWTadj

Table B-2: Fetal and neonatal characteristics[1]

Variable	All Women (n=124)	≤ 22 y (n=48)	23-30 y (n=41)	>31 y (n=35)	P value for energy tertiles
Birth weight (g)	3311 ± 440	3314 ± 459	3389 ± 500	3311 ± 440	0.391
Birth length (cm)	50.7 ± 2.3	50.8 ± 2.2	50.8 ± 2.2	50.3 ± 2.7	0.623
BWTadj	19.6 ± 430.5	-62.2 ± 350.3	31.0 ± 457.6	121.8 ± 486.2	0.160
Placental weight (g)	655.9 ± 126.2	646.5 ± 117.3	642.0 ± 132.5	685.0 ± 126.2	0.289
Δ EFW (g)	2796 ± 465.1	2771 ± 394.9	2816 ± 470	2802 ± 641	0.944
EFW/week (g)	159.6 ± 28.3	151.9 ± 22.1	162.1 ± 32.7	166.8 ± 28.9	0.168
Δ FL (cm)	3.3 ± 0.6	3.3 ± 0.5	3.4 ± 0.5	3.0 ± 0.7	0.132

[1]Analysis was performed using one-way ANOVA with post-hoc testing (Tukey's method).
Abbreviations: BMI : BWTadj : birth weight adjusted for gestational age, EFW : estimated fetal weight, FL : fetal length

Table B-3: Multiple regression model analyzing %FM and %FFM as predictors of fetal growth, birth and placental weight in all women *

All women N=124				
	%FM		%FFM	
Variable	**Standardized β coefficient**	**P value**	**Standardized β coefficient**	**P value**
A. BWTadj **Model R^2= 0.53**	0.5	<0.001	-0.3	0.02
B. Placental weight (g) **Model R^2= 0.56**	0.5	<0.001	-0.4	0.001
C. Δ EFW (g) **Model R^2= 0.56**	0.6	0.01	-0.4	0.014
D. EFW/week (g) **Model R^2= 0.50**	0.5	0.004	-0.3	0.06
E. Δ FL (cm) **Model R^2= 0.39**	0.3	0.09	-0.3	0.143

*Controlled for gender, maternal glucose, age, energy intake, and exercise level

Abbreviations: %FM : percent fat mass, BWTadj : birth weight adjusted for gestational age, EFW : estimated fetal weight, FL : fetal length

Figure B-1: Linear regression plot of %FM (adjusted for gender and maternal glucose) versus birth weight (adjusted for gestational age)

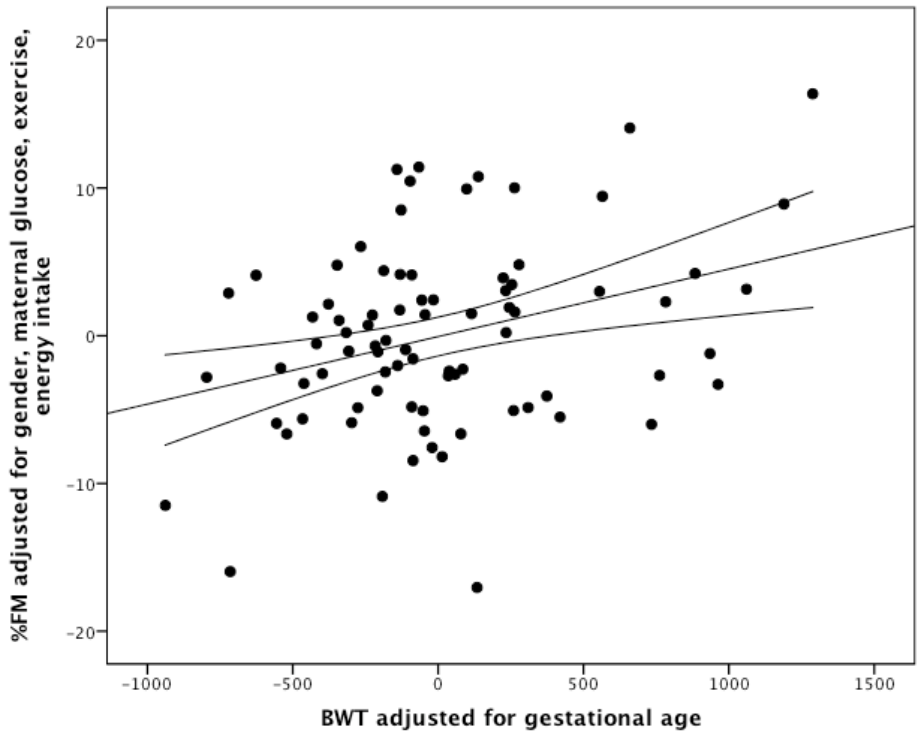


Table B-4: Multiple regression model analyzing (A) %FM and (B) %FFM as predictors of birth weight adjusted for gestational age (BWTadj) by predetermined groups of energy

A

	Standardized β coefficient	**p-value**
Model BWT LoE group		
R^2 = 0.52		
%FM	0.02	0.966
Model BWT NL group		
R^2 = 0.52		
%FM	0.35	0.003
Model BWT HiE group		
R^2 = 0.55		
%FM	0.36	0.175

B

	Standardized β coefficient	**p-value**
Model BWT LoE group		
R^2 = 0.52		
%FFM	-0.03	0.901
Model BWT NL group		
R^2 = 0.37		
%FFM	-0.35	0.003
Model BWT HiE group		
R^2 = 0.55		
%FFM	-0.36	0.176

*Controlled for gender, maternal glucose, energy intake, and exercise level

Table B-5: Multiple regression model analyzing (A) %FM and (B) %FFM as predictors of placental weight by predetermined groups of energy intake

A

	Standardized β coefficient	**p-value**
Model Placenta LoE group		
$R^2 = 0.63$		
%FM	-0.21	0.667
Model Placenta NL group		
$R^2 = 0.32$		
%FM	0.32	0.005
Model Placenta HiE group		
$R^2 = 0.43$		
%FM	0.3	0.242

B

	Standardized β coefficient	**p-value**
Model Placenta LoE group		
$R^2 = 0.63$		
%FFM	0.20	0.674
Model Placenta NLgroup		
$R^2 = 0.32$		
%FFM	-0.33	0.001
Model Placenta HiE group		
$R^2 = 0.43$		
%FFM	-0.30	0.243

*Controlled for gender, maternal glucose, energy intake, and exercise level

Table B-6: Multiple regression model analyzing (A) %FM and (B) %FFM as predictors of estimated fetal weight change by predetermined groups of energy

A

	Standardized β coefficient	**p-value**
Model ∆EFW LoE group		
R^2 = 0.88		
%FM	0.7	0.542
Model ∆EFW NL group		
R^2 = 0.45		
%FM	0.4	0.010
Model ∆EFW HiE group		
R^2 = 0.88		
%FM	0.8	0.021

B

	Standardized β coefficient	**p-value**
Model ∆EFW LoE group		
R^2 = 0.88		
%FFM	-0.75	0.531
Model ∆EFW NL group		
R^2 = 0.45		
%FFM	-0.37	0.010
Model ∆EFW HiE group		
R^2 = 0.88		
%FFM	-0.81	0.021

*Controlled for gender, maternal glucose, energy intake, and exercise level

Discussion

While the relationship of GWG with fetal growth and birth weight is clear (149, 150, 213, 214, 217, 218), the association between specific components of maternal body composition, especially FM, is less clear. A positive association between near-term FFM and TBW and BWT has been reported (136, 154, 155, 158), but data are conflicting regarding the association between FM before the third trimester of pregnancy and BWT (153-155, 219). Therefore, this study sought to better understand how maternal body composition during the second trimester of pregnancy is related to fetal growth in low-income Brazilian women. Briefly, we found that %FM in the second trimester was positively associated with BWTadj, placental weight, and change in estimated fetal weight, independent of dietary intake.

It is well established that pre-pregnancy body mass index (BMI) and gestational weight gain (GWG) are important determinants of fetal growth and birth weight (149, 150, 217, 218). For example, a woman who is underweight or overweight before becoming pregnant and does not attain an adequate GWG has an increased risk of giving birth to an underweight or overweight baby, respectively (151, 218). However, the use of GWG to assess neonatal health outcome independent of pre-pregnancy BMI may result in biased interpretations (220). Furthermore, the revised guidelines for GWG from the Institute of Medicine (IOM) (220), based on pre-gestational BMI (World Health Organization (WHO) BMI classification), were developed from studies with white and black women from developed countries. Thus, these recommendations may not apply for

women from other ethnicities and/or developing countries (220), such as low-income Brazilian women of mixed ethnicity. Maternal dietary intake and body composition assessment, especially FM, may be more useful than GWG to make nutritional and weight gain recommendations during pregnancy since GWG could be influenced by water retention and pre-pregnancy weight is often unknown.

Given that GWG has limitations, it may be useful to consider the assessment of maternal body composition as a complement to GWG recommendations, as maternal body composition is an important determinant of the nutrient supply to the fetus (161, 221). The relationship between FFM and fetal growth is possibly mediated by TBW and by protein turnover (45, 161). The increase in TBW is accounted for mainly by maternal plasma volume expansion, which is accompanied by a higher concentration of red blood cells that carry more oxygen to developing tissues (222, 223) promoting fetal growth. The increased fat deposition during pregnancy supports fetal growth directly and allows energy from the diet to be diverted to support the growth of maternal tissues and the fetus (224). However, the influence of maternal FM during the second trimester of gestation on fetal growth is still unclear.

While in obese women, maternal FM is related to neonatal adiposity (225), no such relationship has been reported for lean or normal-weight women. In our cohort, maternal %FM during the second trimester was positively associated with BWTadj, placental weight, ΔEFW, and ΔEFW/week. However, when multivariate analyses were done by predetermined groups of energy intake, %FM predicted BWTadj and placental weight only in the NL group, and ΔEFW in both NL and

HiE groups while %FM did not have a significant association with ΔEFW/week and ΔFL in any of the groups of energy intake.

The positive association of %FM with BWTadj and placental weight in the NL groups is consistent with the concept that fat deposition during the second trimester of pregnancy is favored due to increased insulin sensitivity (44, 55, 56) while the association between %FM and EFW in women with normal to high-energy intakes points to the crucial role of FM for fetal growth. The increased insulin sensitivity (44) triggers a high rate fat deposition (45, 46) and is followed by a progressive insulin resistance in the late third trimester (47, 48). The insulin response in late pregnancy decreases by 50-75% (49), improving the mobilization of glucose, lipoproteins and FFA, and amino acids to the fetus after a meal (45). Therefore, the mother can use FFA as a fuel source and glucose is preserved for fetal development. Further studies need to address the relationship between maternal body composition and metabolic turnover with fetal growth across different ranges of maternal body weight, GWG, and dietary intake.

Schaefer-Graf (226) compared the influence of maternal lipids on fetal growth between normal women and women with gestational diabetes mellitus (GDM) and found that in women without GDM maternal lipids did not influence neonatal weight. Furthermore, they found increased levels of cord free fatty acids in the women with GDM, which could lead to increased fetal weight. In our study, %FM was related to EFW in the HiE group indicating a possible initial state of insulin resistance before the third trimester in this group of women, leading to an increased fetal size. Another possible explanation is that most women in our

study were overweight, and FM in overweight women and increased waist circumference is positively associated with fetal adiposity (225) and large size at birth (227).

There are some limitations in this study that need to be discussed. First, the assessment of body composition requires the use of techniques that can accurately detect changes in TBW, and the accuracy of BIA during pregnancy has been questioned (135). However, Lukaski (144) showed that BIA might accurately predict TBW during pregnancy and BIA has commonly been used and validated in other studies of maternal body composition throughout pregnancy (137, 144, 153, 156, 158). Furthermore, BIA was conducted during the second trimester of pregnancy, where the influence of amniotic fluid on the body composition assessment is minimal. Segmental BIA could be used to account for variations in body composition during pregnancy, but we found that whole body BIA performed better than segmental BIA (unpublished findings). Also, energy intake was estimated using one FFQ, and pregnant women tend to under-report energy intake (63, 64). The use of more than one FFQ may minimize dietary variations. Strengths of this study include the fact that height and weight were accurately measured and not self-reported. Confounding was minimized by excluding multiple pregnancies and infants with suspected or born with malformation. Furthermore, our findings are important because they fill a clear gap of knowledge on the influence of maternal FM on fetal growth during the second trimester of pregnancy, especially in healthy, well-nourished women.

Based on the results of this study, we conclude that maternal FM during the second trimester of gestation is associated with fetal growth and BWT, independent of diet. In women who have a daily caloric intake of ≤ 1500 kcal/d an increase in %FM might be more necessary to achive optimal fetal growth compared to women who have an adequate caloric intake. Thus maternal %FM during the second trimester needs further evaluation as a possible modifiable and reliable predictor of fetal growth and birth weight as a complement to GWG recommendations for women in developing countries. While larger studies of body composition assessment during pregnancy are necessary, maternal body composition may be used in clinical settings as a complement to GWG to make weight gain recommendations.

SECTION C EFFECTS OF POLYUNSATURATED FATTY ACIDS ON COGNITIVE-LINGUISTIC DEVELOPMENT OF PREMATURE INFANTS

Abstract

Background: Premature infants are at high-risk to develop health problems throughout their lives, as they skip a period of increased nutrient transfer, particularly of LCPUFA during the last trimester of gestation. These fatty acids are important for the development of the retina, the brain, and body tissues, resulting in mental and/or motor limitations if not in adequate amounts. A balance between omega-6 (n-3) and omega-3 (n-6) FA is essential for normal development and homeostasis. However, many reproductive age women do not consume enough LCPUFA, which may result in LCPUFA deprivation during pregnancy.

Objective: To evaluate the relationship between maternal breast milk FA composition and the cognitive, linguistic, and motor development of premature infants.

Methods: Breast milk samples from 25 low-income mothers with premature infants were obtained the first week postpartum and analyzed EFA and LCPUFA composition. The relationship between breast milk FA concentrations and Bayley-III developmental scores of infants between 9-12 months corrected age was assessed using multiple linear regression analysis.

Results: Arachidonic acid had a significant positive relationship (β, 0.6, p = 0.003) with cognition and DHA had a positive relationship with expressive language (β, 0.4, p = 0.03) while LA had a negative relationship (β, -0.6, P =

0.002) with expressive language. The ratios LA: AA and ALA: DHA had a significant negative relationship (β, -0.5, $p < 0.009$ and β, -0.4, $p < 0.04$, respectively) with cognition, while the relationship of AA: ALA with cognition was positive (β, 0.6, $p < 0.002$).

Conclusion: Maternal breast milk from the first week postpartum had AA and DHA concentrations positively associated with cognition, DHA with associated with expressive language, and LA levels associated with lower expressive language scores in premature infants, suggesting no developmental delays in these areas and adequate availability of EFA and LCPUFA in breast milk during the first week after preterm labor (<37 weeks).

Introduction

Premature infants, boys in particular, are at an increased risk of suffering from many disabilities (228), such as motor impairment, attention and learning disabilities, speech and language delay, attachment disturbances, and behavioral problems during infancy and childhood (117-120). The second and third trimesters of gestation are periods of vulnerability to nutritional insults. However, while there are a number of nutrient deficiencies in premature infants (229), LCPUFA deprivation is of particular importance, as there is an accelerated rate of LCPUFA accretion in the central nervous system (CNS) and organ membranes (95). Therefore, potential disabilities may be associated with disturbances in nutrient transfer prenatally and to essential fatty acid-poor milk during breastfeeding.

Maternal LCPUFA are delivered to the fetus during the third trimester of pregnancy when the brain is developing rapidly and during breastfeeding when they are needed for development of the central nervous system, retinal cells, and organs (95). ALA and LA are precursors of DHA and AA, respectively, where LA downregulates the conversion of ALA to DHA (230). DHA and AA both accumulate rapidly in grey matter during the third trimester of gestation and up to the second year of life (96, 97). DHA is crucial for synaptogenesis and myelination (the formation of the lipid myelin bilayer that surrounds axons, which is essential for the development of behavioral, cognitive, and affective functions) (98). Thus, an optimal maternal EFA and LCPUFA status are essential prenatally

and during breastfeeding for proper brain maturation, development, and visual acuity of premature infants (95, 121, 122).

With premature delivery, the LCPUFA supply is interrupted, placing a newborn at risk for developmental impairments. However, whether premature infants benefit from receiving LCPUFA-supplemented formula is unclear. A systematic review of randomized clinical trials (RCT) found that mental development (231) and visual acuity (232) for pre-term infants fed LCPUFA-supplemented formula was significantly greater compared to control groups. At the same time, one RCT of high DHA formula supplementation of preterm infants did not find any clinically significant results on language, behavior, and temperament using the MacArthur Communicative Development Inventory (233). Another trial did not find any associations between maternal DHA supplementation and neurodevelopmental scores assessed by the Bayley-III (234). Even though these RCTs were not conclusive on DHA intakes during pregnancy and lactation, recommendations were made in the absence of large-scale RCTs with an appropriate design. Consequently, further studies are needed to determine more specific dietary recommendations during pregnancy and lactation, particularly for premature infants.

Presently, the adequate intake (AI) of DHA for pregnant and lactating women is 200-300 mg/ DHA per day (109, 235), with an adequate ratio of n-6 to n-3 of 4:1 or less for appropriate interconversion to LCPUFA (109, 236) and sufficient supply to the infant. Still, some studies show that women with premature infants have higher breast milk LCPUFA during the first week

postpartum compared to breast milk of women with full-term births (123-125). For example, mothers of pre-term infants had twice the concentration of AA (0.82 wt%) and DHA (0.33 wt%) compared to full-term infants (AA, 0.44 wt% and DHA 0.15, wt%, respectively) in one study (124) and 1.5 more AA and twice the DHA than full-term infants in another study (125). A recent review concluded that further studies of formula supplementation with LCPUFA for premature infants are necessary and breastfeeding is associated with higher infant IQ and sight skills (126). Together, these findings further heighten the need to establish different DRIs for lactating women with preterm rather than term infants. Therefore, the objective of this study was to assess the fatty acid composition of breast milk in low-income women and to determine the relationship between the FA composition and cognitive, linguistic, and motor development in premature infants.

Methods

Study design and subjects

The sample consisted of 25 healthy preterm infants born adequate for gestational age (AGA) at the Instituto Fernandes Figueira (IFF) between March 2005 and November 2007. The Institutional Review Board/ Research Ethics Committee of the IFF approved the study and written informed consent was obtained from the children's parents or guardians before their participation in the study.

Mother's milk sample collection

Breast milk was manually expressed after breastfeeding in the morning between the 2nd and 7th day postpartum. A minimum of 1 ml of breast milk was collected from each subject in Eppendorf tubes that were labeled with the subject ID. All samples were stored in a freezer at 4°C for no more than 5 days before being transported on ice to the laboratory where they were stored at -70°C until analysis.

Fatty acid analysis

The total lipid fatty acid content of the breast milk samples was analyzed using gas-liquid chromatography (GLC) as described previously (174). Lepage and Roy's (175) transesterification method was used to prepare the fatty acid methyl esters (FAME). FAME were measured in a PerkinElmer chromatography autosystem provided with a hydrogen flame ionization detector and a capillary column (60m x 0.30mm i.d.), packed with 10% SP 2330 (Supelco Inc, Bellefonte, PA, USA) as a stationary phase. Nitrogen was used as a carrier gas. Injection and detection temperatures were both at 220°C, and oven temperature was programmed to vary 5°C/min from 40 to 225 °C. Esters were identified by comparing retention times with known standards (Sigma Aldrich, St Louis, MO, USA) and quantification was done by calculating peak areas with an integrator. Results were expressed as relative area percentage (percentage of total FAME).

Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III)

Trained psychologists applied the Bayley-III on the same day of the medical appointment 9-12 months after birth in a separate room with the infant

and the parent or guardian. After explaining the process of the test, the parent was interviewed and the physical state, the daily routine, and care and environment that surrounded the infant where assessed. Immediately after the interview and assessment, the test was administered. The obtained raw scores were corrected based on the age of the infant according to the instructions, scales, and tables of the manual (176). For this study sample of premature infants, age was corrected for gestational age.

Statistical analysis

The subjects were analyzed together because no significant differences were found between boys and girls. FA values were skewed and were log transformed, so the analyses are derived from transformed data. Multiple linear regression analyses were conducted to assess the association between the FA content in colostrum and the Bayley-III outcome. Some infants underwent the test at either 9 or 12 months or at both time points; therefore, time between birth and date of the test was adjusted for. The Bayley-III scores at 12 mo of age were used except for those infants for whom Bayley-III scores were available from 9 mo of age only (n=8). All values were controlled for sex, gestational age, and length of breast feeding and formula feeding. Additional simple linear regression analyses were conducted to solely assess the relationship between time of breastfeeding and the Bayley-III scores without controlling for any other variable. Exact age at day of the test was calculated as the time between term and date of the test and was used in the analyses that included expressive and receptive language and gross and fine motor development. Cognitive, general language

and motor variables are adjusted for age at the moment of the test. A sensitivity analysis was done including and excluding outliers (values that fell under the 5th (n=1) or above the 95th percentiles (n=2) and results were materially unchanged, therefore all values were kept for analysis. The SAS program (Statistic Analysis System) version 8.0 (SAS Institute, Cary, NC, USA) was used for all analyses and statistical significance was set at $p < 0.05$.

Results

A total of 25 mothers with premature infants born <35 weeks gestational age participated in the study and provided a breast milk sample during the first week postpartum. Infants returned at 9 and/or 12 months corrected age to take the Bayley-III cognitive, language, and motor development test. Only one infant with cerebral palsy was excluded from the analyses. Reference scales and infant scores are shown on **Table 1**.

Physical characteristics are presented on **Table 2**. The mean maternal age was 24.77 years (17-38 years), SD = 6.08. The mean gestational age was 29.60 weeks (24-35 weeks), SD = 2.88. Mean birth weight was 1344.55 g (660-1920 g), SD = 338.57. Thirteen infants were male (41.9%) and 18 infants were female (58.1%). Two infants were exclusively breastfed and 6 infants received exclusive formula feeding. On average breastfed infants received their own mother's milk until 5 months of age, varying between 2 to 16 months; 6 of them being breastfed over 6 months and 2 of them being breastfed over 12 months. Basic perinatal characteristics of the study cohort are presented on **Table 3**.

EFA and LCPUFA breast milk composition

Breast milk composition of EFA, LCPUFA, and ratios are summarized in **Table 4**. The concentration of LA was 17.5% ± 5.2 and 0.81% ± 0.25 for ALA. The mean percent AA was 1.48% ± 0.51 and 0.83% ± 0.35 for DHA. The ratio of LA to ALA was 22 to 1.

Cognitive and Language Development at 9-12 months

The standardized beta coefficients and p-values of EFA, LC-PUFA, and important ratios of these FA as predictors of the Bayley-III are shown in **Table 5**. AA had a significant positive association with infant's cognition, where a one standard deviation increase in AA led to a 0.6 standard deviation in cognition. All ratios (LA: AA, AA: ALA, ALA: DHA) except LA: ALA had a significant effect on cognition. No FA significantly predicted general language development and receptive language development. LA had a significant negative effect on expressive language, with a standard deviation increase leading to a 0.6 standard deviation decrease in expressive language, while DHA had a significant positive relationship (one standard deviation increase in DHA led to a 0.4 standard deviation increase in expressive language). There were not any significant effects of FA percent on motor development; therefore this data is not shown.

Time of breastfeeding alone (without controlling for any variable) was regressed against the Bayley-III scores (**Table 5**). There was a significant negative effect ($p = 0.03$) on receptive language development. This effect

remained similar in the multiple regression models for receptive language development when breastfeeding was controlled for.

Discussion

In Brazil, women of reproductive age or during pregnancy and lactation have LCPUFA intakes that are below the recommended levels (106). Furthermore, infants that receive adequate LCPUFA concentrations during gestation and from breast milk have better mental and motor development than infants who do not receive sufficient LCPUFA or none (96, 114). This study was done to assess the PUFA status in early breast milk of lactating low-income women and the cognitive, linguistic, and motor development of their premature infants.

While breast milk AA levels are normally ~0.4% and DHA levels are ~0.2% of total FA (236), varying concentrations of early breast milk EFA and LCPUFA exist between similar populations of women (237). This study showed higher levels consistent with the higher concentration of LCPUFA in breast milk of women with premature babies (124). Furthermore, the DHA concentration was positively associated with expressive language development and AA with cognitive development. This study sheds light into the n-3 and n-6 fatty acid status of low-income women, which is of major importance given the low intakes of LC-PUFA among this population of women (106), especially women with premature babies that are at risk for nutritional deficiencies and developmental disabilities and the lack of studies using the Bayley-III for this purpose.

Compared to this study, in another (237), breast milk samples from one week postpartum (N=37) of women with pre-term babies from Rio de Janeiro had similar LA levels (16.6% LA). However, DHA and AA were 0.5% and 0.8% higher in our study, respectively, compared to the same cohort. In a study of Hungarian mothers with preterm babies (N=8) (124), 4th day breast milk LA (16.2% LA) was also similar to our cohort, but ALA was 0.42% lower compared to our cohort samples. DHA and AA were also higher in our study by 0.5% and 0.66%, respectively. While Swedish mothers with preterm babies (N=51) had 7% lower LA and 0.49% ALA higher in colostrum samples from the first week postpartum, and they had 0.95% lower AA and 0.38% lower DHA values (238) compared to our study.

Breast milk fatty acid variations are normal between women from different countries (238) and women with preterm and term babies (124) and such variations are highly dependent on a woman's diet. Variations in LA tend to be higher compared to ALA, AA, and DHA, especially from colostrum to mature milk, averaging a 3% increase (237, 239). Variations in ALA average a 0.11% increase from colostrum to mature milk (237, 239). AA and DHA can vary from 0.52% to a 0.21% decrease and 0.07% to 0.09% decrease, respectively from colostrum to mature milk (237, 239). Day to day variations are small and can range from 0.03% decreases every 3 days for ALA and 0.09% from day 1 to day 4 and for LA up to 3% from day 14 to day 21 in mothers with preterm babies (124). For AA day-to-day variations range from -0.04% from day 1 to day 4 post partum to -0.03 from day 14 to day 21 postpartum (124). DHA also decreases by 0.03% from day

1 to day 4 and stabilizes from day 14 to day 21 (124). Considering such small normal changes, when one analyzes our results, a standard deviation increase in any one fatty acid (a considerable amount) would lead to a large change in Bayley scores such as a 0.4 standard deviation increase in expressive language by DHA or a 0.6 standard deviation decrease for expressive language by LA, an area that is affected in premature infants (240).

Our results are in agreement with a previous study in this population and with the increased consumption of oils and processed foods high in n-6 FAs (106, 241) and the decreasing consumption of DHA in Brazilian women (106). It is important to point out that these studies had small cohorts, and this can explain the variability found across different populations of women from the same or from a different country, suggesting that larger population studies could give more accurate information about breast milk EFA and LCPUFA composition. Furthermore, dietary patterns are one important factor that needs to be taken into account and one that was not assessed in the present study. Nonetheless, breast milk FAs were associated with no developmental delays in premature infant cognitive and expressive language as assessed by the Bayley-III.

In our study the breast milk ratio of LA to ALA was 22 to 1, far from the ideal dietary ratio (4:1) for achieving 20% higher plasma and erythrocyte levels of eicosapentaenoic acid (EPA), precursor of DHA (242), which might explain the higher DHA and AA levels. This is in agreement with others that found variable ratios of LA to ALA in colostrums from mothers of preterm and full-term babies (230, 237). Excess LA downregulates ALA metabolism into its LCPUFA because

it competes with ALA for desaturation (243) negatively impacting neurodevelopment (244). Therefore, preformed LCPUFA in the diet of the mother may be a better source for the infant, and when the diet lacks LCPUFA, an adequate LA to ALA ratio is very important to sustain an efficient conversion rate into EPA and DHA for neural tissue and retinal accretion, and adequate levels of AA for growth (245, 246). Not surprisingly, LA was negatively associated with expressive language development and DHA had a significant positive association, consistent with the fact that premature infants may have an expressive language delay (240). These results are in agreement with the negative effects of excessive LA on neurodevelopment (244) and the positive effects of DHA and AA given their rapid accretion to neural tissues (97, 245, 247).

The fact that AA: ALA had a significant positive effect and ALA: DHA and LA: AA had a significant negative effect on cognition is interesting. In particular, the positive effects of DHA and AA acid independently on cognition might explain the negative association of the latter two ratios. The positive association of AA: ALA with cognition might be attributed to the importance of AA as a main component of rapidly growing neural tissues during the first year of life (97, 245, 247-249).

Breastfeeding had a significant negative relationship with receptive language in simple and multiple regression models. One previous study found that there were no differences on receptive language in children who were breastfed or folmula fed (100) while others found that breastfeeding may be protective against receptive and expressive language development delays (250). Because there

was also formula fed babies in our sample, our contrasting finding could possibly be related to the small sample size (n=25). Few studies looked at the effect of breastfeeding on receptive language development in infants (100). While one study had a large population (n=22,400), receptive language was evaluated based on mother's concern about her baby's development (250). Another study used the Peabody Picture Vocabulary Test-Revised to assess receptive language development and compared exclusively breastfed with formula-fed babies (100). Therefore, further studies need to evaluate infant language development directly with standard tests of language development.

Some limitations in our study that deserve consideration include the fact that we did not examine the maternal diet during gestation, which is the main factor influencing breast milk composition (251, 252). Moreover, this study's cohort was small as in previous studies (237), and factors other than diet need to be considered, since ethnic differences in larger cohorts may influence breast milk FA composition (253). Thus, larger, longitudinal studies that control for potential confounders and assess the maternal diet during pregnancy, particularly during the third trimester of gestation and lactation, will lead to a clearer picture of the breast milk composition of this population of women and its association to developmental indices during infancy, in particular during the ages when behavioral and language difficulties become more apparent. Nonetheless, this study is important for various reasons. First, to our knowledge, it is the first study where the Bayley-III is applied to study preterm infant development and breast milk composition. Second, it is known that the EFA and LCPUFA content in

breast milk of Brazilian women is lower than other countries and that breastfeeding indices may be low in Brazil (106), and it is of outmost importance to monitor these changes. Finally, the issue of the insufficient and highly prized DHA and AA supplemented formulas in Brazil must be brought forward.

Provided the maternal diet is optimal, breast milk provides sufficient EFA and LCPUFA for premature infants, and breastfeeding should always be promoted. However, careful attention should be given to the maternal diet to determine the need for supplementation, and because many women who give birth prematurely cannot breastfeed, formula fortified with DHA and AA must be made available to the general population.

In summary, women with preterm babies had higher breast milk AA and DHA levels and lower LA than previous studies, thus healthy n-6 to n-3 ratios that could explain the positive effect on cognitive and expressive language development. We found no evidence of decreased EFA and LCPUFA levels in breast milk or any associations with developmental delays. For women who are able to breastfeed, presuming there is a balanced FA intake, maternal milk provides sufficient EFA and LCPUFA to favor cognitive and expressive development in premature babies.

Table C-1 Bayley-III scoring scale and infant scores

Bayley-III Parameter	**Scales**	**Scores (mean ± SD)**
Cognitive	40-160	101 ± 16.8
Linguistic	40-160	87 ± 9.9
Receptive	1-19	8 ± 2.4
Expressive	1-19	2.8 ± 0.47
Motor	40-160	94 ± 17.5
Fine	1-19	2.9 ± 0.44
Gross	1-19	9.2 ± 3.9

Table C-2 Physical information of the cohort sample (n = 25)

Demographic Factor	Mean ± SD
Maternal Age (years)	24.8 ± 6.1
Gestational Age (weeks)	29.6 ± 2.9
Birth Weight (g)	1344.6 ± 338.6
Duration of breastfeeding	4.0 ± 4.3
Gender	
Male %	41.9
Female %	58.1

Table C-3 Perinatal factors of mothers and preterm infants of the cohort (n=25)

Perinatal Factors	Yes N (%)	No N (%)
Caesarian surgery	16 (61)	9 (39)
APGAR 1^{o} min ≤ 6	8 (37)	17 (63)
APGAR 5^{o} min ≤ 6	1 (4)	24 (96)
Resuscitation in the delivery room	8 (36)	17 (64)
Intubation in the delivery room	7 (28)	18 (72.0)
Twin pregnancy	5 (20)	20 (80)
Smoking during pregnancy	2 (8)	23 (92)
Alcohol during pregnancy	3 (12)	22 (88)
Hypertension	10 (32)	21 (68)
Diabetes	1 (3)	30 (97)

Table C-4 Breast milk essential fatty acid and long-chain polyunsaturated fatty acid concentration during the first week postpartum (n = 25).

Fatty Acid	Area Percent (%) Mean ± SD
C18:2n-6 (LA)	17.5 ± 5.2
C18:3n-3 (ALA)	0.8 ± 0.3
C20:4n-6 (AA)	1.5 ± 0.5
C22:6n-3 (DHA)	0.8 ± 0.3
LA:ALA	22.2 ± 7.2
LA:AA	12.3 ± 6.1
AA:ALA	2.0 ± 1.1
ALA:DHA	1.2 ± 0.7

Table C-5 Multiple linear regression analyses of the relationship between breast milk FA composition (LA, ALA, AA, DHA) and the Bayley-III test

Dependent*	Cognition		Language Total		Receptive Language		Expressive Language	
	Standardized Coefficients		Standardized Coefficients		Standardized Coefficients		Standardized Coefficients	
Independent	β	p-value	β	p-value	β	p-value	β	p-value
LA	-0.2	0.4	0.07	0.8	0.2	0.4	-0.6	0.002
ALA	-0.3	0.7	-0.1	0.5	-0.1	0.5	-0.3	0.2
AA	0.6	0.003	0.2	0.4	0.3	0.2	-0.2	0.5
DHA	0.3	0.08	0.3	0.2	0.1	0.5	0.4	0.03
LA/ALA	0.1	0.6	0.2	0.4	0.4	0.07	-0.4	0.037
LA/AA	-0.5	0.009	-0.07	0.7	-0.04	0.8	-0.3	0.1
AA/ALA	0.6	0.002	0.7	0.5	0.2	0.3	-0.05	0.8
ALA/DHA	-0.4	0.04	-1.5	0.2	-0.2	0.3	-0.4	0.06

*Adjusted for sex, gestational age, length of breastfeeding, and age at day of test

LA= Linoleic acid (C18:2n-6); ALA= Alpha-linolenic acid (C18:3n-3); AA= Arachidonic acid (C20:4n-6); DHA= Docosahexaenoic acid (C22:6n-3).

Table C-6 Simple linear regression analysis of the relationship between duration of breastfeeding and the Bayley-III test

Dependent*	**Cognition**		**Language Total**		**Receptive Language**		**Expressive Language**	
Independent	β	p-value	β	p-value	β	p-value	β	p-value
Length of breastfeeding	0.33	0.069	-0.17	0.10	-0.056	0.03	-0.002	0.58

7 SUMMARY AND CONCLUSION

Based on the results from this study, the assessment of body composition using BIA during pregnancy can shed light into the relationship between the components of GWG and fetal growth in low-income Brazilian women. The assessment of body composition requires the use of techniques that can accurately detect changes in TBW, and while the accuracy of BIA during pregnancy has been questioned (135), Lukaski (144) showed that BIA might accurately predict TBW during pregnancy. Interestingly, the use of segmental BIA was significantly in disagreement with whole body BIA. Using whole body BIA, %FM significantly predicted fetal growth and birth weight of term infants in well-fed women. In a secondary part of this study, a subsample of women with preterm infants provided adequate breast EFA and LCPUFA concentrations positively associated to infant cognitive and expressive language development.

Previous studies of body composition during pregnancy (154, 155, 157, 158) have shown that FFM and TBW towards the end of gestation are associated with birth weight, suggesting that the FFM component of GWG might be more important for fetal development. GWG during the second trimester of pregnancy is a significant predictor of fetal growth and birth weight (156, 213, 254). However, such associations are unclear regarding the components of GWG, particularly FM (153, 154) during the second trimester of pregnancy, what this study sought to understand. Based on the experiments of this research, with the use of whole body BIA, FM was significantly related to fetal growth and birth weight. The consequences of inadequate maternal weight gain and dietary intake

are well documented (35, 149, 162, 217, 218, 254). Therefore, if maternal diet and body composition can be optimized before pregnancy or early in gestation (1^{st}-2^{nd} trimester), the baby's future health and productivity can potentially be spared.

7.1 Bioimpedance analysis during pregnancy

Bioimpedance analysis is a safe, portable, inexpensive, quick and easy to use technique of body composition assessment that is highly correlated with other techniques such as deuterium dilution and hydrodensitometry (127-131). However, the accuracy of BIA may be restricted to healthy, euvolemic populations rather than clinical ones. Specifically, during pregnancy, the validity of whole body BIA may be questioned due to the assumption that the body is a single conducting cylinder. Therefore, we postulated that a segmental approach might be more accurate, as it considers the limbs and trunk separately.

Data from this study suggests that whole body BIA is more accurate than segmental BIA during pregnancy. Briefly, there was clinically significant disagreement between methods based on Bland-Altman plots. The two sets of prediction equations based on segment length and impedance parameters, such as R, overpredicted FM and underpredicted FFM compared to the measured whole body BIA. This disagreement could have stemmed from the fact the prediction equations we used for the segmental measurements were developed from healthy non-pregnant women.

7.1.1 Future studies

While we found such significant differences between methods, we recognize that the BIA technique may not be valid in clinical populations due to the differences in body proportions, water distribution, and varying hydration factor. However, during pregnancy the hydration factor was found to be similar to the pre-pregnancy hydration status (203), and BIA, using prediction equations developed from another study's own sample, accurately predicted TBW compared to deuterium dilution (144). Buchholz (135) recommended that the use of BIA in clinical settings should be restricted to studies that follow groups of patients, but not at the level of the individual. Together, this sheds light into the importance of developing accurate, longitudinally evaluated methods of body composition during pregnancy. Future studies should develop prediction equations for Brazilian pregnant women and test them using BIA before, during, and after pregnancy while comparing the measurements to more accurate methods of body composition to evaluate the reliability of the measurement.

7.2 Maternal body composition and birth weight

The relationship between maternal body composition and birth weight has been studied extensively (44, 45, 149-151, 217, 218), yet studies are unclear with regards to the possible influence of FM during gestation (153-155, 219), particularly the second trimester when maternal fat stores increase the most (44, 55, 56). Furthermore, it is important to study body composition, as GWG has its limitations (192).

We found that %FM during the second trimester of gestation was positively associated with birth weight and placental weight and fetal growth in our study population. There were significant differences between the means of the maternal characteristics after dividing each variable into tertiles of age. Maternal body composition is an important determinant of the nutrient supply to the fetus (161, 221). Therefore, the increased fat mass deposition during pregnancy may support fetal growth directly or allow energy (glucose and amino acids) from the diet to be diverted to support fetal growth (45, 224).

7.2.1 Future studies

While our findings are significant, we used BIA, which might have introduced bias in our measurements. Furthermore, we assessed body composition cross-sectionally, and studies with more measurements throughout pregnancy should be done to confirm our findings. Therefore, further studies should evaluate maternal nutrition status longitudinally, including body composition and dietary assessment, and macronutrient turnover rate pre-pregnancy, during gestation, and postpartum using more accurate techniques of body composition assessment. Parallel studies should be done in developed vs. developing countries.

7.3 Breast milk fatty acid composition and infant development

The intake of EFA and LCPUFA is essential for proper growth and for the homeostasis of the body (109, 246, 255). During pregnancy, the third trimester of gestation in particular, there is an increased transfer rate and fetal tissue accretion of these FA (95). Brazilian reproductive age, pregnant, and lactating

women have a low intake of EFA and LCPUFA (106). Furthermore, premature babies skip this period of growth and may be at increased risk of potential cognitive and motor disabilities compared to babies born at term (117-120, 256). Previous studies of preterm babies analyzed the concentration of EFA and LCPUFA in different countries and found variable levels (106, 124, 237, 238). In this study we sought to analyze the EFA and LCPUFA concentration in the breast milk of women with premature babies in Rio de Janeiro, Brazil and subsequently assess the babies' development.

Briefly, we did not find any developmental delays in the study sample. We found LA, ALA, DHA, and AA concentrations that were positively associated to cognitive and expressive language development. Compared to previous studies, our sample had higher LCPUFAS and ALA, which could have conferred a possible protective effect against developmental delays. Such a finding is important because of the increased intake of n-6 fatty acids found in most processed foods and oils (106, 241).

7.3.1 Future areas of research

Larger population studies could give more accurate information about breast milk EFA and LCPUFA composition. It is clearly necessary to further assess the diet of pregnant women using food diaries at different stages of pregnancy and then evaluate infant development. One factor that could explain the variability found across studies and ours is that we had a small N. The infants in our study should be followed-up until older age and be tested longitudinally to study potential long-term effects of breastfeeding. Furthermore, to our

knowledge, this is the first study to use the Bayley-III test for premature infants and associate the outcome to breast milk composition, thus future studies should further confirm our findings.

7.4 SUMMARY

The maternal nutritional status during pregnancy can determine the future health the baby, therefore it is of utmost importance to study which factors may be involved and how they can be improved benefitting fetal/infant development, the purpose of this study. Briefly we found that FM during the second trimester of pregnancy is associated with increased fetal growth, placental and birth weight. In this population of low-income pregnant women it is not only crucial to understand how body composition relates to fetal development, but also it is critical to evaluate the composition of breast milk, as the quality of the breast milk that infants receive may be as important as the maternal environment during gestation, especially for premature infants. We found higher levels of EFA and LCPUFA than other studies in a similar population, and found no associations with developmental delays in premature infants. Based on the data from this dissertation, further studies are necessary, as the Brazilian diet is transitioning into a Westernized one, and the study of maternal diet, body composition, and breast milk quality is more important now than ever.

8 APPENDIX

8.1 Appendix A: Consent form

Purpose

You are being invited to participate from a study to test if the body composition and diet of pregnant women is related to the health of your newborn. This study includes evaluations of the mother during pregnancy and after delivery and evaluations of the infant after birth. This research is a partnership of the State University of Rio de Janeiro with Rutgers University at the United States.

Procedures that will be used

At the beginning of the monitoring you will answer a questionnaire with information about your pregnancy, weight, diet, and personal information like age, income, etc. A body composition analysis will be conducted.

After the birth of your son or daughter, information about the delivery, length, weight, presence of possible genetic diseases and illnesses that occurred due to the delivery, contained in medical records will be recorded.

Discomforts and possible risks of participating from this research

The electrodes used to assess body composition may cause discomfort during removal after the test. Trained professionals will conduct the test.

Payment, costs or compensation of expenses and possible damages

If you were to suffer any harm provided for in this term of consent and that is resultant of your participation, along with full assistance, you have the right to compensation.

Expected benefits

Smaller number of children with low weight, lower incidence of malnutrition, better development and growth of the child.

Voluntary participation / Withdrawal from the study

Participation in this study is completely voluntary. You can decide not to participate from this study or withdraw from the study at any time, without any prejudice in the monitoring and care provided to you and you newborn in maternity.

Confidentiality

Your identity will be kept confidential. Your identifications will be removed from all analyses and no researcher will provide any information about your data.

Other general information

The results from the analyses and from the study will be available and will be published, be them favorable or not, at the end of the study. You will not receive any special medical care except for those related to the study.

Who to contact in case of doubt

If you have any question or doubt about this research, you can contact the coordinator, Tatiana Toro-Ramos or Dr. Rosely Sichieri at the Institute of Social Medicine of the State University of Rio de Janeiro, São Francisco Xavier St. n° 524, floor 7º, room 7002, block E, Telephone: 2587-7303 (turnouts 244 or 255), or by e-mail: tatiana.ttr@gmail.com or sichieri@ims.uerj.br.

In case you need to contact the Ethics Commission in Research of the UERJ: São Francisco Xavier St., 524, room 3020, block E, floor 3°, - Maracanã - Rio de Janeiro, RJ, e-mail: etica@uerj.br - Telephone: (021) 2569-3490.

I have read and understood what was explained to me about my participation and my son or daughter's participation in the research to take place in this maternity. I had the opportunity to ask about the study and all my questions and doubts were clearly answered. I voluntarily authorize my inclusion and that of my son or daughter in the collection of all the information above. I will receive a signed and dated copy of this document.

Rio de Janeiro,_____ of 20( ).

Participant's name:

_____

Participant's signature:

_____

Professional that explained the informed consent:

_____

8.2 Appendix B: Demographic questionnaire

02.1 Nome:

_____

02.2 Data de Nascimento: ____ / _____ / _____

02.3 Endereço:

_____

02.4 Bairro: _____ 02.5 CEP: _____ 02.6 Município:

_____

02.7 Telefone: _____ - _____ 02.6 Telefone de contato:

_____ - _____

02.8 Nome para contato:

_____

02.9 Ponto de referência:

_____

02.10 Você comeria 1 ovo diariamente:

1. ( ) sim (Punção para colesterol: se maior que 200 mg/dl -NOEGG)
2. ( ) não (Vai para NOEGG)

02.11 Estado civil:

1. ( ) Solteiro (a)
2. ( ) Casado (a) ou vive com companheira (o)
3. ( ) Separado (a), divorciado (a) ou desquitado (a)
4. ( ) Viúvo (a)

02.12 Cor da pele (segundo o entrevistador)**:**

1. ( ) branca 2. ( ) parda 3. ( ) negra
4. ( ) outra

02.13 Das pessoas que moram na sua residência, qual aquela que você considera que seja chefe da família?

1. ( ) A própria entrevistada

2. ( ) Outro: _____

02.14 Qual foi a última série que o (a) CHEFE DA FAMILIA cursou com aprovação? (caso a entrevistada seja a chefe da família, Ir para 02.15)

SÉRIE	GRAU	INFORMAÇÕES ADICIONAIS			
		Sem escolar.	0 0	Sup. incomp.	0 3
		Pós-grad.	4 4	Sup. compl.	3 3

1º GRAU: ENSINO FUNDAMENTAL; 2º GRAU: ENSINO MÉDIO; 3º GRAU: ENSINO SUPERIOR

02.15 Qual foi a última série que você cursou com aprovação?

SÉRIE	GRAU	INFORMAÇÕES ADICIONAIS			
		Sem escolar.	0 0	Sup. incomp.	0 3
		Pós-grad.	4 4	Sup. compl.	3 3

1º GRAU: ENSINO FUNDAMENTAL; 2º GRAU: ENSINO MÉDIO; 3º GRAU: ENSINO SUPERIOR

02.16 Qual era seu peso antes de ficar grávida? _____ Kg

03.1. Nesta residência tem...	**1. Sim**	**2. Não**
1 Geladeira simples?		
2 Geladeira duplex ou freezer?		
3 Máquina de lavar roupa?		
4 Aspirador de pó?		
5 Vídeo cassete ou DVD?		

03.2. Quantos(as) ... existem nesta residência?	**Número**
1 Rádios	
2 Banheiros	
3 TV a cores	
4 Empregadas mensalistas	
5 Automóveis	

04.1 Você trabalha atualmente? 0. ( ) sim 1. ( ) não (Vá para 04.5)

04.2 Qual é a sua ocupação atual?

_____

04.3 Você já trabalhou antes? 0. ( ) sim 1. ( ) não (Vá para 04.7)

04.4 Qual foi a sua ocupação anterior?

_____

04.5 Qual foi a renda total da sua família no último mês? Conte os salários, aposentadorias, pensões e outros rendimentos.

R$_____

04.6. Quantas pessoas incluindo o sr. (ou a sra.) dependeram desta renda no último mês? _____ pessoas

04.7 Quantos cômodos da casa são utilizados para dormir?_____

04.8 Quantas pessoas dormem na casa? _____ pessoas

04.9 A sua casa é:

1. própria
2. alugada
3. outro: _____

_____

8.3 Appendix C: Food frequency questionnaire

Com que freqüência você comeu estes alimentos nos últimos dois meses?

Este questionário foi desenhado para conhecer o consumo habitual de alguns alimentos. Essas informações são muito importantes para nós! Agradecemos a sua colaboração!

Para cada alimento listado abaixo, marque a opção que melhor descreve o seu consumo médio nos últimos **dois meses**. Por favor, tome a porção indicada como uma referência para relatar o seu consumo.

Veja o exemplo dado nas duas primeiras linhas. Se você, usualmente, come arroz duas vezes por dia, sendo uma colher de servir em cada refeição, faça um círculo em torno da **opção de QUANTIDADE que melhor descreve** a quantidade média que v. consome a cada vez e assinale a **FREQÜÊNCIA mais próxima do seu hábito**, no caso, de **2 a 3 vezes** ao dia.

Ainda no exemplo: se você, geralmente, tem por hábito comer meia concha de feijão três vezes por semana, proceda da mesma forma, circule a **opção de QUANTIDADE que melhor descreve** a quantidade média que v. consome a cada vez (no caso, meia concha) e assinale a **FREQÜÊNCIA mais próxima do seu hábito**, no caso, de **2 a 4 vezes** por semana.

No caso de não comer o alimento em questão, assinale “Nunca ou quase nunca”.

Estas duas primeiras linhas representam os exemplos citados:

PRODUTO	QUANTIDADE			Freqüência							
				Mais de 3 vezes por dia	2 a 3 vezes por dia	1 vez por dia	5 a 6 vezes por semana	2 a 4 vezes por semana	1 vez por semana	1 a 3 vezes por mês	Nunca ou quase nunca
Arroz	1 colher	1 colher de	2 colheres de servir ou mais		X						
Feijão	½ concha	1 concha	2 conchas ou mais					X			

PRODUTO	QUANTIDADE			TABLE OF CONTENTS							
				Mais de 3 vezes por dia	2 a 3 vezes por dia	1 vez por dia	5 a 6 vezes por semana	2 a 4 vezes por semana	1 vez por semana	1 a 3 vezes por mês	Nunca ou quase nunca
Arroz	1-2 colheres de sopa	1 colher de servir	2 colheres de servir ou mais								
Macarrão	1 pegador	2 pegadores	3 pegadores ou mais								
Farinha de mandioca	1 colher	2 colheres	3 colheres ou mais								
Polenta ou angu	1 pedaço	2 pedaços	3 pedaços ou mais								
Batata cozida ou purê	1 unidade ou 1 colher de sopa	2 unidades 2 colheres de sopa	3 unidades/ 3colheres ou mais								
Mandioca ou aipim	1 pedaço	2 pedaços	3 pedaços ou mais								
Lasanha, Nhoque, Ravióli	**Marque só a freqüência**										
Feijão	½ concha	1 concha	2 conchas ou mais								
Lentilha, ervilha ou grão de bico	1 colher	2 colheres	3 colheres ou mais								
Bolo	1 fatia	2 fatias	3 fatias ou mais								
Biscoito recheado	1-2 unidades	3-5 unidades	6 unidades ou mais								
Biscoito doce	1-2 unidades	3-5 unidades	6 unidades ou mais								
Biscoito salgado	1-2 unidades	3-5 unidades	6 unidades ou mais								
Pão francês ou pão de forma	1-2 unidades /fatias	2-4 unidades /fatias	3-6 unidades /fatias								

PRODUTO	QUANTIDADE			TABLE OF CONTENTS							
				Mais de 3 vezes por dia	2 a 3 vezes por dia	1 vez por dia	5 a 6 vezes por semana	2 a 4 vezes por semana	1 vez por semana	1 a 3 vezes por mês	Nunca ou quase nunca
Manteiga ou margarina	**Marque só a freqüência**										
Queijo	1 fatia	2 fatias	3 fatias ou mais								
Requeijão	**Marque só a freqüência**										
Leite	1 copo	2 copos									
Iogurte	1 copo ou unidade	2 copos ou unidades ou mais									
Alface	1 folha	3-4 folhas	5 folhas ou mais								
Couve	1 colher	2 colheres	3 colheres ou mais								
Repolho	1 colher	2 colheres	3 colheres ou mais								
Couve-flor ou Brócolis	1 ramo	2 ramos	3 ramos ou mais								
Tomate	½ unidade	1-2 unidades	3 unidades ou mais								
Pepino	1-2 fatias	3-4 fatias	5 fatias ou mais								
Chuchu	1 colher	2 colheres	3 colheres ou mais								
Abobrinha	1 colher	2 colheres	3 colheres ou mais								
Abóbora	1 pedaço	2 pedaços	3 pedaços ou mais								

PRODUTO	QUANTIDADE			TABLE OF CONTENTS							
				Mais de 3 vezes por dia	2 a 3 vezes por dia	1 vez por dia	5 a 6 vezes por semana	2 a 4 vezes por semana	1 vez por semana	1 a 3 vezes por mês	Nunca ou quase nunca
Cenoura	1 colher	2 colheres	3 colheres ou mais								
Beterraba	1-2 fatias	3-4 fatias	5 fatias ou mais								
Quiabo	1 colher	2 colheres	3 colheres ou mais								
Vagem	1 colher	2 colheres	3 colheres ou mais								
Pimentão	**Marque só a freqüência**										
Alho	**Marque só a freqüência**										
Cebola	**Marque só a freqüência**										
Laranja ou tangerina	1 média	2 médias	3 méd. ou mais								
Banana	1 média	2 médias	3 méd. ou mais								
Mamão	1 fatia ou ½ papaia	2 fatias ou 1 papaia ou mais									
Maçã	1 unidade	2 unidades ou mais									
Melancia ou melão	1 fatia	2 fatias									
Abacaxi	1 fatia	2 fatias	3 fatias ou mais								
Manga	1 unidade	2 unidades ou mais									
Uva	1/2 cacho	1 cacho	2 cachos ou mais								

PRODUTO	QUANTIDADE			TABLE OF CONTENTS							
				Mais de 3 vezes por dia	2 a 3 vezes por dia	1 vez por dia	5 a 6 vezes por semana	2 a 4 vezes por semana	1 vez por semana	1 a 3 vezes por mês	Nunca ou quase nunca
Ovo frito, mexido, fritada	1 ovo	2 ovos	3 ovos ou mais								
Ovo cozido	1 ovo	2 ovos	3 ovos ou mais								
Peixe fresco	1 filé ou posta	2 filés ou 2 postas ou mais									
Carne de porco	1 pedaço	2 pedaços ou mais									
Frango	1 pedaço	2 pedaços ou mais									
Carne de boi	1 bife ou 1 pedaço médio, 3 colheres de sopa de carne ensopada ou de carne moída	2 bifes ou 2 pedaços médio, 6 colheres de sopa de carne ensopada ou de carne moída									
Hambúrguer	1 hambúrguer	2 hambúrgueres ou mais									
Sardinha ou atum (lata)	**Marque só a freqüência**										
Bucho, fígado, moela, coração	**Marque só a freqüência**										
Salsicha	1 unidade média	2 unidades médias	3 unidades médias ou mais								
Lingüiça	1 unidade média	2 unidades médias	3 unidades médias ou mais								
Frios como mortadela, presunto, apresuntado, salame,	**Marque só a freqüência**										

PRODUTO	QUANTIDADE			TABLE OF CONTENTS							
				Mais de 3 vezes por dia	2 a 3 vezes por dia	1 vez por dia	5 a 6 vezes por semana	2 a 4 vezes por semana	1 vez por semana	1 a 3 vezes por mês	Nunca ou quase nunca
Bacon ou toucinho	**Marque só a freqüência**										
Carnes ou peixes conservados em sal; bacalhau, carne seca, etc.	**Marque só a freqüência**										
Churrasco	**Marque só a freqüência**										
Pizza	1 pedaço	2 ou mais pedaços									
Batata frita, chips ou palha	1 pacote pequeno de chips ou o equivalente a 1 porção pequena de batata frita do McDonald's	2 pacotes pequenos de chips ou o equivalente a 2 porções pequenas de batata frita do McDonald's	3 ou mais pacotes pequenos de chips ou o equivalente a 3 ou mais porções pequenas de batata frita do McDonald's								
Salgadinhos tipo Cheetos, Fofura, Torcida	1 pacote	2 pacotes	3 pacotes ou mais								
Pipoca (saco)	**Marque só a freqüência**										
Salgados tipo risoli, coxinha, pastel, kibe	1 unidade	2 unidades ou mais									
Amendoim (saco)	**Marque só a freqüência**										

PRODUTO	QUANTIDADE			TABLE OF CONTENTS							
				Mais de 3 vezes por dia	2 a 3 vezes por dia	1 vez por dia	5 a 6 vezes por semana	2 a 4 vezes por semana	1 vez por semana	1 a 3 vezes por mês	Nunca ou quase nunca
Alimentos enlatados: ervilha, azeitona, palmito, etc.	**Marque só a freqüência**										
Maionese	1 colher de chá	2 colheres de chá ou mais									
Sorvete	1 bola	2 bolas ou mais									
Balas	**Marque só a freqüência**										
Chocolate em pó ou Nescau	1 colher	2 colheres	3 colheres ou mais								
Chocolate barra (30g) ou bombom	1 unidade	2 unidades	3 unidades ou mais								
Doce à base de leite	1 pedaço	2 pedaços	3 pedaços ou mais								
Doce à base de fruta	1 pedaço	2 pedaços	3 pedaços ou mais								
Quindim	1 und	2 und	3 und ou mais								
Quindão	1 pedaço	2 pedaços	3pedaços ou mais								
Açúcar	1 colher de sobremesa	2 colheres de sobremesa ou mais									
Café	1 xícara	2 xícaras	3 xícaras ou mais								
Chá ou Mate	1 copo	2 copos	3 copos ou								

PRODUTO	QUANTIDADE			Mais de 3 vezes por dia	2 a 3 vezes por dia	1 vez por dia	5 a 6 vezes por semana	2 a 4 vezes por semana	1 vez por semana	1 a 3 vezes por mês	Nunca ou quase nunca
			mais								
Refrigerantes à base de cola	1 copo	2 copos	3 copos ou mais								
Outros refrigerantes e guaranás	1 copo	2 copos	3 copos ou mais								
Suco da fruta ou da polpa	1 copo	2 copos	3 copos ou mais								
Vinho	1 copo	2 copos	3 copos ou mais								
Cerveja	1-2 copos	3-4 copos	5 copos ou mais								
Outras bebidas alcoólicas	1 dose	2 doses	3 doses ou mais								

05.1 O que o sr. (ou sra.) utiliza com maior freqüência para passar no pão ou em biscoitos:

1. ( ) Margarina, por favor, especifique a marca: _____

2. ( ) Manteiga (**Pule para a questão 06.3**)

3. ( ) Ambas

4. ( ) Não utiliza nem manteiga e nem margarina (**Pule para a questão 06.3**)

05.2 Se o sr. (ou sra.) utiliza margarina, usualmente, utiliza margarina *light*?

0. ( ) Não

1. ( ) Sim

2. ( ) Não sei informar

05.3 Que tipo de leite o sr. (ou sra.) bebe com maior freqüência?

1. ( ) Leite desnatado

2. ( ) Leite semi-desnatado

3. ( ) Leite integral

4. ( ) Leite C (do saquinho de plástico)

5. ( ) Não bebe leite

05.4 Quando o sr. (ou sra.) consome queijo, requeijão ou iogurte, na maior parte das vezes esses produtos são:

1. ( ) *diet* ou *light*

2. ( ) normal

3. ( ) utiliza os dois tipos na mesma proporção

4. ( ) não sabe informar

5. ( ) Não consome queijo, requeijão ou iogurte

05.5 Que tipo de refrigerante o sr. (ou a sra.) costuma beber?

1. ( ) *diet* ou *light*

2. ( ) normal

3. ( ) utiliza os dois tipos na mesma proporção

4. ( ) não sabe informar

5. ( ) Não bebe refrigerante

05.6 Com que freqüência o sr. (ou a sra.) coloca sal na comida servida no prato?

1. ( ) Nunca
2. ( ) Algumas vezes
3. ( ) Sempre

05.7 Com que freqüência o sr. (ou a sra.) retira a pele do frango ou a gordura visível da carne?

1. ( ) Nunca
2. ( ) Algumas vezes
3. ( ) Sempre

05.8 Com que freqüência o sr. (ou a sra.) utiliza adoçante em café, chá, sucos, frutas, etc.?

1. ( ) Nunca
2. ( ) Algumas vezes
3. ( ) Sempre

05.9 Qual tipo de suco de fruta o sr. (ou a sra.) utiliza mais freqüentemente? (marcar no máximo duas respostas)

1. ( ) Feito com a própria fruta natural
2. ( ) Feito com polpa congelada
3. ( ) Suco de garrafa
4. ( ) Pó para preparar em água
5. ( ) Suco de caixa pronto para beber
6. ( ) Não toma suco de fruta

05.10 O sr. (ou a sra.) come frutas todos os dias ou pelo menos 5 vezes por semana? (sem contar sucos e refrescos)

1. ( ) Sim **(Vá para 05.12)**

0. ( ) Não

05.11 Qual é o principal motivo de o sr. (ou a sra.) não comer frutas todos os dias ou pelo menos 5 vezes por semana?

1. ( ) Não gosto de frutas

2. ( ) Frutas são caras

3. ( ) Frutas são difíceis de comprar

4. ( ) Não tenho o costume

5. ( ) Frutas são difíceis de comer

6. ( ) Não tenho tempo

7. ( ) Frutas são difíceis de preparar

05.12 O sr. (ou a sra.) come verduras ou legumes todos os dias ou pelo menos 5 vezes por semana? (sem ser batata, inhame, aipim)

1. ( ) Sim **(Vá para 05.14)**

0. ( ) Não

05.13 Qual é o principal motivo de o sr. (ou a sra.) não comer verduras ou legumes todos os dias ou pelo menos 5 vezes por semana?

1. ( ) Não gosto de verduras ou legumes

2. ( ) Verduras ou legumes são caros

3. ( ) Verduras ou legumes são difíceis de comprar

4. ( ) Não tenho o costume

5. ( ) Verduras ou legumes são difíceis de comer

6. ( ) Não tenho tempo

7. ( ) Verduras ou legumes são difíceis de preparar

05.14 Quantas dúzias de ovos , aproximadamente, são comprados por mês para o consumo da família? _____

05.15 Quantos quilos de sal, aproximadamente, são comprados por mês para o consumo da família? _____ kg

05.16 Quantos quilos de açúcar, aproximadamente, são comprados por mês para o consumo da família? _____ kg

05.17 Em média, com que freqüência o sr. (ou a sra.) fez as seguintes refeições nos últimos seis meses (marque com um X no local apropriado):

	Todos os dias	5-6 vezes na semana	3-4 vezes na semana	1-2 vezes na semana	Nunca
Café da manhã					
Lanche no lugar de almoço					
Almoço					
Lanche da tarde					
Lanche no lugar de jantar					
Jantar					

05.18 Onde o sr. (ou a sra.) realizou, usualmente, as seguintes refeições nos últimos seis meses (marque com um X no local apropriado):

	0.Usualmente não realiza	1.Casa	2.No trabalho, mas leva de casa	3.Fornecida pelo trabalho	4.Lanchonete, bar, restaurante	5.Outros
Café da manhã						
Almoço						
Lanche da tarde						

Jantar						

06.1 Comparando-se com as pessoas da sua idade, o sr. (ou a sra.) considera o seu estado de saúde como...

1. ( ) Muito bom
2. ( ) Bom
3. ( ) Regular
4. ( ) ou Ruim?

06.2 Você está com algum problema de saúde atualmente?

0. **( )** sim
1. **( )** não

06.3 Na sua opinião, a sua alimentação é saudável?

0. ( ) Não
1. ( ) Sim

8.4 Appendix D: Activity Questionnaire

Nós queremos saber quanto tempo você gasta fazendo atividade física em uma semana NORMAL. Por favor, responda cada questão *mesmo* que considere que não seja ativo. Para responder considere as atividades como meio de transporte, no trabalho, exercício e esporte.

07.1. Em quantos dias de uma semana normal, você realiza atividades LEVES ou MODERADAS por pelo menos 10 minutos, que façam você suar POUCO ou aumentam LEVEMENTE sua respiração ou batimentos do coração, como nadar, pedalar ou varrer:

1 ( ) _____ dias por **SEMANA**

2 ( ) Não quero responder

3 ( ) Não sei responder

07.2 Nos dias em que você faz este tipo de atividade, quanto tempo você gasta fazendo essas atividades POR DIA?

1 ( ) _____ horas _____ minutos

2 ( ) Não quero responder

3 ( ) Não sei responder

07.3 Em quantos dias de uma semana normal, você realiza atividades VIGOROSAS por pelo menos 10 minutos, que façam você suar BASTANTE ou aumentem MUITO sua respiração ou batimentos do coração, como correr e nadar rápido ou fazer jogging:

1 ( ) _____ dias por **SEMANA**

2 ( ) Não quero responder

3 ( ) Não sei responder

07.4 Nos dias que você faz este tipo de atividades quanto tempo você gasta fazendo essas atividades POR DIA?

1 ( ) _____ horas _____ minutos

2 ( ) Não quero responder

3 ( ) Não sei responder

ATIVIDADE FÍSICA NO TRABALHO

07.5 Atualmente você trabalha ou faz trabalho voluntário fora de sua casa?

Sim ( ) Não ( )

07.6 Quantos dias de uma semana normal você trabalha? _____ dias

Durante um dia normal de trabalho, quanto tempo você gasta:

07.7. Andando rápido:_____ horas**_____** minutos

07.08 Fazendo atividades de esforço moderado como subir escadas ou carregar pesos leves: ____ horas**____** minutos

07.9 Fazendo atividades vigorosas como trabalho de construção pesada ou trabalhar com enxada, escavar: ___ horas**_____** minutos

ATIVIDADE FÍSICA EM CASA

Agora, pensando em todas as atividades que você tem feito em casa durante uma semana normal:

07.10 Em quantos dias de uma semana normal você faz atividades dentro da sua casa por pelo menos 10 minutos de esforço moderado como aspirar, varrer ou esfregar:

1 ( ) _____ dias por **SEMANA**

2 ( ) Não quero responder

3 ( ) Não sei responder

07.11 Nos dias que você faz este tipo de atividades quanto tempo você gasta fazendo essas atividades POR DIA? _____ horas **_____** minutos

07.12 Em quantos dias de uma semana normal você faz atividades no jardim ou quintal por pelo menos 10 minutos de esforço *moderado* como varrer, rastelar, podar:

1 ( ) **_____** dias por **SEMANA**

2 ( ) Não quero responder

3 ( ) Não sei responder

07.13 Nos dias que você faz este tipo de atividades quanto tempo você gasta POR DIA? _____ horas **_____** minutos

07.14 Em quantos dias de uma semana normal você faz atividades no jardim ou quintal por pelo menos 10 minutos de esforço *vigoroso* ou forte como carpir, arar, lavar o quintal:

1 ( ) **_____** dias por **SEMANA**

2 ( ) Não quero responder

3 ( ) Não sei responder

07.15 Nos dias que você faz este tipo de atividades quanto tempo você gasta POR DIA? _____ horas **_____** minutos

ATIVIDADE FÍSICA COMO MEIO DE TRANSPORTE

Agora pense em relação a caminhar ou pedalar para ir de um lugar a outro em uma semana normal.

07.16 Em quantos dias de uma semana normal você caminha de forma rápida por pelo menos 10 minutos para ir de um lugar para outro? (Não inclua as caminhadas por prazer ou exercício)

1 ( ) _____ dias por **SEMANA**

2 ( ) Não quero responder

3 ( ) Não sei responder

07.16 Nos dias que você caminha para ir de um lugar para outro quanto tempo POR DIA você gasta caminhando? (Não inclua as caminhadas por prazer ou exercício) _____ horas _____ minutos

07.17 Em quantos dias de uma semana normal você pedala rápido por pelo menos 10 minutos para ir de um lugar para outro? (Não inclua o pedalar por prazer ou exercício)

1 ( ) _____ dias por **SEMANA**

2 ( ) Não quero responder

3 ( ) Não sei responder

07.18 Nos dias que você pedala para ir de um lugar para outro quanto tempo POR DIA você gasta pedalando? (Não inclua o pedalar por prazer ou exercício) _____ horas _____ minutos

8.5 Appendix E: Bayley-III Sample Report

Bayley-III Scales of Infant Development Caregiver Report

EXAMINEE:	Sam Weisinger	REPORT DATE:	10/6/2005
AGE:	37 months 20 days	ETHNICITY:	<Not Specified>
DATE OF BIRTH:	8/17/2002	EXAMINER:	Maggie Young
EXAMINEE ID:	123-45-678	Age Adjusted for Prematurity?	No
GENDER:	Male		

Test Administered: Bayley Scales of Infant and Toddler Development, Third Edition (Bayley–III)

About the Bayley–III

Your child was just tested using the *Bayley Scales of Infant and Toddler Development, Third Edition* (Bayley–III). He or she was asked to do a number of activities to see if your child's thinking, language, and moving (sitting, walking) skills are similar to children his or her own age. Some of the activities your child was asked to perform may have seemed very easy while some of the activities may have seemed very hard. No child is expected to do well on every activity.

You may also have been asked questions about your child's social skills (such as expressing emotions or talking with others) and behaviors (such as playing with others or dressing). These questions help us find out your child's range of skills. No child is expected to successfully show every skill.

What does the Bayley–III measure?

The Bayley–III has three major parts that are tested with the child: Cognitive, Language, and Motor. The Questionnaire that you completed looks at your child's Social-Emotional and Adaptive Behavior development.

* The Cognitive Scale (Cog) looks at how your child thinks, reacts, and learns about the world around him or her.
    * Infants are given tasks that measure their interest in new things, their attention to familiar and unfamiliar objects, and how they play with different kinds of toys.
    * Toddlers are given items that examine how they explore new toys and experiences, how they solve problems, and their ability to complete puzzles.
    * Preschool-age children are given items that measure pretend play and activities such as building with blocks, color matching, counting, and solving more complex puzzles.
* The Language Scale (Lang) has two parts.

9 REFERENCES

1. Monteiro CA, D'A Benicio MH, Conde WL, Popkin BM. Shifting obesity trends in Brazil. Eur J Clin Nutr. 2000 Apr;54:342-6.

2. Lof M, Olausson H, Bostrom K, Janerot-Sjöberg B, Sohlstrom A, Forsum E. Changes in basal metabolic rate during pregnancy in relation to changes in body weight and composition, cardiac output, insulin-like growth factor I, and thyroid hormones and in relation to fetal growth. Am J Clin Nutr. 2005 Mar;81:678-85.

3. Duncan BB, Rumel D, Zelmanowicz A, Mengue SS, dos Santos S, Dalmáz A. Social inequality in mortality in São Paulo State, Brazil. Int J Epidemiol. 1995 Apr;24:359-65.

4. Pelletier DL, Frongillo EA, Schroeder DG, Habicht JP. The effects of malnutrition on child mortality in developing countries. Bull World Health Organ. 1995;73:443-8.

5. de Onis M, Frongillo EA, Blössner M. Is malnutrition declining? An analysis of changes in levels of child malnutrition since 1980. Bull World Health Organ. 2000;78:1222-33.

6. (FAO) FaAO. The state of food insecurity in the world.; 2006.

7. Brown JL, Pollitt E. Malnutrition, poverty and intellectual development. Sci Am. 1996 Feb;274:38-43.

8. Nutrition UNSCo. 31st Annual Report. 2004.

9. Organization WH. Obesity and overweight. 2011.

10. Popkin BM. Global nutrition dynamics: the world is shifting rapidly toward a diet linked with noncommunicable diseases. Am J Clin Nutr. 2006 Aug;84:289-98.

11. NHLBI Obesity Education Initiative Expert Panel on the Identification E, and Treatment of Overweight and Obesity in Adults. Clinical Guidelines on the Identification. Evaluation, and Treatment of Overweight and Obesity in Adults. Bethesda (MD): National Heart, Lung, and Blood Institute; September 1998.

12. Monteiro CA, Moura EC, Conde WL, Popkin BM. Socioeconomic status and obesity in adult populations of developing countries: a review. Bull World Health Organ. 2004 Dec;82:940-6.

13. Bank W. Brazil; a poverty assessment. Main Report; 1995.

14. Bank W. Inequality and economic development in Brazil. World Bank Country Studies. 2004.

15. Bank W. Brazil Measuring Poverty Using household Consumption. 2007.

16. Popkin B. Nutritional patterns and transitions. Population and Development Review. 1993;19:138-97.

17. Popkin BM. The nutrition transition in low-income countries: an emerging crisis. Nutr Rev. 1994 Sep;52:285-98.

18. Estatística /BdGe. Pesquisa de Orçamentos Familiares 2002-2003: análise da disponibilidade domiciliar de alimentos e do estado nutricional no Brasil. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística; 2004.

19. Coutinho JG, Gentil PC, Toral N. [Malnutrition and obesity in Brazil: dealing with the problem through a unified nutritional agenda]. Cad Saude Publica. 2008;24 Suppl 2:S332-40.

20. Monteiro CA, Conde WL. [Secular trends in malnutrition and obesity among children in the city of São Paulo, Brazil (1974-1996)]. Rev Saude Publica. 2000 Dec;34:52-61.

21. Monteiro CA, Benicio MH, Iunes R, Gouveia NC, Taddei JA, Cardoso MA. Nutritional status of Brazilian children: trends from 1975 to 1989. Bull World Health Organ. 1992;70:657-66.

22. Oliveira VA, Assis AM, Pinheiro SM, Barreto ML. [Determinants of weight and linear growth deficits in children under two years of age]. Rev Saude Publica. 2006 Oct;40:874-82.

23. Gigante DP, Victora CG, Araújo CL, Barros FC. [Trends in the nutritional profile of children born in 1993 in Pelotas, Rio Grande do Sul, Brazil: longitudinal analyses]. Cad Saude Publica. 2003;19 Suppl 1:S141-7.

24. Monteiro CA, Conde WL, Popkin BM. The burden of disease from undernutrition and overnutrition in countries undergoing rapid nutrition transition: a view from Brazil. Am J Public Health. 2004 Mar;94:433-4.

25. DC M, AC C, L M, OL MN, JB SJ. A construção da vigilância e prevenção das doenças crônicas não transmissíveis no contexto do Sistema Único de Saúde. Epidemiol Serv Saúde. 2006;15:47-65.

26. Som S, Pal M, Bharati P. Role of individual and household level factors on stunting: a comparative study in three Indian states. Ann Hum Biol. 2007 2007 Nov-Dec;34:632-46.

27. Waterlow JC. Protein-energy malnutrition: the nature and extent of the problem. Clin Nutr. 1997 Mar;16 Suppl 1:3-9.

28. Sawaya AL, Martins PA, Baccin Martins VJ, Florêncio TT, Hoffman D, do Carmo P Franco M, das Neves J. Malnutrition, long-term health and the effect of nutritional recovery. Nestle Nutr Workshop Ser Pediatr Program. 2009;63:95-105; -8, 259-68.

29. Hoffman DJ, Martins PA, Roberts SB, Sawaya AL. Body fat distribution in stunted compared with normal-height children from the shantytowns of São Paulo, Brazil. Nutrition. 2007 Sep;23:640-6.

30. Hoffman DJ, Sawaya AL, Coward WA, Wright A, Martins PA, de Nascimento C, Tucker KL, Roberts SB. Energy expenditure of stunted and nonstunted boys and girls living in the shantytowns of São Paulo, Brazil. Am J Clin Nutr. 2000 Oct;72:1025-31.

31. Monteiro CA, Benicio MH, Conde WL, Konno S, Lovadino AL, Barros AJ, Victora CG. Narrowing socioeconomic inequality in child stunting: the Brazilian experience, 1974-2007. Bull World Health Organ. 2010 Apr;88:305-11.

32. Traill WB. Trends towards overweight in lower- and middle-income countries: some causes and economic policy options. The double burden of malnutrition Case studies from six developing countries; 2006. p. 350.

33. Stovitz SD, Demerath EW, Hannan PJ, Lytle LA, Himes JH. Growing into obesity: patterns of height growth in those who become normal weight,

overweight, or obese as young adults. Am J Hum Biol. 2011 2011 Sep-Oct;23:635-41.

34. Oken E, Rifas-Shiman SL, Field AE, Frazier AL, Gillman MW. Maternal gestational weight gain and offspring weight in adolescence. Obstet Gynecol. 2008 Nov;112:999-1006.

35. Dabelea D, Crume T. Maternal environment and the transgenerational cycle of obesity and diabetes. Diabetes. 2011 Jul;60:1849-55.

36. Broughton-Pipkin F. Maternal Physiology: Blackwell Publishing; 2008.

37. Artal R, Platt LD, Sperling M, Kammula RK, Jilek J, Nakamura R. I. Maternal cardiovascular and metabolic responses in normal pregnancy. Am J Obstet Gynecol. 1981 May;140:123-7.

38. Paisley TS, Joy EA, Price RJ. Exercise during pregnancy: a practical approach. Curr Sports Med Rep. 2003 Dec;2:325-30.

39. Robson SC, Hunter S, Boys RJ, Dunlop W. Serial study of factors influencing changes in cardiac output during human pregnancy. Am J Physiol. 1989 Apr;256:H1060-5.

40. Pivarnik JM. Cardiovascular responses to aerobic exercise during pregnancy and postpartum. Semin Perinatol. 1996 Aug;20:242-9.

41. Coolen JC, Verhaeghe J. Physiology and clinical value of glycosuria after a glucose challenge during pregnancy. Eur J Obstet Gynecol Reprod Biol. 2010 Jun;150:132-6.

42. Mshelia DS, Kullima A, Gali RM, Kawuwa MB, Mamza YP, Habu SA, Dangaji U. The use of plasma lipid and lipoprotein ratios in interpreting the hyperlipidaemia of pregnancy. J Obstet Gynaecol. 2010;30:804-8.

43. Catalano PM, Tyzbir ED, Wolfe RR, Roman NM, Amini SB, Sims EA. Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. Am J Obstet Gynecol. 1992 Oct;167:913-9.

44. Catalano PM, Tyzbir ED, Wolfe RR, Calles J, Roman NM, Amini SB, Sims EA. Carbohydrate metabolism during pregnancy in control subjects and women with gestational diabetes. Am J Physiol. 1993 Jan;264:E60-7.

45. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. Am J Clin Nutr. 2000 May;71:1256S-61S.

46. Brelje TC, Scharp DW, Lacy PE, Ogren L, Talamantes F, Robertson M, Friesen HG, Sorenson RL. Effect of homologous placental lactogens, prolactins, and growth hormones on islet B-cell division and insulin secretion in rat, mouse, and human islets: implication for placental lactogen regulation of islet function during pregnancy. Endocrinology. 1993 Feb;132:879-87.

47. Barbour LA, McCurdy CE, Hernandez TL, Kirwan JP, Catalano PM, Friedman JE. Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. Diabetes Care. 2007 Jul;30 Suppl 2:S112-9.

48. Buchanan TA, Metzger BE, Freinkel N, Bergman RN. Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. Am J Obstet Gynecol. 1990 Apr;162:1008-14.

49. Ryan EA, O'Sullivan MJ, Skyler JS. Insulin action during pregnancy. Studies with the euglycemic clamp technique. Diabetes. 1985 Apr;34:380-9.

50. Homko CJ, Sivan E, Reece EA, Boden G. Fuel metabolism during pregnancy. Semin Reprod Endocrinol. 1999;17:119-25.

51. Catalano PM, Nizielski SE, Shao J, Preston L, Qiao L, Friedman JE. Downregulated IRS-1 and PPARgamma in obese women with gestational diabetes: relationship to FFA during pregnancy. Am J Physiol Endocrinol Metab. 2002 Mar;282:E522-33.

52. Lesser KB, Carpenter MW. Metabolic changes associated with normal pregnancy and pregnancy complicated by diabetes mellitus. Semin Perinatol. 1994 Oct;18:399-406.

53. Herrera E, Ortega H, Alvino G, Giovannini N, Amusquivar E, Cetin I. Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. Eur J Clin Nutr. 2004 Sep;58:1231-8.

54. Butte NF, King JC. Energy requirements during pregnancy and lactation. Public Health Nutr. 2005 Oct;8:1010-27.

55. Forsum E, Löf M. Energy metabolism during human pregnancy. Annu Rev Nutr. 2007;27:277-92.

56. Prentice AM, Spaaij CJ, Goldberg GR, Poppitt SD, van Raaij JM, Totton M, Swann D, Black AE. Energy requirements of pregnant and lactating women. Eur J Clin Nutr. 1996 Feb;50 Suppl 1:S82-110; discussion S10-1.

57. Butte NF, Wong WW, Treuth MS, Ellis KJ, O'Brian Smith E. Energy requirements during pregnancy based on total energy expenditure and energy deposition. Am J Clin Nutr. 2004 Jun;79:1078-87.

58. E. HF. Clinical Physiology in Obstetrics. Oxford: Blackwell Scientific Publications; 1980.

59. Prentice AM, Poppitt SD, Goldberg GR, Murgatroyd PR, Black AE, Coward WA. Energy balance in pregnancy and lactation. Adv Exp Med Biol. 1994;352:11-26.

60. Liberman CS, Pino SC, Fang SL, Braverman LE, Emerson CH. Circulating iodide concentrations during and after pregnancy. J Clin Endocrinol Metab. 1998 Oct;83:3545-9.

61. Bronstein MN, Mak RP, King JC. Unexpected relationship between fat mass and basal metabolic rate in pregnant women. Br J Nutr. 1996 May;75:659-68.

62. Prentice AM, Goldberg GR, Poppitt SD. Reproductive stresses in undernourished and well-nourished women. Bibl Nutr Dieta. 1996:1-10.

63. Goldberg GR, Black AE, Jebb SA, Cole TJ, Murgatroyd PR, Coward WA, Prentice AM. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. Eur J Clin Nutr. 1991 Dec;45:569-81.

64. Hill RJ, Davies PS. The validity of self-reported energy intake as determined using the doubly labelled water technique. Br J Nutr. 2001 Apr;85:415-30.

65. Koletzko B, Lien E, Agostoni C, Böhles H, Campoy C, Cetin I, Decsi T, Dudenhausen JW, Dupont C, et al. The roles of long-chain polyunsaturated fatty

acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med. 2008;36:5-14.

66. Gibson RS, Abebe Y, Stabler S, Allen RH, Westcott JE, Stoecker BJ, Krebs NF, Hambidge KM. Zinc, gravida, infection, and iron, but not vitamin B-12 or folate status, predict hemoglobin during pregnancy in Southern Ethiopia. J Nutr. 2008 Mar;138:581-6.

67. Ma AG, Chen XC, Wang Y, Xu RX, Zheng MC, Li JS. The multiple vitamin status of Chinese pregnant women with anemia and nonanemia in the last trimester. J Nutr Sci Vitaminol (Tokyo). 2004 Apr;50:87-92.

68. Aris A, Leblanc S, Ouellet A, Moutquin JM. Detrimental effects of high levels of antioxidant vitamins C and E on placental function: considerations for the vitamins in preeclampsia (VIP) trial. J Obstet Gynaecol Res. 2008 Aug;34:504-11.

69. C N, DM H, LM R. Contribution of animal source foods in improving diet quality and function in children in the developing world. Nutrition Research. 2002;22:193-220.

70. Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, Jiménez M, Gil A, Rivero M, Veszprémi B, et al. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. Am J Clin Nutr. 2007 May;85:1392-400.

71. Innis SM. Essential fatty acid transfer and fetal development. Placenta. 2005 Apr;26 Suppl A:S70-5.

72. Helland IB, Saugstad OD, Smith L, Saarem K, Solvoll K, Ganes T, Drevon CA. Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. Pediatrics. 2001 Nov;108:E82.

73. Malcolm CA, McCulloch DL, Montgomery C, Shepherd A, Weaver LT. Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial. Arch Dis Child Fetal Neonatal Ed. 2003 Sep;88:F383-90.

74. Lieu PT, Heiskala M, Peterson PA, Yang Y. The roles of iron in health and disease. Mol Aspects Med. 2001 2001 Feb-Apr;22:1-87.

75. Prasad AS, Bao B, Beck FW, Sarkar FH. Zinc enhances the expression of interleukin-2 and interleukin-2 receptors in HUT-78 cells by way of NF-kappaB activation. J Lab Clin Med. 2002 Oct;140:272-89.

76. Kräutler B. Biochemistry of B12-cofactors in human metabolism. Subcell Biochem. 2012;56:323-46.

77. See AW, Clagett-Dame M. The temporal requirement for vitamin A in the developing eye: mechanism of action in optic fissure closure and new roles for the vitamin in regulating cell proliferation and adhesion in the embryonic retina. Dev Biol. 2009 Jan;325:94-105.

78. Chambon P. The nuclear receptor superfamily: a personal retrospect on the first two decades. Mol Endocrinol. 2005 Jun;19:1418-28.

79. Black MM. Micronutrient deficiencies and cognitive functioning. J Nutr. 2003 Nov;133:3927S-31S.

80. Dijkhuizen MA, Wieringa FT, West CE, Muherdiyantiningsih, Muhilal. Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia. Am J Clin Nutr. 2001 Apr;73:786-91.

81. Keen CL, Hanna LA, Lanoue L, Uriu-Adams JY, Rucker RB, Clegg MS. Developmental consequences of trace mineral deficiencies in rodents: acute and long-term effects. J Nutr. 2003 May;133:1477S-80S.

82. Keen CL, Clegg MS, Hanna LA, Lanoue L, Rogers JM, Daston GP, Oteiza P, Uriu-Adams JY. The plausibility of micronutrient deficiencies being a significant contributing factor to the occurrence of pregnancy complications. J Nutr. 2003 May;133:1597S-605S.

83. Dunstan JA, Simmer K, Dixon G, Prescott SL. Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed. 2008 Jan;93:F45-50.

84. Bouwstra H, Dijck-Brouwer DA, Boehm G, Boersma ER, Muskiet FA, Hadders-Algra M. Long-chain polyunsaturated fatty acids and neurological

developmental outcome at 18 months in healthy term infants. Acta Paediatr. 2005 Jan;94:26-32.

85. Dewey KG. The challenges of promoting optimal infant growth. J Nutr. 2001 Jul;131:1879-80.

86. Winick M. Nutrition and cell growth. Nutr Rev. 1968 Jul;26:195-7.

87. Kunz LH, King JC. Impact of maternal nutrition and metabolism on health of the offspring. Semin Fetal Neonatal Med. 2007 Feb;12:71-7.

88. Gopalakrishnan GS, Gardner DS, Dandrea J, Langley-Evans SC, Pearce S, Kurlak LO, Walker RM, Seetho IW, Keisler DH, et al. Influence of maternal pre-pregnancy body composition and diet during early-mid pregnancy on cardiovascular function and nephron number in juvenile sheep. Br J Nutr. 2005 Dec;94:938-47.

89. Cleal JK, Poore KR, Newman JP, Noakes DE, Hanson MA, Green LR. The effect of maternal undernutrition in early gestation on gestation length and fetal and postnatal growth in sheep. Pediatr Res. 2007 Oct;62:422-7.

90. Rasmussen KM. Effects of under- and overnutrition on lactation in laboratory rats. J Nutr. 1998 Feb;128:390S-3S.

91. Dewey KG, Lovelady CA, Nommsen-Rivers LA, McCrory MA, Lönnerdal B. A randomized study of the effects of aerobic exercise by lactating women on breast-milk volume and composition. N Engl J Med. 1994 Feb;330:449-53.

92. Agale S, Kulkarni A, Ranjekar P, Joshi S. Maternal caloric restriction spares fetal brain polyunsaturated fatty acids in Wistar rats. Brain Dev. 2010 Feb;32:123-9.

93. Das UN. Long-chain polyunsaturated fatty acids in memory formation and consolidation: further evidence and discussion. Nutrition. 2003 2003 Nov-Dec;19:988-93.

94. Innis SM. Fatty acids and early human development. Early Hum Dev. 2007 Dec;83:761-6.

95. Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and

their relationship to the neonatal essential fatty acid status. Br J Nutr. 1995 Jul;74:55-68.

96. Agostoni C. Role of long-chain polyunsaturated fatty acids in the first year of life. J Pediatr Gastroenterol Nutr. 2008 Nov;47 Suppl 2:S41-4.

97. Innis SM. Dietary omega 3 fatty acids and the developing brain. Brain Res. 2008 Oct;1237:35-43.

98. Joardar A, Sen AK, Das S. Docosahexaenoic acid facilitates cell maturation and beta-adrenergic transmission in astrocytes. J Lipid Res. 2006 Mar;47:571-81.

99. Champeil-Potokar G, Chaumontet C, Guesnet P, Lavialle M, Denis I. Docosahexaenoic acid (22:6n-3) enrichment of membrane phospholipids increases gap junction coupling capacity in cultured astrocytes. Eur J Neurosci. 2006 Dec;24:3084-90.

100. Auestad N, Scott DT, Janowsky JS, Jacobsen C, Carroll RE, Montalto MB, Halter R, Qiu W, Jacobs JR, et al. Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. Pediatrics. 2003 Sep;112:e177-83.

101. Cheruku SR, Montgomery-Downs HE, Farkas SL, Thoman EB, Lammi-Keefe CJ. Higher maternal plasma docosahexaenoic acid during pregnancy is associated with more mature neonatal sleep-state patterning. Am J Clin Nutr. 2002 Sep;76:608-13.

102. SanGiovanni JP, Parra-Cabrera S, Colditz GA, Berkey CS, Dwyer JT. Meta-analysis of dietary essential fatty acids and long-chain polyunsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants. Pediatrics. 2000 Jun;105:1292-8.

103. Cheatham CL, Nerhammer AS, Asserhøj M, Michaelsen KF, Lauritzen L. Fish oil supplementation during lactation: effects on cognition and behavior at 7 years of age. Lipids. 2011 Jul;46:637-45.

104. Kris-Etherton PM, Grieger JA, Etherton TD. Dietary reference intakes for DHA and EPA. Prostaglandins Leukot Essent Fatty Acids. 2009 2009 Aug-Sep;81:99-104.

105. Nochera CL, Goossen LH, Brutus AR, Cristales M, Eastman B. Consumption of DHA + EPA by low-income women during pregnancy and lactation. Nutr Clin Pract. 2011 Aug;26:445-50.

106. Torres AG, Trugo NM. Evidence of inadequate docosahexaenoic acid status in Brazilian pregnant and lactating women. Rev Saude Publica. 2009 Apr;43:359-68.

107. da Cunha J, Macedo da Costa TH, Ito MK. Influences of maternal dietary intake and suckling on breast milk lipid and fatty acid composition in low-income women from Brasilia, Brazil. Early Hum Dev. 2005 Mar;81:303-11.

108. Torres MA, Braga JA, Taddei JA, Nóbrega FJ. Anemia in low-income exclusively breastfed infants. J Pediatr (Rio J). 2006 2006 Jul-Aug;82:284-7.

109. Simopoulos AP. Human requirement for N-3 polyunsaturated fatty acids. Poult Sci. 2000 Jul;79:961-70.

110. Schanler RJ, Shulman RJ, Lau C. Feeding strategies for premature infants: beneficial outcomes of feeding fortified human milk versus preterm formula. Pediatrics. 1999 Jun;103:1150-7.

111. Schanler RJ. Outcomes of human milk-fed premature infants. Semin Perinatol. 2011 Feb;35:29-33.

112. Schanler RJ, Hurst NM, Lau C. The use of human milk and breastfeeding in premature infants. Clin Perinatol. 1999 Jun;26:379-98, vii.

113. Chessex P, Reichman B, Verellen G, Putet G, Smith JM, Heim T, Swyer PR. Quality of growth in premature infants fed their own mothers' milk. J Pediatr. 1983 Jan;102:107-12.

114. Lundqvist-Persson C, Lau G, Nordin P, Strandvik B, Sabel KG. Early behaviour and development in breast-fed premature infants are influenced by omega-6 and omega-3 fatty acid status. Early Hum Dev. 2010 Jul;86:407-12.

115. Heiman H, Schanler RJ. Benefits of maternal and donor human milk for premature infants. Early Hum Dev. 2006 Dec;82:781-7.

116. Schanler RJ, Atkinson SA. Effects of nutrients in human milk on the recipient premature infant. J Mammary Gland Biol Neoplasia. 1999 Jul;4:297-307.

117. Bhutta AT, Cleves MA, Casey PH, Cradock MM, Anand KJ. Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. JAMA. 2002 Aug;288:728-37.

118. Luoma L, Herrgård E, Martikainen A, Ahonen T. Speech and language development of children born at < or = 32 weeks' gestation: a 5-year prospective follow-up study. Dev Med Child Neurol. 1998 Jun;40:380-7.

119. Anderson PJ, Doyle LW, Group VICS. Executive functioning in school-aged children who were born very preterm or with extremely low birth weight in the 1990s. Pediatrics. 2004 Jul;114:50-7.

120. van Baar AL, van Wassenaer AG, Briët JM, Dekker FW, Kok JH. Very preterm birth is associated with disabilities in multiple developmental domains. J Pediatr Psychol. 2005 2005 Apr-May;30:247-55.

121. Hadders-Algra M. Prenatal long-chain polyunsaturated fatty acid status: the importance of a balanced intake of docosahexaenoic acid and arachidonic acid. J Perinat Med. 2008;36:101-9.

122. Hulzebos CV, Sauer PJ. Energy requirements. Semin Fetal Neonatal Med. 2007 Feb;12:2-10.

123. Bitman J, Wood L, Hamosh M, Hamosh P, Mehta NR. Comparison of the lipid composition of breast milk from mothers of term and preterm infants. Am J Clin Nutr. 1983 Aug;38:300-12.

124. Kovács A, Funke S, Marosvölgyi T, Burus I, Decsi T. Fatty acids in early human milk after preterm and full-term delivery. J Pediatr Gastroenterol Nutr. 2005 Oct;41:454-9.

125. Luukkainen P, Salo MK, Nikkari T. Changes in the fatty acid composition of preterm and term human milk from 1 week to 6 months of lactation. J Pediatr Gastroenterol Nutr. 1994 Apr;18:355-60.

126. Schulzke SM, Patole SK, Simmer K. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev. 2011:CD000375.

127. Bhati CS, Silva MA, Wigmore SJ, Bramhall SR, Mayer DA, Buckels JA, Neil DA, Murphy N, Mirza DF. Use of bioelectrical impedance analysis to assess liver steatosis. Transplant Proc. 2009 Jun;41:1677-81.

128. Choi SJ, Kim NR, Hong SA, Lee WB, Park MY, Kim JK, Hwang SD, Lee HK. Changes in body fat mass in patients after starting peritoneal dialysis. Perit Dial Int. 2011 Jan;31:67-73.

129. da Silva EG, Carvalhaes MA, Hirakawa HS, Peraçoli JC. Bioimpedance in pregnant women with preeclampsia. Hypertens Pregnancy. 2010;29:357-65.

130. Woodrow G, Oldroyd B, Turney JH, Smith MA. Segmental bioelectrical impedance in patients with chronic renal failure. Clin Nutr. 1996 Oct;15:275-9.

131. Panella C, Guglielmi FW, Mastronuzzi T, Francavilla A. Whole-body and segmental bioelectrical parameters in chronic liver disease: effect of gender and disease stages. Hepatology. 1995 Feb;21:352-8.

132. Kyle UG, Genton L, Lukaski HC, Dupertuis YM, Slosman DO, Hans D, Pichard C. Comparison of fat-free mass and body fat in Swiss and American adults. Nutrition. 2005 Feb;21:161-9.

133. Cornish B. Bioimpedance analysis: scientific background. Lymphat Res Biol. 2006;4:47-50.

134. Hoffer EC, Meador CK, Simpson DC. Correlation of whole-body impedance with total body water volume. J Appl Physiol. 1969 Oct;27:531-4.

135. Buchholz AC, Bartok C, Schoeller DA. The validity of bioelectrical impedance models in clinical populations. Nutr Clin Pract. 2004 Oct;19:433-46.

136. Butte NF, Ellis KJ, Wong WW, Hopkinson JM, Smith EO. Composition of gestational weight gain impacts maternal fat retention and infant birth weight. Am J Obstet Gynecol. 2003 Nov;189:1423-32.

137. Larciprete G, Valensise H, Vasapollo B, Altomare F, Sorge R, Casalino B, De Lorenzo A, Arduini D. Body composition during normal pregnancy: reference ranges. Acta Diabetol. 2003 Oct;40 Suppl 1:S225-32.

138. Barker DJ, Gelow J, Thornburg K, Osmond C, Kajantie E, Eriksson JG. The early origins of chronic heart failure: impaired placental growth and initiation of insulin resistance in childhood. Eur J Heart Fail. 2010 Aug;12:819-25.

139. Barker DJ. Fetal programming of coronary heart disease. Trends Endocrinol Metab. 2002 Nov;13:364-8.

140. Barker DJ. The origins of the developmental origins theory. J Intern Med. 2007 May;261:412-7.

141. Barker DJ, Thornburg KL, Osmond C, Kajantie E, Eriksson JG. The prenatal origins of lung cancer. II. The placenta. Am J Hum Biol. 2010 2010 Jul-Aug;22:512-6.

142. Goldberg GR. Nutrition in pregnancy: the facts and fallacies. Nurs Stand. 2003 2003 Jan 22-28;17:39-42.

143. Lukaski HC, Hall CB, Siders WA. Assessment of change in hydration in women during pregnancy and postpartum with bioelectrical impedance vectors. Nutrition. 2007 2007 Jul-Aug;23:543-50.

144. Lukaski HC, Siders WA, Nielsen EJ, Hall CB. Total body water in pregnancy: assessment by using bioelectrical impedance. Am J Clin Nutr. 1994 Mar;59:578-85.

145. Ghezzi F, Franchi M, Balestreri D, Lischetti B, Mele MC, Alberico S, Bolis P. Bioelectrical impedance analysis during pregnancy and neonatal birth weight. Eur J Obstet Gynecol Reprod Biol. 2001 Oct;98:171-6.

146. Wells JC, Fewtrell MS. Measuring body composition. Arch Dis Child. 2006 Jul;91:612-7.

147. Wotton MJ, Thomas BJ, Cornish BH, Ward LC. Comparison of whole body and segmental bioimpedance methodologies for estimating total body water. Ann N Y Acad Sci. 2000 May;904:181-6.

148. Thomas BJ, Cornish BH, Ward LC, Patterson MA. A comparison of segmental and wrist-to-ankle methodologies of bioimpedance analysis. Appl Radiat Isot. 1998 1998 May-Jun;49:477-8.

149. Viswanathan M, Siega-Riz AM, Moos MK, Deierlein A, Mumford S, Knaack J, Thieda P, Lux LJ, Lohr KN. Outcomes of maternal weight gain. Evid Rep Technol Assess (Full Rep). 2008 May:1-223.

150. Thorsdottir I, Birgisdottir BE. Different weight gain in women of normal weight before pregnancy: postpartum weight and birth weight. Obstet Gynecol. 1998 Sep;92:377-83.

151. Frederick IO, Williams MA, Sales AE, Martin DP, Killien M. Pre-pregnancy Body Mass Index, Gestational Weight Gain, and Other Maternal Characteristics in Relation to Infant Birth Weight. Maternal and Child Health Journal. 2008 Sep;12:557-67.

152. Hure AJ, Collins CE, Giles WB, Paul JW, Smith R. Greater Maternal Weight Gain During Pregnancy Predicts a Large but Lean Fetal Phenotype: A Prospective Cohort Study. Matern Child Health J. 2011 Nov.

153. Villar J, Cogswell M, Kestler E, Castillo P, Menendez R, Repke JT. Effect of fat and fat-free mass deposition during pregnancy on birth weight. Am J Obstet Gynecol. 1992 Nov;167:1344-52.

154. Mardones-Santander F, Salazar G, Rosso P, Villarroel L. Maternal body composition near term and birth weight. Obstet Gynecol. 1998 Jun;91:873-7.

155. Lederman SA, Paxton A, Heymsfield SB, Wang J, Thornton J, Pierson RN. Maternal body fat and water during pregnancy: do they raise infant birth weight? Am J Obstet Gynecol. 1999 Jan;180:235-40.

156. Farah N, Stuart B, Donnelly V, Kennelly MM, Turner MJ. The influence of maternal body composition on birth weight. Eur J Obstet Gynecol Reprod Biol. 2011 Feb.

157. Thame M, Trotman H, Osmond C, Fletcher H, Antoine M. Body composition in pregnancies of adolescents and mature women and the relationship to birth anthropometry. Eur J Clin Nutr. 2007 Jan;61:47-53.

158. Larciprete G, Valensise H, Vasapollo B, Di Pierro G, Menghini S, Magnani F, De Lorenzo A, Arduini D. Maternal body composition at term gestation and birth weight: is there a link? Acta Diabetol. 2003 Oct;40 Suppl 1:S222-4.

159. Mathews F, Yudkin P, Neil A. Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. BMJ. 1999 Aug;319:339-43.

160. Godfrey K, Robinson S, Barker DJ, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. BMJ. 1996 Feb;312:410-4.

161. Duggleby SL, Jackson AA. Relationship of maternal protein turnover and lean body mass during pregnancy and birth length. Clin Sci (Lond). 2001 Jul;101:65-72.

162. Hales CN, Barker DJ. The thrifty phenotype hypothesis. Br Med Bull. 2001;60:5-20.

163. Lee YY, Park KS, Pak YK, Lee HK. The role of mitochondrial DNA in the development of type 2 diabetes caused by fetal malnutrition. J Nutr Biochem. 2005 Apr;16:195-204.

164. Fernandez-Twinn DS, Ozanne SE. Mechanisms by which poor early growth programs type-2 diabetes, obesity and the metabolic syndrome. Physiol Behav. 2006 Jun;88:234-43.

165. NEEL JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet. 1962 Dec;14:353-62.

166. Wells JC. The thrifty phenotype hypothesis: thrifty offspring or thrifty mother? J Theor Biol. 2003 Mar;221:143-61.

167. Jones RH, Ozanne SE. Fetal programming of glucose-insulin metabolism. Mol Cell Endocrinol. 2009 Jan;297:4-9.

168. Phillips DI, Bennett FI, Wilks R, Thame M, Boyne M, Osmond C, Forrester TE. Maternal body composition, offspring blood pressure and the hypothalamic-pituitary-adrenal axis. Paediatr Perinat Epidemiol. 2005 Jul;19:294-302.

169. Barker DJ. The fetal and infant origins of disease. Eur J Clin Invest. 1995 Jul;25:457-63.

170. Barker DJ. Fetal origins of coronary heart disease. BMJ. 1995 Jul;311:171-4.

171. Green AS, Rozance PJ, Limesand SW. Consequences of a compromised intrauterine environment on islet function. J Endocrinol. 2010 Jun;205:211-24.

172. Pettitt DJ, Jovanovic L. Birth weight as a predictor of type 2 diabetes mellitus: the U-shaped curve. Curr Diab Rep. 2001 Aug;1:78-81.

173. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. Diabetes Care. 2009 Jun;32:1076-80.

174. Azara CR, Maia IC, Rangel CN, Silva-Neto MA, Serpa RF, De Jesus EF, Do Carmo MG, Fialho E. Ethanol intake during lactation alters milk nutrient composition and growth and mineral status of rat pups. Biol Res. 2008;41:317-30.

175. Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res. 1986 Jan;27:114-20.

176. Bayley N. Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III). San Antonio, TX: Harcourt Assessment; 2006.

177. Sichieri R, Siqueira KS, Pereira RA, Ascherio A. Short stature and hypertension in the city of Rio de Janeiro, Brazil. Public Health Nutr. 2000 Mar;3:77-82.

178. Vintzileos AM, Campbell WA, Neckles S, Pike CL, Nochimson DJ. The ultrasound femur length as a predictor of fetal length. Obstet Gynecol. 1984 Dec;64:779-82.

179. Department of Obstetrics and Gynaecology CS.

180. Lapillonne A, Braillon P, Claris O, Chatelain PG, Delmas PD, Salle BL. Body composition in appropriate and in small for gestational age infants. Acta Paediatr. 1997 Feb;86:196-200.

181. Krebs NF, Westcott JL, Rodden DJ, Ferguson KW, Miller LV, Hambidge KM. Exchangeable zinc pool size at birth is smaller in small-for-gestational-age than in appropriate-for-gestational-age preterm infants. Am J Clin Nutr. 2006 Dec;84:1340-3.

182. Mantzoros CS, Varvarigou A, Kaklamani VG, Beratis NG, Flier JS. Effect of birth weight and maternal smoking on cord blood leptin concentrations of full-term and preterm newborns. J Clin Endocrinol Metab. 1997 Sep;82:2856-61.

183. Gupta P, Ray M, Dua T, Radhakrishnan G, Kumar R, Sachdev HP. Multimicronutrient supplementation for undernourished pregnant women and the

birth size of their offspring: a double-blind, randomized, placebo-controlled trial. Arch Pediatr Adolesc Med. 2007 Jan;161:58-64.

184. Bracco D, Thiébaud D, Chioléro RL, Landry M, Burckhardt P, Schutz Y. Segmental body composition assessed by bioelectrical impedance analysis and DEXA in humans. J Appl Physiol. 1996 Dec;81:2580-7.

185. Baumgartner RN, Chumlea WC, Roche AF. Estimation of body composition from bioelectric impedance of body segments. Am J Clin Nutr. 1989 Aug;50:221-6.

186. Kushner RF, Schoeller DA. Estimation of total body water by bioelectrical impedance analysis. Am J Clin Nutr. 1986 Sep;44:417-24.

187. Tanaka NI, Miyatani M, Masuo Y, Fukunaga T, Kanehisa H. Applicability of a segmental bioelectrical impedance analysis for predicting the whole body skeletal muscle volume. J Appl Physiol. 2007 Nov;103:1688-95.

188. Ishiguro N, Kanehisa H, Miyatani M, Masuo Y, Fukunaga T. Applicability of segmental bioelectrical impedance analysis for predicting trunk skeletal muscle volume. J Appl Physiol. 2006 Feb;100:572-8.

189. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986 Feb;1:307-10.

190. Hollander FM, De Roos NM, De Vries JH, Van Berkhout FT. Assessment of nutritional status in adult patients with cystic fibrosis: whole-body bioimpedance vs body mass index, skinfolds, and leg-to-leg bioimpedance. J Am Diet Assoc. 2005 Apr;105:549-55.

191. Lehnert ME, Clarke DD, Gibbons JG, Ward LC, Golding SM, Shepherd RW, Cornish BH, Crawford DH. Estimation of body water compartments in cirrhosis by multiple-frequency bioelectrical-impedance analysis. Nutrition. 2001 Jan;17:31-4.

192. Guidelines IoMUaNRCUCtRIPW. Weight Gain During Pregnancy: Reexamining the Guidelines. Washington (DC): National Academic Press (US); 2009.

193. Kinoshita T, Itoh M. Longitudinal variance of fat mass deposition during pregnancy evaluated by ultrasonography: the ratio of visceral fat to subcutaneous fat in the abdomen. Gynecol Obstet Invest. 2006;61:115-8.

194. Hediger ML, Scholl TO, Belsky DH, Ances IG, Salmon RW. Patterns of weight gain in adolescent pregnancy: effects on birth weight and preterm delivery. Obstet Gynecol. 1989 Jul;74:6-12.

195. Fischer RL. Amniotic Fluid: Physiology and Assessment. The Global Library of Women's Medicine; 2008.

196. Jensen RK, Doucet S, Treitz T. Changes in segment mass and mass distribution during pregnancy. J Biomech. 1996 Feb;29:251-6.

197. Culver CC, Viano DC. Anthropometry of seated women during pregnancy: defining a fetal region for crash protection research. Hum Factors. 1990 Dec;32:625-36.

198. Organ LW, Bradham GB, Gore DT, Lozier SL. Segmental bioelectrical impedance analysis: theory and application of a new technique. J Appl Physiol. 1994 Jul;77:98-112.

199. Baumgartner RN. Electrical impedance and total body electrical conductivity. Champaign, IL; 1996.

200. Richardson T. Art Student's Guide to the Proportions of the Human Form: Tom Richardson; 2008.

201. Morita H, Takeuchi K, Funakoshi T, Mizutori M, Maruo T. Potential use of bioelectrical impedance analysis in the assessment of edema in pregnancy. Clin Exp Obstet Gynecol. 1999;26:151-4.

202. Yasuda R, Takeuchi K, Funakoshi T, Maruo T. Bioelectrical impedance analysis in the clinical management of preeclamptic women with edema. J Perinat Med. 2003;31:275-80.

203. Lof M, Forsum E. Evaluation of bioimpedance spectroscopy for measurements of body water distribution in healthy women before, during, and after pregnancy. J Appl Physiol. 2004 Mar;96:967-73.

204. Haroun D, Taylor SJ, Viner RM, Hayward RS, Darch TS, Eaton S, Cole TJ, Wells JC. Validation of bioelectrical impedance analysis in adolescents across different ethnic groups. Obesity (Silver Spring). 2010 Jun;18:1252-9.

205. Kushner RF. Bioelectrical impedance analysis: a review of principles and applications. J Am Coll Nutr. 1992 Apr;11:199-209.

206. Bartok C, Atkinson RL, Schoeller DA. Measurement of nutritional status in simulated microgravity by bioelectrical impedance spectroscopy. J Appl Physiol. 2003 Jul;95:225-32.

207. Rush EC, Crowley J, Freitas IF, Luke A. Validity of hand-to-foot measurement of bioimpedance: standing compared with lying position. Obesity (Silver Spring). 2006 Feb;14:252-7.

208. Gatford KL, Simmons RA, De Blasio MJ, Robinson JS, Owens JA. Review: Placental programming of postnatal diabetes and impaired insulin action after IUGR. Placenta. 2010 Mar;31 Suppl:S60-5.

209. Norman M. Low birth weight and the developing vascular tree: a systematic review. Acta Paediatr. 2008 Sep;97:1165-72.

210. Barker DJ. Intrauterine programming of adult disease. Mol Med Today. 1995 Dec;1:418-23.

211. Plagemann A, Harder T, Kohlhoff R, Rohde W, Dörner G. Glucose tolerance and insulin secretion in children of mothers with pregestational IDDM or gestational diabetes. Diabetologia. 1997 Sep;40:1094-100.

212. Eriksson JG, Kajantie E, Thornburg KL, Osmond C, Barker DJ. Mother's body size and placental size predict coronary heart disease in men. Eur Heart J. 2011 Jun.

213. Neufeld LM, Haas JD, Grajéda R, Martorell R. Changes in maternal weight from the first to second trimester of pregnancy are associated with fetal growth and infant length at birth. Am J Clin Nutr. 2004 Apr;79:646-52.

214. Hickey CA, Cliver SP, McNeal SF, Hoffman HJ, Goldenberg RL. Prenatal weight gain patterns and birth weight among nonobese black and white women. Obstet Gynecol. 1996 Oct;88:490-6.

215. Reynolds RM, Osmond C, Phillips DI, Godfrey KM. Maternal BMI, parity, and pregnancy weight gain: influences on offspring adiposity in young adulthood. J Clin Endocrinol Metab. 2010 Dec;95:5365-9.

216. IPAQ. Scoring Protocol: Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) 2005.

217. Rode L, Hegaard HK, Kjaergaard H, Møller LF, Tabor A, Ottesen B. Association between maternal weight gain and birth weight. Obstet Gynecol. 2007 Jun;109:1309-15.

218. Ochsenbein-Kölble N, Roos M, Gasser T, Zimmermann R. Cross-sectional study of weight gain and increase in BMI throughout pregnancy. Eur J Obstet Gynecol Reprod Biol. 2007 Feb;130:180-6.

219. Kopp-Hoolihan LE, van Loan MD, Wong WW, King JC. Fat mass deposition during pregnancy using a four-component model. J Appl Physiol. 1999 Jul;87:196-202.

220. Medicine. Io. Weight gain during pregnancy: reexamining the guidelines. 500 Fifth Stret, N.W., Lockbox 285, Washington, DC 20055: National Academic Press; 2009.

221. White CL, Purpera MN, Morrison CD. Maternal obesity is necessary for programming effect of high-fat diet on offspring. Am J Physiol Regul Integr Comp Physiol. 2009 May;296:R1464-72.

222. Rosso P. Nutrition and Metabolism in Pregnancy: Mother anf Fetus: Oxford University Press, USA; 1990.

223. Hytten F. Blood volume changes in normal pregnancy. Clin Haematol. 1985 Oct;14:601-12.

224. King JC, Bronstein MN, Fitch WL, Weininger J. Nutrient utilization during pregnancy. World Rev Nutr Diet. 1987;52:71-142.

225. Sewell MF, Huston-Presley L, Super DM, Catalano P. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. Am J Obstet Gynecol. 2006 Oct;195:1100-3.

226. Schaefer-Graf UM, Meitzner K, Ortega-Senovilla H, Graf K, Vetter K, Abou-Dakn M, Herrera E. Differences in the implications of maternal lipids on

fetal metabolism and growth between gestational diabetes mellitus and control pregnancies. Diabet Med. 2011 Sep;28:1053-9.

227. Salem W, Adler A, Lee C, Smith G. Maternal waist to hip ratio is a risk factor for macrosomia. BJOG. 2011 Oct.

228. Wood NS, Marlow N, Costeloe K, Gibson AT, Wilkinson AR. Neurologic and developmental disability after extremely preterm birth. EPICure Study Group. N Engl J Med. 2000 Aug;343:378-84.

229. Shah MD, Shah SR. Nutrient deficiencies in the premature infant. Pediatr Clin North Am. 2009 Oct;56:1069-83.

230. Gibson RA, Makrides M, Neumann MA, Simmer K, Mantzioris E, James MJ. Ratios of linoleic acid to alpha-linolenic acid in formulas for term infants. J Pediatr. 1994 Nov;125:S48-55.

231. Smithers LG, Gibson RA, McPhee A, Makrides M. Effect of long-chain polyunsaturated fatty acid supplementation of preterm infants on disease risk and neurodevelopment: a systematic review of randomized controlled trials. Am J Clin Nutr. 2008 Apr;87:912-20.

232. Smithers LG, Gibson RA, McPhee A, Makrides M. Higher dose of docosahexaenoic acid in the neonatal period improves visual acuity of preterm infants: results of a randomized controlled trial. Am J Clin Nutr. 2008 Oct;88:1049-56.

233. Smithers LG, Collins CT, Simmonds LA, Gibson RA, McPhee A, Makrides M. Feeding preterm infants milk with a higher dose of docosahexaenoic acid than that used in current practice does not influence language or behavior in early childhood: a follow-up study of a randomized controlled trial. Am J Clin Nutr. 2010 Mar;91:628-34.

234. Makrides M, Gibson RA, McPhee AJ, Yelland L, Quinlivan J, Ryan P, Team DI. Effect of DHA supplementation during pregnancy on maternal depression and neurodevelopment of young children: a randomized controlled trial. JAMA. 2010 Oct;304:1675-83.

235. Makrides M. Is there a dietary requirement for DHA in pregnancy? Prostaglandins Leukot Essent Fatty Acids. 2009 2009 Aug-Sep;81:171-4.

236. Makrides M, Neumann MA, Jeffrey B, Lien EL, Gibson RA. A randomized trial of different ratios of linoleic to alpha-linolenic acid in the diet of term infants: effects on visual function and growth. Am J Clin Nutr. 2000 Jan;71:120-9.

237. Tinoco SM, Sichieri R, Setta CL, Moura AS, do Carmo MG. Trans fatty acids from milk of Brazilian mothers of premature infants. J Paediatr Child Health. 2008 Jan;44:50-6.

238. Sabel KG, Lundqvist-Persson C, Bona E, Petzold M, Strandvik B. Fatty acid patterns early after premature birth, simultaneously analysed in mothers' food, breast milk and serum phospholipids of mothers and infants. Lipids Health Dis. 2009;8:20.

239. Martins Laurindo V, Calil T, Rodrigues Leone C, Araújo Ramos JL. Nutritional composition of colostrum from mothers delivering term infants appropriate or small for gestational age. II - Nutritional composition of human milk at different lactation phases and its advantages over cow's milk: Faculdade de Medicina da Universidade de São Paulo; 1991.

240. Siegel LS. The long-term prognosis of pre-term infants: conceptual, methodological, and ethical issues. Hum Nat. 1994;5:103-26.

241. Levy-Costa RB, Sichieri R, Pontes NoS, Monteiro CA. [Household food availability in Brazil: distribution and trends (1974-2003)]. Rev Saude Publica. 2005 Aug;39:530-40.

242. Clark KJ, Makrides M, Neumann MA, Gibson RA. Determination of the optimal ratio of linoleic acid to alpha-linolenic acid in infant formulas. J Pediatr. 1992 Apr;120:S151-8.

243. Gibson RA, Muhlhausler B, Makrides M. Conversion of linoleic acid and alpha-linolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life. Matern Child Nutr. 2011 Apr;7 Suppl 2:17-26.

244. Novak EM, Dyer RA, Innis SM. High dietary omega-6 fatty acids contribute to reduced docosahexaenoic acid in the developing brain and inhibit secondary neurite growth. Brain Res. 2008 Oct;1237:136-45.

245. Innis SM. Omega-3 Fatty acids and neural development to 2 years of age: do we know enough for dietary recommendations? J Pediatr Gastroenterol Nutr. 2009 Mar;48 Suppl 1:S16-24.

246. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002 Oct;56:365-79.

247. Makrides M, Smithers LG, Gibson RA. Role of long-chain polyunsaturated fatty acids in neurodevelopment and growth. Nestle Nutr Workshop Ser Pediatr Program. 2010;65:123-33; discussion 33-6.

248. Koletzko B, Larqué E, Demmelmair H. Placental transfer of long-chain polyunsaturated fatty acids (LC-PUFA). J Perinat Med. 2007;35 Suppl 1:S5-11.

249. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. J Pediatr. 1992 Apr;120:S129-38.

250. Dee DL, Li R, Lee LC, Grummer-Strawn LM. Associations between breastfeeding practices and young children's language and motor skill development. Pediatrics. 2007 Feb;119 Suppl 1:S92-8.

251. Imhoff-Kunsch B, Stein AD, Villalpando S, Martorell R, Ramakrishnan U. Docosahexaenoic acid supplementation from mid-pregnancy to parturition influenced breast milk fatty acid concentrations at 1 month postpartum in Mexican women. J Nutr. 2011 Feb;141:321-6.

252. Jensen CL, Maude M, Anderson RE, Heird WC. Effect of docosahexaenoic acid supplementation of lactating women on the fatty acid composition of breast milk lipids and maternal and infant plasma phospholipids. Am J Clin Nutr. 2000 Jan;71:292S-9S.

253. Su LL, S K TC, Lim SL, Chen Y, Tan EA, Pai NN, Gong YH, Foo J, Rauff M, Chong YS. The influence of maternal ethnic group and diet on breast milk fatty acid composition. Ann Acad Med Singapore. 2010 Sep;39:675-5.

254. Strauss RS, Dietz WH. Low maternal weight gain in the second or third trimester increases the risk for intrauterine growth retardation. J Nutr. 1999 May;129:988-93.

255. Simopoulos AP. n-3 fatty acids and human health: defining strategies for public policy. Lipids. 2001;36 Suppl:S83-9.

256. Wood NS, Costeloe K, Gibson AT, Hennessy EM, Marlow N, Wilkinson AR, Group ES. The EPICure study: associations and antecedents of neurological and developmental disability at 30 months of age following extremely preterm birth. Arch Dis Child Fetal Neonatal Ed. 2005 Mar;90:F134-40.

10 CURRICULUM VITAE

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PhD, Department of Nutritional Sciences, GPA: 3.834

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RESEARCH and CLINICAL EXPERIENCE

Doctoral Research: Department of Nutritional Sciences, Rutgers University – New Brunswick, 2009 – 2012 (research adviser: Dr. Daniel J. Hoffman). "The relationship between maternal body composition and diet with fetal development in low-income women in Brazil."

Supervisor of Undergraduate Honors Thesis Project, Rutgers University – New Brunswick, 2011 (research adviser: Dr. Daniel J. Hoffman). "Racial differences in body composition and energy expenditure in college students"

Supervisor of Undergraduate Honors Thesis Project, Rutgers University, 2011 (research adviser: Dr. Daniel J. Hoffman). "Energetic costs of mastication and model hominid food processing behaviors"

Pre-Doctoral Research: Department of Nutritional Sciences, Rutgers University – New Brunswick, 2007 - 2008 (research adviser: Dr. Daniel J. Hoffman). "Development of population specific anthropometric prediction equations for children in Brazil."

Undergraduate Research: Department of Biology, University of Puerto Rico, Mayagüez, PR, 2005 – 2007 (research adviser: Dr. Inés Sastre). "The competitive hability of Thuidium tomentosum Schimp. under different light conditions in the forest of Toro Negro, Puerto Rico."

Undergraduate Internship: Boyce Thompson Institute for Plant Research, Cornell University, Ithaca, NY, Summer 2005 (research advisers: Dr. Minsang Lee and Dr. Georg Jander). "The Role of Homocysteine Methyltransferase and Methionine Methyltransferase in Methionine Synthesis and Transport. Plant Genome Research Projects(PGRP)."

Undergraduate Research: Department of Biology, University of Puerto Rico, Mayagüez, PR, 2004 – 2005 (research adviser: Dr. Juan Carlos Martínez Cruzado). "The mutational rate of the two hypervariable regions from the mitochondrial DNA of families living in Vieques versus families living in Puerto Rico."

TEACHING EXPERIENCE

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PUBLICATIONS

Toro-Ramos T., Barbosa Baker Meio M.D., Streit Morsch D., Lopes Moreira M.E., Tavares do Carmo M.G., Sichieri R, Hoffman D.J. “Effects of polyunsaturated fatty acids on cognitive-linguistic development of premature infants” 2011 (in progress for submission).

Toro-Ramos T., Sichieri R., Hoffman D.J. “Estimation of body fat and fat free mass from bioelectrical impedance analysis of body segments during pregnancy” 2011 (in progress for submission).

Toro-Ramos T., Sichieri R., Hoffman D.J. “The impact of maternal body composition on fetal growth” 2011 (in progress for submission).

Lee M, Huang T., **Toro-Ramos T.**, Fraga M., Last R.L., Jander G. “Reduced activity of Arabidopsis thaliana HMT2, a methionine biosynthetic enzyme, increases seed methionine content.” The Plant journal: for cell and molecular biology. 2008;54(2):310-20.

Toro-Ramos T., Rondo P., Sawaya A.L., Hoffman D. “Development of population-specific anthropometric prediction equations for children in Brazil.” Submitted for publication in Annals of Human Biology. Aug 2011.