

**Associations of Maternal Diet with Infant Growth Outcomes in Eight Indigenous Communities
Of Guatemala: Effect Modification by Maternal Age**

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ABSTRACT

Background

The *Mam*-Mayan Indigenous communities living in the Western Highlands of Guatemala have the highest prevalence of infant stunting and one of the highest rates of adolescent pregnancies in Latin America. There is limited research on the adequacy of maternal diet, especially among lactating *Mam*-Mayan women, and how this may relate to infant growth outcomes and the role of maternal age on these relationships.

Hypothesis & objectives

Our study aimed to explore the associations among maternal macro and micronutrient intakes, maternal age, and infant growth. This research 1) investigated the adequacy of nutrient intakes of the pregnant and lactating *Mam*-Mayan women, 2) explored the associations between nutrient intakes and early infant growth outcomes, and 3) assessed how maternal age (adolescent vs. adults) modified the relationship between maternal nutrient intakes and infant growth outcomes.

Methods

This study included dietary data from pregnant (n=117) and lactating women during early (n=164), late lactation (n=168) (N=449). We calculated the nutrient intakes of the lactating mothers from 24-hour recalls and compared them to INCAP (Institute of Nutrition of Central American and Panama) Recomendaciones Dietéticas Diarias (RDD) (INCAP, 2012) and IOM (Institute of Medicine) Dietary Reference Intake (IOM, 2005) recommendations to assess the nutrient adequacy of the mothers. Infant growth outcomes included weight-for-age-z-scores (WAZ), length-for-age-z-scores (LAZ), head circumference-for-age-z-score (HCAZ). Multiple linear regressions were used to explore the associations between maternal nutrient intakes during early and late lactation on infant WAZ, LAZ, and HCAZ, whereas multiple logistic regressions were used to explore their associations with infant underweight (WAZ<-2SD), stunting (LAZ<-2SD), and low cranial growth (HCAZ<-1SD). Maternal age (adolescents (≤ 19 years) vs. adults (>19 years)) was investigated as an effect modifier of the relationship between maternal nutrients and infant

growth outcomes. We adjusted for height, weight, parity, and infant sex. $P < 0.05$ was considered statistically significant.

Major results

First, average intakes of carbohydrates, fiber, sugars, magnesium, phosphorous and sodium were adequate when evaluated using IOM DRI and INCAP RDD, but all other nutrients fell below the DRI and RDD. Second, in early lactation, higher intakes of energy, carbohydrates, fiber, and seven micro-nutrients (calcium, magnesium, selenium, folate, vitamin A, retinol, PUFA) were associated with increased WAZ. However, only higher intakes of folate, DFE and PUFAs lowered the odds of underweight ($WAZ < -2SD$). In early lactation, higher intakes MUFA & PUFA, selenium, and vitamin E were associated with higher LAZ, whereas no nutrient lowered the odds of stunting ($LAZ < -2SD$). However, in early lactation, higher intakes of energy, carbohydrates, fiber, PUFA, vitamin B6, and folate were associated with increased HCAZ but only fiber, PUFA and folate were associated with reduced odds of low head circumference ($HCAZ < -1SD$). In late lactation, retinol was negatively associated with WAZ and LAZ and only cholesterol was associated with higher LAZ and with reduced odds of infant underweight.

Lastly, maternal age modified the association between nutrient intakes and infant growth during lactation. Higher intakes of vitamin A and retinol were associated with higher WAZ scores for adolescents in early lactation (positive association) whereas higher lycopene intakes in late lactation were associated with lower WAZ scores for adolescent mothers in late lactation (negative association). Higher intakes of selenium, vitamin E, SFA and MUFA were associated with higher LAZ for adolescent mothers in early lactation (positive association), whereas higher lycopene intakes in late lactation was associated with lower LAZ scores for adolescent mothers in late lactation (negative association). Higher intakes of lipids, vitamin E, SFA, and MUFA significantly lowered the odds of stunting among infants of adolescent mothers in early lactation. Higher intakes of calcium, iron, and folate (DFE) significantly lowered the odds of low cranial growth among infants of adult mothers in early lactation. No significant modification by age was observed in the relationship between the maternal nutrients and infant growth outcomes in late lactation.

Conclusion

To our knowledge, this is the first study to explore the nutrient adequacy of the maternal *Mam-Mayan* Guatemalan traditional diet and infant growth during the first six months of lactation. We found that maternal intakes during the first six weeks of breastfeeding had greater impacts on infant growth outcomes compared to nutrient intakes at 4-6 months. Higher nutrient intakes of specific nutrients among adolescent mothers were associated with improved infant growth outcomes. The early lactation period could be a critical point for nutritional interventions for improving infant growth outcomes.

Résumé

Contexte

Les communautés autochtones Mam-Mayas vivant dans les hautes terres occidentales du Guatemala ont la prévalence la plus élevée de retard de croissance infantile et l'un des taux les plus élevés de grossesse chez les adolescentes en Amérique latine. À l'heure actuelle, il existe peu de recherches sur l'adéquation de l'alimentation maternelle, en particulier chez les femmes mam-mayas allaitantes, et sur la façon dont elle est liée à la croissance du nourrisson et au rôle de l'âge maternel dans ces relations.

Hypothèse et objectifs

Notre étude visait à explorer les associations entre les apports maternels en macro et micronutriments, l'âge maternel et la croissance du nourrisson. Cette recherche 1) a étudié la pertinence des apports nutritionnels des femmes Mam-Maya enceintes et allaitantes, 2) a exploré les associations entre les apports en nutriments et les résultats de croissance précoce du nourrisson, et 3) a évalué comment l'âge maternel (adolescent versus adultes) a modifié la relation entre les apports nutritionnels maternels et les résultats de croissance du nourrisson.

Méthodes

Cette étude comprenait des données alimentaires de femmes enceintes ($n = 117$) et allaitantes au début ($n = 164$), à la fin de l'allaitement ($n = 168$) ($N = 449$). Nous avons calculé les apports nutritionnels des mères allaitantes à partir de rappels de 24 heures et les avons comparé aux recommandations de l'INCAP (Institute of Nutrition of Central American and Panama), Des Recomendaciones Dietéticas Diarias (RDD) (INCAP, 2012) et de l'IOM (Institute of Medicine) Dietary Reference Intakes (IOM, 2005) pour évaluer l'adéquation nutritionnelle des mères. Les résultats de la croissance du nourrisson comprenaient les scores z du poids pour l'âge (WAZ), les scores z de taille pour l'âge (LAZ), le score z de la circonférence de la tête pour l'âge (HCAZ). Des régressions linéaires multiples ont été utilisées pour explorer les associations entre les apports nutritionnels maternels au début et à la fin de l'allaitement et les WAZ, LAZ et HCAZ,

des nourrissons, tandis que des régressions logistiques multiples ont été utilisées pour explorer leurs associations avec l'insuffisance pondérale du nourrisson ($WAZ < -2SD$), le retard de croissance ($LAZ < -2SD$) et la faible croissance crânienne ($HCAZ < -1SD$). L'âge maternel (adolescentes (≤ 19 ans) vs adultes (> 19 ans)) a été étudié comme un modificateur d'effet de la relation entre les nutriments maternels et les résultats de croissance du nourrisson. Nous avons ajusté pour la taille, le poids, la parité et le sexe du nourrisson. Une valeur de $P < 0,05$ a été considérée comme statistiquement significatif.

Principaux résultats

Tout d'abord, les apports moyens en glucides, fibres, sucres, magnésium, phosphore et sodium étaient adéquats lorsqu'ils étaient évalués à l'aide de l'IOM DRI et de l'INCAP RDD, mais tous les autres nutriments étaient inférieurs à l'ANREF (Apports nutritionnels de référence) et au RDD (Recommandations diététiques quotidiennes). Deuxièmement, au début de la lactation, des apports plus élevés en énergie, en glucides, en fibres et en sept micronutriments (calcium, magnésium, sélénium, folate, vitamine A, rétinol, acides gras polyinsaturés [AGPI]) ont été associés à une augmentation de la WAZ. Cependant, seuls des apports plus élevés de folate, de DFE et de AGPI ont réduit les risques d'insuffisance pondérale ($WAZ < -2SD$). Au début de l'allaitement, des apports plus élevés en acide gras monoinsaturés et AGPI, en sélénium et en vitamine E étaient associés à une LAZ plus élevée, tandis qu'aucun nutriment ne réduisait les risques de retard de croissance ($LAZ < -2SD$). Cependant, au début de la lactation, des apports plus élevés en énergie, en glucides, en fibres, en AGPI, en vitamine B6 et en folate étaient associés à une augmentation du HCAZ, mais seules les fibres, les AGPI et le folate étaient associés à une réduction du risque de faible circonférence de la tête ($HCAZ < -1SD$). À la fin de l'allaitement, le rétinol était négativement associé à WAZ et la LAZ et seul le cholestérol était associé à une LAZ plus élevée et à une réduction des risques d'insuffisance pondérale chez le nourrisson.

Enfin, l'âge maternel a modifié l'association entre les apports nutritionnels et la croissance du nourrisson pendant l'allaitement. Des apports plus élevés en vitamine A et en rétinol étaient

associés à des scores WAZ plus élevés chez les adolescentes en début de lactation (association positive), tandis que des apports plus élevés en lycopène en fin de lactation étaient associés à des scores WAZ plus faibles chez les mères adolescentes en fin de lactation (association négative). Des apports plus élevés en sélénium, en vitamine E, en AGS et en AGMI étaient associés à des taux de LAZ plus élevés chez les mères adolescentes en début d'allaitement (association positive), tandis que des apports plus élevés en lycopène en fin de lactation étaient associés à des scores LAZ plus faibles chez les mères adolescentes en fin de lactation (association négative). Des apports plus élevés en lipides, en vitamine E, en AGS et en AGMI ont considérablement réduit les risques de retard de croissance chez les nourrissons de mères adolescentes au début de l'allaitement. Des apports plus élevés en calcium, en fer et en folate (DFE) ont considérablement réduit les risques de faible croissance crânienne chez les nourrissons de mères adultes en début de lactation. Aucune modification significative selon l'âge n'a été observée dans la relation entre les nutriments maternels et les résultats de croissance du nourrisson à la fin de la lactation.

Conclusion

À notre connaissance, il s'agit de la première étude à explorer l'adéquation nutritionnelle du régime alimentaire traditionnel guatémaltèque Mam-Maya maternel et la croissance du nourrisson au cours des six premiers mois de la lactation. Nous avons constaté que les apports maternels au cours des six premières semaines d'allaitement avaient des impacts plus importants sur les résultats de croissance du nourrisson par rapport aux apports nutritionnels à 4-6 mois. Des apports nutritionnels plus élevés de nutriments spécifiques chez les mères adolescentes ont été associés à une amélioration des résultats de croissance du nourrisson. La période de lactation précoce pourrait être un point critique pour les interventions nutritionnelles visant à améliorer les résultats de croissance du nourrisson.

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CONTRIBUTION OF AUTHORS

My thesis was written in the traditional monograph style according to the Guidelines Concerning Thesis Preparation stipulated by McGill University. Malathi Kanapuram wrote this thesis.

The study design was a collaborative effort, with myself and my supervisors, Drs. Koski and Jock, who guided Malathi in the development of the conceptual framework, hypotheses, and specific objectives. The study design was developed based on an evolving understanding that maternal age might be an important effect modifier of the relationship between maternal diet and infant growth.

Drs. Solomons and Vossenaar trained and coordinated the team that collected the 24-hour recalls from the field and assembled the dataset.

As first author, I was responsible for data analysis, and data interpretation of the maternal nutrient intakes and their association with infant anthropometry, and preparation of the written thesis. Initially, I took the data sets of maternal nutrient intakes, and infant anthropometry, used these data sets to assess the nutrient adequacy of the Guatemalan pregnant and lactating women and the effect modification of age on infant anthropometry using STATA.

Dr. Brittany Wenniser: iostha Jock taught me STATA and introduced me to the concept of effect modification. She worked with me in the development of the STATA analysis and also guided me through the interpretation of the results.

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LIST OF ABBREVIATIONS

BMI	Body Mass Index
CRONOS	Cross-Cultural Research on the Nutrition of Older Subjects Study
DRI	Dietary Reference Intakes
HCAZ	Head-circumference-for-age-z-scores
LMIC	Low-and-middle-income countries
LBW	Low birth weight
LAZ	Length-for-age-z-score
PP	Postpartum
PCP	Pasta, cheese, and processed meat
PTB	Pre-term birth
RDD	<i>Recomendaciones Dietéticas Diarias</i>
SGA	Small-for-gestational age
SfN	Seafood and noodles
VFR	Vegetable, fruit, and white rice
WAZ	Weight-for-age-z-score

CHAPTER 1. OVERVIEW

Background

Historical Perspective

The Guatemalan diet is one of the most thoroughly studied diets compared to other developing countries (Solomons, 2000). This body of research began with the interest of the Jesuit priests to document the post-Mayan culture over the years. The Spanish Conquistadores continued this study in 1524, followed by anthropologists and archeologists in the 19th century (Solomons, 2000). Institute of Nutrition of Central America and Panama (INCAP) was founded in 1949 by Nevin S. Scrimshaw and has conducted an extensive study on the Guatemalan dietary pattern and its nutritional value. Today Center for Studies of Sensory Impairment, Aging and Metabolism (CESSIAM) continues this tradition, focussing on Indigenous communities in the Western highlands of Guatemala (Solomons, 2000).

The Indigenous village of Santa Maria has changed little since the Spaniards encountered them in the 16th century (Mata, Simhon, Urrutia, & Kronmal, 1983). But currently, the exposure of the people to more diverse and inexpensive foods may modify their traditional diet; however, there is little recent documentation available on these changes and how the traditional plant-based diets have changed (Bermudez, Hernandez, Mazariegos, & Solomons, 2008). Bermudez et al. have compared the consumption of foods and beverages from the different food groups over six decades to document the disappearance of some foods and the emergence of new foods (Bermudez et al., 2008). She also compared the micro and macronutrient intakes over six decades with the nutrient intakes reported in INCAP studies (1950 to 1969), including Cross-Cultural Research on the Nutrition of Older Subjects Study, CRONOS Metro (1999 and 2000), and CRONOS Maya (2001 and 2002) (Bermudez et al., 2008). The CRONOS Metro and Maya studies together are called 'contemporary studies'. She compared the dietary surveys conducted in the INCAP studies, CRONOS Metro and Maya and classified them into "old foods" and "new foods" ; 110 "old foods" and 108 "new foods" for a total of 210 foods were used to describe the changes. Subjects from the INCAP studies consumed 108 (51%) of these 210 foods, and subjects from the CRONOS Metro and Maya studies consumed 138 (66%) and 162 (77%) of these foods,

respectively (Bermudez et al., 2008). She concludes that the Guatemalan people's food consumption and dietary patterns have changed by increased food variety at the expense of reduction in the consumption of nutrient dense foods and increased the consumption of processed foods from the middle of the 20th century to the beginning of the 21st century (Bermudez et al., 2008). Despite these historical studies to date, only a few studies examined the diet patterns of Guatemalan Indigenous communities during lactation, but none have examined its impact on infant growth.

Rationale for Thesis Hypotheses

Previous research has shown that maternal diet impacts infant growth outcomes (Chia et al., 2016). However, few studies have been conducted to examine this issue in pregnant and lactating women living in low-middle income countries, and none have been conducted among the Indigenous population of Guatemala. No recent studies have assessed the maternal intake of pregnant and lactating women living in Guatemala. There is a need to understand the dietary intake of the *Mam*-Mayan Indigenous pregnant and lactating women of Guatemala to prevent impaired infant growth outcomes. Guatemala has the sixth-highest rate of chronic undernutrition and the third-highest adolescent birthrate in Central America - 114 births for every 1000 women aged 15-19 years of age each year (Iniciativa Guatemala, 2017).

Previous research has also shown the association of maternal age (adolescents versus adults) and the growth of infants (Akseer et al., 2022), but none have examined this issue in Guatemala. Pregnancy in girls aged 10-19 years is defined as adolescent pregnancy (Chen et al., 2007; Ganchimeg et al., 2014). Declining age at menarche is one among some important factors that have strongly influenced the teenage pregnancy in the recent years (Chen et al., 2007). Approximately 11% of the births worldwide are to adolescents and about 90% of the adolescent pregnancies occur in low- and middle-income countries (Ganchimeg et al., 2014). Pregnancy at an early age of life, increases the likelihood of a high risk of pregnancy outcomes such as adverse growth and development of the offspring and will have a negative impact on the future well-being of the mother and infant (Ganchimeg et al., 2014) (Johnson & Moore, 2016). Low birth weight (LBW), preterm delivery, perinatal death, small for gestational age were some of the

adverse pregnancy outcomes reported in the previous studies (Ganchimeg et al., 2014; Johnson & Moore, 2016; Yu, Mason, Crum, Cappa, & Hotchkiss, 2016). The goal of this research was to investigate interrelationships among maternal diet and maternal age on infant growth and development in the first six months postpartum.

Hypotheses

1. *Mam*-Mayan pregnant and lactating women will have inadequate intakes of selected nutrients (based on RDD and DRI);
2. Infant growth will be associated with maternal macro and micronutrient intakes during lactation; and
3. The association between maternal nutrient intakes and infant growth outcomes will differ between adolescent and adult mothers.

Specific Objectives

1. To describe the nutrient intakes of the pregnant and lactating Indigenous *Mam*-Mayan women of Guatemala and compare them to RDDs and DRIs
2. To explore the associations between nutrient intakes and early infant growth outcomes; and
3. To assess the effect modification of maternal age (adolescent vs adults) on the relationship between maternal nutrient intakes and infant growth outcomes.

CHAPTER 2 – LITERATURE REVIEW

1. Characteristics of the Traditional Diet of Guatemala – Foods and Nutrient Composition

The traditional Mayan diet of Guatemala is composed of the 'triad' consisting of corn, beans, and squashes because these foods compliment each other nutritionally (Solomons, 2000), and traditional vegetables and fruits complementing it includes *busnay*, tomatoes, green leafy vegetables, potatoes, root crops, onions, carrots, cucumber, cabbage, and fruits including mango, apple, orange, and watermelon. Low amounts of animal products which include 'game meat' (meat from wild animals hunted for food, as well as mollusks and reptiles), fish and shellfish, chicken and turkey, milk, dairy products, and eggs (Bermudez et al., 2008; Orozco, Solomons, & Briend, 2006) are eaten.

1.1. Corn

Corn is central aspect of Mayan culture and diet. Mayan creation stories describe that man was created from the corn dough called 'masa' by the gods and that corn should be consumed as an essential nutrient for the body. It is reported that about 70% of the energy intake is obtained from the corn in a standard form of a tortilla; however, there are other forms of corn eaten less frequently (Solomons, 2000). The corn kernels may be dried and used as the starting ingredient for preparing the tortilla, which is flat and pancake-like. There is also a long and traditional process called nixtamalization involved in preparing the dough for the tortillas. Traditionally, the process was performed by stone-grinding and milling, but with the availability of electricity, electric-powered mills are used in most villages. The corn grains are soaked overnight in water with lime (CaO) added. This process makes the corn soft, with its outer skin loosened from the endosperm. Nixtamalization changes the nutrition profile of the corn, increasing calcium, zinc, and niacin content. (Solomons, 2000).

Tortillas are consumed at every meal by everyone in the family as it is their staple food. The whole day's supply of tortillas is prepared in the predawn hours and is eaten hot and fresh in the morning. They are reheated for their midday and evening meals. Among the white, yellow, and blue corn varieties that are grown, white corn is widely consumed. In some parts of the highlands, the corn dough was wrapped in leaves to produce a dense corn cake called *tomalitos*. There are

some other varieties prepared from this corn dough in traditional Guatemalan cuisine like corn tamales (corn dough filled with meat or fowl and then wrapped with a leaf), atol de elote (warm sweet corn porridge), atol de maiz or atol blanco (seasoned gruel consumed with beans) and elotes asados (charcoal-broiled corn on the cob). With the introduction of rice, Sometimes the consumption of corn tortillas was replaced by boiled white rice (Bermudez et al., 2008; Solomons, 2000).

Corn, consumed in the form of tortillas, contributes about 64% of the total caloric intake, about 75% of the protein intake and also acts as a significant source of calcium as the consumption of dairy products is low(Solomons, 2000). A survey conducted by the National Nutrition Survey of Guatemala showed that about 68% of the total calcium reported was from tortilla consumption. Only 16% of calcium is reported from the intake of dairy products. Soaking the corn grains overnight in lime water softens the corn and increases the nutritive value of the corn (Solomons, 2000). Evidence shows that the Guatemalan tortillas contain 200mg/100g of dry matter and contributes about 640 mg of calcium per day to the adult women's diet (Krause et al., 1992). One hundred grams of raw corn provides approximately 86 kcal of energy, 3.27g of protein, 1.35g of total fat, 18.7g carbohydrates, 2g of fiber, 2mg of calcium, 0.53mg of iron. But 100g of tortillas provides 218 kcal of energy, 5.7g of protein, 2.85g of total fat, 44.64g of carbohydrates, 6.3g of fiber, 81mg of calcium, 1.23mg of iron (USDA, 2017). The Guatemalan calcium intake, which is about 1100 mg, is considered the highest in the world. Vitamin E is also acquired from the corn oil and is normal to high. The same study also confirmed that the intakes of folic acid, thiamine, and vitamin B6 are adequate to maintain the sufficient status (Solomons, 2000). The tortillas are also an essential source of iron, copper, and zinc (Solomons, 1997). From the daily ration of about 580 g of tortillas, the women in the rural villages ingest about 8.3 mg of iron and 2 mg per 100 g of beans (Solomons, 2000).

1.2. Beans

Beans are one of the essential foods of the traditional Guatemalan diet. There are a variety of the common bean (*Phaseolus vulgaris*) that are used in Guatemala, including black (negros), red (Colorados), and white (Blancos). Some varieties like Broad beans (*Vicia faba*) and the lentils

(*Lens* spp.) are also grown, but they are minimally consumed (Solomons, 2000). The Black beans are preferred, soaked overnight for cleaning and softening, and boiled in earthen crocks. They are served in different forms like whole (*parados*), or mashed and refried (*volteados*), or combined with their broth as black bean soup (*sopa de frijol*)(Solomons, 2000).

100 grams of black beans provides 180 kcal of energy, 8.23 grams of protein, 7.01g of total fat, 22.04g of carbohydrates, 8.1g of fiber, 25mg of calcium, 1.96mg of iron (USDA, 2017). Legumes are a rich source of protein and fiber. A significant portion of the dietary energy is acquired from legumes (Solomons, 2000). 23% of the weight of the edible portion of the beans is a protein that approaches an egg's protein quality by the principle of complementation of essential amino acids if the ratio of protein and cereal is about 30 to 70 percent. Methionine poor legumes are complemented by the methionine-adequate cereal, corn, and the lysine-poor corn is complemented by the lysine-rich legumes (Solomons, 2000).

1.3. Squashes

The squash family includes *Cucurbita maxima*, *Cucurbita pepo*, and *Sechium edule*. The traditional orange pumpkins were called Calabaza. *Cucurbita maxima* is a green-skinned, pumpkin-like squash with two varieties. The pale green small variety tastes like zucchini, and these variety is ingested often compared to other varieties. *Perulero* and *Guisquil* are white and green-skinned of the same variety. The leaves are used as herbs in Guatemalan cuisine(Solomons, 2000). Squashes are a fair source of phosphorous, potassium, calcium, manganese, and a good source of vitamin C; 100 grams of squash consists of 19 kcal of energy, 1.01g of protein, 0.27g of total fat, 3.88g of carbohydrates, 1g of fiber, 21mg of calcium, 0.44mg of iron, 20mg of manganese, 32mg of phosphorous, 222mg of potassium, 2mg of sodium, 0.29mg of zinc, 0.092mg of copper, 19.3mg of vitamin C (USDA, 2017).

1.4. Fruits and Vegetables

Tomatoes and bananas are popular fruits. They are consumed in both raw and cooked forms. Fried plantain is another source of charred tissue among the Guatemalan diet (Solomons, 2000). Mango, apple, orange, and watermelon are also consumed (Enneman, Hernandez, Campos, Vossenaar, & Solomons, 2009). Green leafy vegetables, potatoes, onions, carrots, root crops,

cucumber, cabbage, and plantain are consumed by the people of Guatemala (Bermudez et al., 2008; Enneman et al., 2009; Fitzgerald et al., 1992). Squashes, tomatoes, potatoes, and bananas along with the herbs in stews and seasonal fresh foods provide a generous amount of Vitamin C. The herbs (leaves of squash plants, tomatoes) used in the cooking also contain this vitamin (Solomons, 2000).

1.5. Animal products

A variety of animal products including game meat, fish and shellfish, chicken and turkey, milk, dairy products, and eggs are commonly eaten (Bermudez et al., 2008; Orozco et al., 2006). A survey reported that, on average, less than 5% of the daily energy is reported from red meat (Valdes-Ramos, Cervantes, Mendoza-Perdomo, Anderson, & Solomons, 2006). A survey of children living in the Yepocapa area revealed that more than 30% of children had not reported a single source of red meat in two weeks. The consumption of red meat is so minimal that often in the questionnaire, the meat consumption was classified on a times-per-year rather than daily, weekly, or monthly basis (Solomons, 2000). Due to the lack of availability, milk and milk products are also notably under-consumed in Guatemala (Valdés-Ramos, Mendoza, & Solomons, 2001). When comparing meat consumption to milk products, cheese and milk were consumed more than the pork or fish by the pregnant women in Gujitos, Guatemala (Fitzgerald et al., 1992).

2. Nutrition transition

2.1. Historical overview

The nutrition transition results from the dynamics of urbanization and globalization (Bermudez et al., 2008). The nutrition transition is defined as “a situation in which the foods in the diet tend to shift from a large plant-based and low energy density fare to one with increasing sources of animal protein and foods with greater energy density from sugars and fats” (Bermudez et al., 2008). Guatemala is one of the countries undergoing rapid geographical and demographical changes. Currently, people's exposure to more diverse and inexpensive foods is modifying their traditional diet. However, little documentation is available on these changes in Guatemala. Details of what types of foods in their traditional plant-based diets were changing and/or being abandoned were last summarized in 2008 (Bermudez et al., 2008). Others have summarized

changes in patterns of consumption between urban and rural communities in 2011 (Soto-Méndez et al., 2011).

2.2. Differences between rural and urban communities

Differences exist in the pattern of consumption of traditional foods between rural and urban communities. The complete list of foods is listed in **Table 2.1**, and the relative percent contribution of the food's energy is shown in Fig 2.1. In the rural sample, (Soto-Méndez et al., 2011) reported that traditional foods such as tortillas and scrambled eggs were consumed over modern foods. Bottled water, instant coffee, vegetable oil, margarine, and tea were more dominant in the urban sample. The rural sample derived their energy mainly from tortillas, table sugar, sweet rolls, whereas the urban populations' energy mainly was derived from beef, boiled white rice and white rolls (Soto-Méndez et al., 2011). Sugar was one of the most consumed foods in the Guatemalan population. In the previous research, sugar intake was mentioned multiple times after the tortillas (Fitzgerald et al., 1992; Soto-Méndez et al., 2011). Tortillas were in the top fourth position for the urban sample, whereas they were the rural population's first and top energy contributors. Plant-based, non-fortified and processed foods had a more significant contribution than the animal-based, non-processed and fortified foods in the rural sample than the urban sample. The animal to plant-based foods ratio was 1:7 in the rural population and 1:4 in the urban population (Soto-Méndez et al., 2011).

Rural energy intake was higher than the urban samples energy intake. This might be due to the high consumption of plant-based foods in the rural population which included carbohydrate rich foods like corn, fruits, and vegetables. About six times higher energy was consumed by the rural population than the urban population from the traditional foods which are plant-based (Soto-Méndez et al., 2011). The tortillas consumed by the rural women were found to have significantly more concentrations of calcium, iron, and zinc than the tortillas consumed by urban women. Making the tortillas rather than the type of maize used was a more critical determinant of tortilla mineral content for calcium, iron, and zinc. Tortilla prepared using the hand mill, and mano y metate (grinding stones) had a significantly higher iron content. The use of mano y metate was also associated with increased zinc content (Krause et al., 1992). The food variety

and diet diversity of the people was different for the people living in rural areas and urban areas. In a study by Soto-Méndez, there was more variety in the foods consumed by urban women than rural women (Soto-Méndez et al., 2011).

2.3. Assessing the contemporary diet

In 2008 Bermudez et al compared the foods and nutrient intakes reported by the Institute of Nutrition of Central America and Panama including INCAP studies from 1950 to 1969 as well as the CRONOS Metro (1999 and 2000), and CRONOS Maya (2001 and 2002) studies (Bermudez et al., 2008). The CRONOS Metro and Maya studies together are called 'contemporary studies'. She compared the dietary surveys conducted in the INCAP studies, CRONOS Metro and Maya studies and classified them into "old foods" and "new foods" [110 "old" foods and 108 "new" foods. Thus, a total of 210 foods were used for comparative purposes (Bermudez et al., 2008).

Bermudez et al. compared the consumption of foods and beverages from the different food groups over six decades (1950- mid 20th century) in order to describe the disappearance of traditional foods and the emergence of new foods in the Guatemalan diet (Bermudez et al., 2008). She also compared the micro and macronutrient intakes over the six decades. Subjects from the INCAP studies consumed 108 of these 210 foods, and subjects from the CRONOS Metro studies consumed 138 and Maya studies consumed 162 of these foods. The old and new foods list is given in **Table 2.2** (Bermudez et al., 2008).

Meat from the wild animals hunted for food was called "Game meat." Interestingly, the food-group game meat, which was present in the old foods, completely disappeared from the new foods. The consumption of fish and shellfish products decreased drastically in the new foods. Native leafy vegetables like busnay were also absent in the new foods, and new vegetables like broccoli, processed tomatoes like ketchup emerged in the modern dietary patterns of Guatemalans. Vegetables were most significant in the food group with the largest number of products (46 products) followed by fruits (26 foods). The corn products and rice puddings consumption was continued and maintained. By 2008, several game meats had disappeared, including turkey though poultry chicken was reported in the new foods (Bermudez et al., 2008).

Some foods have been added to the new foods list over six decades (1950- mid 20th century) in Guatemala. New products that have been added to the food group fats & oils included from various sources. Based on the limited studies that have been conducted, some new food groups emerged like snacks and soups, new products have been added to the group vegetables like broccoli and processed tomatoes to beans, potatoes, root crops, and plantain. The intake of processed foods also increased. There are now many ready-to-eat breakfast cereals, including other cereals such as pasta and Chinese noodles. Importantly, in 2008, there had already been a significant increase in the number of new beverage products added to the list including sodas and sweet drinks (Bermudez et al., 2008).

There have also been changes in the macro and micronutrient intakes of the people over the years. The previous research showed that the daily energy intake had increased from 2,000 kcal in INCAP studies (1950 -1960) to 2,790 kcal in the CRONOS Metro study (1999 -2000) to 3,200 kcal in the CRONOS Maya (2001-2002) study. However, the contribution of carbohydrates has decreased from 79% in the INCAP studies to 61% in CRONOS Maya and 67% in CRONOS Metro study from. At the same time, fat intake has increased from 9% from the old foods to 27% from the contemporary foods. Protein intake was similar among the old and new foods. The intake of calcium has been gradually increased from 1.1 g/day in the old foods to 1.6 g/day in the contemporary foods. The intake of vitamin C has increased about four-fold from the old to new foods intake. Subjects in the CRONOS Maya study (2001-2002) consumed higher amounts of iron, thiamine, riboflavin, and niacin than those of the INCAP studies(1950-1960). Interestingly, the new foods have contributed to increased intakes iron, thiamin, riboflavin and niacin when compared to old foods. Despite these changes in the intakes of the macro and micronutrients, the people of Guatemala are still dealing with some inadequacies (Bermudez et al., 2008; Solomons, 2000; Valdes-Ramos et al., 2006) as described below.

3. Evidence of adequacy of Guatemalan diet

3.1. General population

The evidence of adequacy of Guatemalan diet in general population is described in **Table 2.3**. Tortillas, beans, and squashes contribute to the Guatemalan diet (Wurtman & Fernstrom, 1979)

(Solomons, 2000), but since the diet is mostly plant-based, the carbohydrate content is high, contributing to 66% of the energy (Bermudez et al., 2008; Gregory, McCullough, Ramirez-Zea, & Stein, 2009). The mean tortilla intake per day for women is 579g which contributes about 245g of carbohydrates to the diet (Krause et al., 1992; USDA, 2017). Because the Guatemalan diet is highly plant-based, it is relatively low in fat content. Moreover, the Guatemalans underconsume both milk and milk products, and animal food sources like red meat which reduces their intakes of fat from these sources (Solomons, 2000). However, with the changes in the diet and addition of more animal foods, the fat content of the diet has increased from 9% reported in the earlier studies, the overall contribution of fat to energy had risen 27% in the CRONOS Maya group (Bermudez et al., 2008; Valdés-Ramos et al., 2001). The Guatemalan diet is also composed of high amounts of legumes or beans which provide about 33g of protein or or 13% of calories to the diet (Krause et al., 1992) (Wurtman & Fernstrom, 1979). Despite the low consumption of the animal products, the traditional diet with considerable amounts of beans is providing moderate protein for the population (Solomons, 2000). The protein content of the Guatemalan diet is unchanged over years and is still providing about 12 to 13% of the total calories (Bermudez et al., 2008).

The Guatemalan diet is associated with both adequate and inadequate intakes of minerals and trace elements. Approximately 300mg of sodium is ingested only through tortillas which is very low resulting in the sodium deficiency in the population (Solomons, 2000); approximately, 100g of tortillas provide 45mg of sodium (USDA, 2017). Despite of the low intakes of milk and milk products, calcium intakes are considered among highest in the world with about 1000mg to 1600mg from lime treated corn tortillas (Bermudez et al., 2008; Solomons, 2000). Rural women ingest about 8.3 mg of iron from the daily ration of 580g of tortillas (Solomons, 2000). Though the iron is high among the population, the tannins in coffee, the phytic acid in corn and beans and the dietary fiber in coarse grains and legumes represent a “cocktail” of inhibitors of iron availability (Solomons, 2000). The groups vulnerable for iron deficiency and anaemia are women of childbearing age and the preschoolers. A previous study conducted on the Guajitos pregnant women revealed that, 20% of them had anemia (Solomons, 1997). The daily ration of tortillas consumption provides about 10.7 mg of zinc, but due to the adverse reactions of the inhibitors

and the absence of zinc in the flesh tissue leads to the poor net absorption of this metal (Solomons, 2000). The first systematic survey in the 1950's had revealed a goiter rate of 38% in Guatemala due to the iodine deficiency. The fortification has been made mandatory to reduce the iodine deficiency of the people (Solomons, 1997).

3.2. Pregnant & lactating women

Literature reviews on the evidence of adequacy of pregnant and lactating women are shown in **Table 2.4**. Adequate nutrition during pregnancy is essential for maternal and child health. Pregnant women are more susceptible to inadequate nutritional status because of the high nutrient demands of the growing fetus (Lee, Talegawkar, Merialdi, & Caulfield, 2013). Poor nutrition of pregnant women can result in multiple long-term adverse effects on the mother and the offspring's health (Lander et al., 2019). Previous research shows that malnutrition in pregnant women is prevalent among the low- and middle-income countries (LMIC) due to various reasons like poor diet quality, high intensity of agricultural labor, socio-economic constraints, and high parity (Lee et al., 2013). Guatemala has the sixth-highest rate of chronic malnutrition in the world (USAID, 2018). Women of reproductive age living in these countries are at high risk of inadequate nutrition, resulting in increased nutritional demands of pregnancy and fetal growth (Lander et al., 2019).

Previous literature shows that pregnant women in low- and middle-income countries suffer from deficiencies due to relatively insufficient dietary intakes (Lander et al., 2019). Lee et al., in their review article, have reviewed the average maternal intake of the macro-nutrients and micro-nutrients by the women living in low- and middle-income countries. The median energy intake was 2055 kcal/day; the protein was 63 g/day, 54 g/day for fat and 323 g/day for carbohydrates (Lee et al., 2013). Several studies have contrasted the intake of macronutrients among the low- and middle-income countries and found that the pregnant women in the Caribbean and Central/South America (LAC) consumed higher amounts of energy, protein, fat, and carbohydrates (Lander et al., 2019) (Lee et al., 2013). Research shows that 42% of pregnant women worldwide and more than 50% of pregnant women in low- and middle-income countries are anemic, mainly due to iron deficiency. Dietary deficiencies in other essential vitamins and minerals, including vitamin A, folate, Ca, and zinc, are also high in low- and middle-income

countries which may contribute to iron deficiency (anemia).. Based on the previous research, the pregnant women in the Caribbean and Central/South America (LAC) consumed higher concentrations of vitamins A, C, and riboflavin when compared with the pregnant women living in Asia and Africa (Lee et al., 2013). Folate intakes are below the recommended values in LAC when compared to Africa. Pregnant women in the Caribbean and Central/South America (LAC), especially in Guatemala, are the only ones to meet the calcium requirements compared to other pregnant women living in other low- and middle-income countries (Lee et al., 2013).

To our knowledge, few have studied the dietary intakes of lactating women in the low-middle-income countries (Michaelsen et al., 2011; Moser et al., 1988) with only one study based in Guatemala in 1980 (Schutz, Lechtig, & Bradfield, 1980). Michaelsen et al., in their review article assessed the intakes of polyunsaturated fatty acids in LMIC's, whereas Moser et al., in their study assessed the dietary intakes of copper, iron, zinc, and selenium of Nepalese lactating mothers. Study by Schutz et al., in Guatemala aimed to estimate the total energy intakes and expenditures of the lactating women and compare the results with the nonpregnant and nonlactating women living in the same villages. The study was conducted in three Ladino villages in the rural highlands of Eastern Guatemala. The criteria for selecting the lactating women were a current lactation of more than six months, an age range of 18 to 38 years, living within 1 km of the field unit and willingness to participate in the study. Eighteen women were included in the lactating group, and six nonpregnant, nonlactating women were selected as a control group. The 24-hour dietary recall was used to study the food intake and the energy expenditure of the lactating mothers living in Guatemala (Schutz et al., 1980). The energy expenditure was measured by monitoring heart rate throughout the day. Then the heart rate was related to oxygen consumption using individually determined regression lines between heart rate and oxygen consumption (Schutz et al., 1980). There is a large gap in the scientific literature providing recent evidence to describe the impact of maternal diet during lactation.

4. Impact of healthy maternal diet on pregnancy outcomes in LMIC's

The associations of maternal diet with the pregnancy outcomes have been described in **Table 2.5**. Nutrition plays a vital role in maternal and child health (Abu-Saad & Fraser, 2010). The

current understanding of biological processes indicates that women's nutritional status before and during early pregnancy may be necessary to determine early developmental processes and ensure successful pregnancy outcomes (Ramakrishnan, Grant, Goldenberg, Zongrone, & Martorell, 2012). Maternal nutrition during pregnancy is a significant determinant for birth outcomes and the offspring's health outcomes in later life (Chia et al., 2019). Poor maternal nutritional status is often associated with adverse infant outcomes (Abu-Saad & Fraser, 2010). The availability and the supply of the nutrients to the developing fetus depend entirely on the maternal nutritional status, which is dependent on the maternal nutritional stores and dietary intakes (Ramakrishnan et al., 2012). Understanding the association between maternal nutrition and birth outcomes can help develop a nutritional intervention that will improve the birth outcomes and long-term quality of life of the infants.

Inadequate maternal nutrition during pregnancy is often associated with adverse infant outcomes like low birth size (LBW; birth weight <2500g), macrosomia (birthweight >4000g), large-for-gestational-age (LGA; birth weight >90th percentile for gestational age), small-for-gestational-age (SGA; birth weight <10th percentile for gestational age), infants with small head circumference, and infants with small length-for-age (LAZ) (Chia et al., 2019) (Colon-Ramos et al., 2015). Globally, 11% of the births are preterm, 15-20% are low in birth weight, and the large-for-gestational-age has been increased by 15-25% in the last few decades (Chia et al., 2019). In addition to the short-term consequences of the low birth weight, such as high infant mortality rates and childhood growth failures among the survivors, low birth weight infants also carry a long-term risk in the form of adult coronary heart disease and type 2 diabetes (Rao et al., 2001). There is reduced birth weight and length in infants born to the mothers whose maternal intake was inadequate especially during the second and third trimester (Papathakis, Singh, & Manary, 2016). Beginning at conception, the first 1000 days of life are considered vital for the prevention of childhood stunting since growth failure begins in the utero and continues until about two years of age (Papathakis et al., 2016).

Poor linear growth or stunting of infants, results primarily from the inadequate nutrient availability of the mother (Papathakis et al., 2016). World Health Organization in a review of trends of stunting among the pre-school children worldwide estimated that there were 171

million children with stunting in 2010, with 97.6% of them living in developing countries (Ramakrishnan et al., 2012). Although stunting percentage globally has been reduced to 26.7% in 2010 from 39.7% in 1996, stunting in Africa has remained unchanged at 40% while it is decreasing in Asia and Latin America (Ramakrishnan et al., 2012).

Few studies have examined the association between dietary pregnancy patterns and birth outcomes which can influence the infant's growth outcomes (Abu-Saad & Fraser, 2010; Chia et al., 2019; Colon-Ramos et al., 2015). Chia et al., in their review article concluded that healthy dietary patterns which is characterized by high intakes of fruits, vegetables, low-fat dairy, lean protein, and wholegrains were significantly associated with reduced risk of preterm birth (PTB) (Chia et al., 2019), whereas unhealthy diet, characterized by high intakes of processed meat, refined grains, and foods rich in sugars or fat were significantly associated with lower birth weight and had a higher risk of PTB (Chia et al., 2019). Colon-Ramos et al., in their article reported that consumption of processed (processed meat, fast food items, snacks, sweets, and soft drinks) and processed-Southern dietary patterns (cooked cereals, peaches, corn, fried fish, beans, greens, pig's feet, tongue, pork, fast food items, snacks, sweets, and soft drinks) significantly decreased infant WAZ and HCAZ but increased infant LAZ (Colon-Ramos et al., 2015). The impact of maternal diets on pregnancy outcomes are described in **Table 2.5**.

Improved maternal nutrition has been associated with increased fetal growth and a reduction in adverse birth outcomes in developing countries and in populations with nutrient deficiencies, but not in well-nourished population (Abu-Saad & Fraser, 2010). Social economic status (SES) factors are an important predictor for nutrient intake and diet quality. Among rural Indian women, intake of dairy products was strongly associated with SES and was also associated with birth size. Women consuming more than 6 glasses of milk daily had a 49% lower risk of SGA and a 108-g increase in birth weight compared with those consuming no milk. There was also increased risk of large-for-gestational-age birth. Higher dairy-product intake in early pregnancy (6–18 weeks) was positively associated with birth weight. Proportion of energy derived from protein in early pregnancy was positively associated with birth weight (1-g increase was associated with 16-g increase in birth weight); among “reliable dietary reporters”, isoenergetic 1% increase in dairy protein was associated with a 25-g increase in birth weight; no detrimental

effects of high protein intake on birth weight (Abu-Saad & Fraser, 2010). Fatty fish intake of >60 g/day was associated with higher SGA birth risk in comparison with <5 g/day intake of fatty fish. Fatty fish intake may be associated with reduced fetal growth due to exposure to persistent organic pollutants. Intake of fish 2 times per week was associated with lower PTB (pre-term birth) risk in comparison with <2 times per week. Folate intake <500 µg/day was associated with increased risk of PTB in multivariate analysis (Abu-Saad & Fraser, 2010).

An observational study was conducted by Chia et al., to investigate the association of the dietary patterns during pregnancy and risk of preterm birth and larger birth size in a multiethnic Asian cohort (Chia et al., 2016). Three distinct maternal dietary patterns were identified: (1) the vegetable, fruit, and white rice (VFR) pattern which was characterized by higher intakes (high positive factor loadings) of vegetables, fruits, plain white rice, whole-grain bread, fish, and nuts and seeds and lower intakes (negative factor loadings) of fried potatoes, burgers, carbonated and sweetened drinks, and flavored rice; (2) the seafood and noodle (SfN) pattern which was characterized by higher intake of soup, seafood, fish and seafood products, noodles (flavored and in soup), and low-fat red meat and lower intakes of legumes, ethnic bread, white rice, and curry-based gravies and the pasta, cheese, and (3) a processed meat (PCP) pattern which was characterized by high intakes of pasta, tomato, and cream-based gravies, cheese, and processed meat (Chia et al., 2016). Greater adherence to the VFR diet pattern was associated with a lower risk of preterm birth. No associations were seen for SfN and PCP patterns in relation to the risk of preterm birth (Chia et al., 2016). In a multivariable model, there were trends towards associations between higher VFR pattern score and higher birth weight. A higher VFR pattern score was associated with a higher ponderal index and an increased risk of having an LGA infant. The VFR pattern score was not associated with birth length or SGA births. There were trends towards associations between a higher SfN pattern score and higher ponderal index but not with birth weight or length (Chia et al., 2016). The PCP pattern score was also not associated with any outcomes related to birth size. In a secondary analysis, the higher VFR pattern score was associated with a larger head circumference and estimated percentage body fat at birth. There were trends toward associations between a higher VFR pattern score and higher abdominal

circumference and a higher sum of triceps and subscapular skinfold thickness. No associations were observed for the SfN and PCP patterns (Chia et al., 2016).

Adequate maternal diet quality during pregnancy, characterised by a high intake of vegetables, fruits, whole grains, fish, and dairy products, may have a beneficial effect on lowering the risk of preterm birth of the infants. Based on the evidence from the previous studies, maternal dietary patterns during pregnancy characterised by a high intake of vegetables, fruits, legumes, fish, and milk products may reduce the risk of having SGA infants.

5. Differential impact of maternal age on birth outcomes in LMIC's

Pregnancy in girls aged 10-19 years is defined as an adolescent pregnancy (WHO, 2011). Today, living cohort of adolescents is the largest in history with 1.2 billion girls and boys aged 10-19 years of which the majority live in low-income or middle-income country (LMIC) (UNICEF, 2019). About 11% of births worldwide are to adolescents and 90% of these births occur in LMIC's (WHO, 2011). Pregnancies in adolescence account for 23% of the disease burden that arises from the pregnancy and childbirth (Johnson & Moore, 2016). Adolescence is a critical period which marks the phenomenal changes that includes rapid psychosocial, physical, sexual, and cognitive maturation, and higher need of nutrients than any other stage of lifecycle (Akseer et al., 2022). However, there is not enough literature to clarify whether pregnancy during adolescence limits the maternal growth or with adequate nutrition the adolescent mothers will continue to grow on a normal trajectory (Rah et al., 2008; Scholl & Hediger, 1993). Some studies have suggested a nutrition partitioning that favours fetus when there is a competition of nutrients between the mother and the fetus (i.e., if mother has inadequate nutrient intakes and stores) (Rah et al., 2008).

The differential impact of maternal age on pregnancy outcomes in LMIC's is described in **Table 2.6**. Rapid growth and development occur during adolescence, approximately 15% of final adult height and 50% of adult body weight is attained during adolescence (Spear, 2002). Previous studies revealed that a large proportion of adolescents enter pregnancy with a poor nutritional status and have inadequate dietary intakes during pregnancy and lactation (Chaturvedi et al., 1996; Pathak, Singh, Kapil, & Raghuvanshi, 2003). In several undernourished adolescent pregnant

women, depletion of maternal nutrients and impaired growth of fetus occur simultaneously due to the competition of nutrients between the growing adolescent mother and the fetus (King, 2003). Rah et al., found that pregnant adolescents had lower weight, BMI, mid-upper-arm-circumference (MUAC), upper-arm-muscle are and percent body fat at 6 months postpartum compared to never-pregnant adolescents (Rah et al., 2008). According to the Rah et al., findings, pregnancy and lactation during adolescence has appeared to halt linear growth and resulted in weight loss, depletion of fat and lean body mass at the end of a year follow-up (Rah et al., 2008). Given the high number of adolescent pregnancies in the LMIC's, it is important to understand the relationship between maternal age, pregnancy, and birth outcomes to prevent the adverse outcomes and also for policy recommendations (Akseer et al., 2022).

Pregnancy outcomes during adolescence are described in **Table 2.6**. Most studies have investigated the relationship between adolescent pregnancies and birth outcomes in developing countries, having observed associations with higher risks of preterm birth (PTB), perinatal and maternal death, preterm delivery (PTB) (Ganchimeg et al., 2013). Chen et al., in their retrospective cohort study observed that the rates of pre-term delivery, very LBW, LBW, small-for-gestational age (SGA), neonatal mortality were higher among the adolescents. They also observed that these birth outcomes consistently increased with decreasing age (Chen et al., 2007). Findings by Ganchimeg et al., also showed that there was higher risks of low birth weight and preterm birth among adolescents (Ganchimeg et al., 2013). Akseer et al., from his research observed that adolescent mothers had 23% increased risk of PTB, 60-63% increased risk of infant mortality, 28% increased risk of LBW when compared to infants of mothers aged 20-24 years (Akseer et al., 2022).

As there are no studies that have studied the impact of maternal age on the infant growth outcomes up to 6 months of age in developing countries and especially in Guatemala, we chose to address this knowledge gap by investigating the role of maternal age on infant growth during the first six months of lactation, given the high rates of infant growth faltering in early lactation in Guatemala. Therefore, we focused our investigation on identifying if maternal age was an effect modifier of the relationship between the maternal diet of lactating mothers and infant

growth in early lactation. This study will be the first study to look at the growth faltering among infants of Guatemala in early lactation.

Figure 2.1- Relative energy contribution from the foods based on residence (urban or rural)

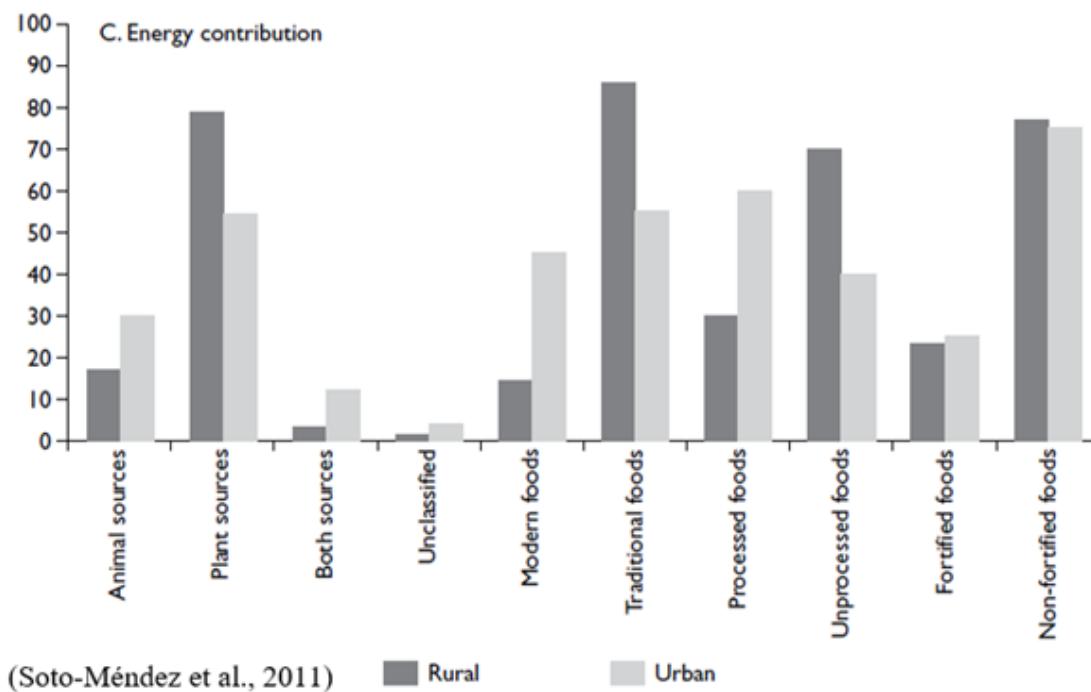


Table 2.1- The top foods by mentions, weight, and energy by the rural and urban population

Mentions		Energy		Weight	
Rural sample (no. of people =20)	Urban sample (no. of people=20)	Rural sample (no. of people =20)	Urban sample (no. of people =20)	Rural sample (no. of people =20)	Urban sample (no. of people =20)
Tap water	Bottled water	Tortillas	Beef	Tap water	Beef
Table sugar	Table sugar	Table sugar	Boiled white rice	Tortilla	Boiled white rice
Tortilla	Instant coffee	Sweet rolls	White rolls	Boiled black beans	Fiambre (traditional dish with meat, cheese, and vegetables)
Instant coffee	White rolls	Beef meat	Tortilla	Boiled white rice	Artificially flavoured fruit beverage
Ground coffee	Tortilla	Boiled black beans	Scrambled eggs	Table sugar	Coca cola
Sweet rolls	Scrambled eggs	Boiled white rice	Incaparina with milk and sugar	Beef stew	Natural fruit-based drink
Vegetable oil	Boiled white rice	Scrambled eggs	Table sugar	Scrambled eggs	Whole milk
Scrambled eggs	Vegetable oil	Vegetable oil	Pork	Sweet rolls	Tortillas
Boiled black beans	Margarine	Mashed black beans	Boiled chicken	Mashed black beans	Scrambled eggs
Boiled white rice	Tea	Pork sausage	Chicken breas	Chicken broth (with vegetables)	White rolls

(Soto-Méndez et al., 2011)

Table 2.2- List of old and new foods consumed by the people of Guatemala

List of “Old foods” consumed by the people of Guatemala				List of “New foods” consumed by the people of Guatemala			
Food group	INCAP studies (1950–60)	CRONOS Metro study (1999–2000)	CRONOS Maya study (2001–02)	Food group	Total	CRONOS Metro study (1999–2000)	CRONOS Maya study (2001–02)
Total	108	78	82	Total	102	60	80
Corn, corn tortilla, and corn atole	3	3	3	Corn, corn tortilla, and corn atole	4	2	2
Corn tamales	2	1	2	Corn tamales	6	2	6
Beans	3	3	4	Rice	4	1	4
Rice	1	1	1	Bread	4	2	4
Bread	3	3	2	Breakfast cereals	4	3	3
Breakfast cereals	1	0	1	Other cereals (e.g., pasta)	4	4	4
Other cereals (e.g., pasta)	2	1	1	Milk, dairy products, and egg	4	4	4
Milk, dairy products, and egg	8	6	7	Meat, beef, and pork	4	3	3
Meat, beef, and pork	11	9	9	Chicken and turkey	3	2	1
Game meat ^a	3	0	0	Fish and shellfish	1	1	1
Chicken and turkey	2	1	1	Green and yellow vegetables	2	2	2
Fish and shellfish	5	1	3	Other vegetables	5	2	5
Green leafy vegetables	11	5	6	Fruit ^b	13	8	11
Green and yellow vegetables	4	4	3	Fats and oils	7	6	7
Other vegetables	17	13	13	Sugar	2	1	1
Potatoes, root crops, and plantain	7	4	6	Beverages ^c	10	9	9
Fruit ^b	13	10	9	Snacks ^d	3	2	3
Fats and oils	2	2	2	Desserts ^e	6	6	5
				Soups	4	3	4
				Other mixed dishes with meat	3	2	1

(Bermudez et al., 2008)

Table 2.3- Evidence of adequacy of Guatemalan diet

Author	Study Design, Population Characteristics & Location	Dietary Methods, Chosen nutrients /Foods	Aim & Main Findings
Wurtman & Fernstrom Early Hum Dev (1979)	Comparative study Finca in Guatemala (experimental) Americal women (Control) n= 39 American = 20 Guatemalan-19	24- hour dietary recall -Protein (amino acids) -Fat -CHO	Aim: To compare the Guatemalan diet to that of American diet. Main-findings: Guatemalans sample diet has 13% of the daily calories from protein which is significantly less protein than the American sample. Tortillas, beans, rice, contributed to the Guatemalan sample whereas American diet was composed of meat, eggs, cheese, fish, and poultry. The fat content of the Guatemalan diet was extremely low with 6% calories. The Guatemalan diet has low levels of tryptophan and lysine. The protein content of the milk samples of the Guatemalan women is significantly lower than the American women. The lipid content with the sub-groups was significantly lower than the American mothers. Except for glycine, phenylalanine, and arginine all the free amino acids are significantly lower in the Guatemalan milk samples.
Krause et al Ecol Food Nutr. (1992)	Cross sectional study Guatemala n=60 Divided into Rural, semi-urban and Urban categories.	Intake of Tortillas the previous day of the data collection -Energy -Protein -Calcium -Iron -Zinc -Copper	Aim: To measure the contents of calcium. Iron, zinc, and copper in tortillas and assessed the daily intake of these minerals by the Guatemalan women Main-findings: The mean mineral content of 100g of tortillas from the white maize was 108 mg of calcium, 1.5 mg of iron, 1.8 mg of zinc and 0.2 mg of copper The mean tortilla intake per day was 579 g which contributed 1,160 kcal, 33 g of protein, 643 mg calcium, 8.3 mg iron, 10.7 mg zinc and 0.89 mg copper. The contribution of calcium, iron and zinc was statistically significant in rural sample than the urban sample Tortilla prepared with combined use of the hand mill and mano y metate (grinding stones) had a significantly higher iron content Dietary fiber, phytic acid and other competing minerals including calcium, have been implicated as anti-bioavailability factors affecting the absorption of zinc.

Author	Study Design, Population Characteristics & Location	Dietary Methods, Chosen nutrients /Foods	Aim & Main Findings
Solomons NW Arch Latinoam Nutr (1997)	Review Guatemala	--Vitamin A -Zinc -Copper -Riboflavin -iron -calcium -iodine	Aim: This is a review study on the globalization and the micronutrient content of the Guatemalan population in general. Main findings: Iodine deficiency disorders have been common in Guatemala and a systematic survey has revealed a goiter rate of 38%. 100 grams of tortillas contains, 108 mg of calcium, 1.5 mg of iron, 1.8 mg of zinc, and 0.2 mg of copper. Consumption of zinc by the Guajitos pregnant women were 11.3 mg. The mean daily intake of zinc in the school children of 6-7 years of age was 10.1 mg/day for boys and 8.4mg/day for girls. Riboflavin deficiency is prevalent in Guatemala due to the low consumption of milk products.
Solomons N W Asia Pac J Clin Nutr (2000)	Review Guatemala	24-hour recalls Food frequency questionnaire Traditional Guatemalan diet Energy -Protein -Fat -Calcium -Iron -Vit A - Vit C - Vit E - Vit B6 -Vit B2 - Vit B12 - Zinc - Folic acid -Thiamine -Sodium	Aim: To describe the characteristics of Guatemalan traditional diet Main findings: Maize (as tortillas), beans, and squashes are staple diet. Calcium intake is highest with 1100mg per day from tortillas. Vit E levels are normal to high Vit A is low as the diet is poor in animal protein sources and bioconversion of carotenoids is low. Riboflavin and vit B12 levels is low due to the low consumption of the dairy products Intake of Iron (8.3 mg) and zinc (10.7 mg) from the daily tortilla's consumption is not low but the bioavailability of the metals is inhibited. Vit C, thiamine, folic acid and vit B6 are adequate Total fat intake is low. (total energy from fat is < 20%) Sodium intake is low. Fiber intake is abundant with 27 grams per day. Legumes serve as a good source of protein. Coffee is the leading export crop and is highly consumed beverage along with cane sugar which is second leading export cultivation.
Valdes-Ramos et al., Nutr Res (2001)	Cross sectional study Guatemala n=269 Four communities from Rural province of Santa Rosa	Food frequency questionnaires (Willett FFQ) 24- hour recalls -Fruits -Vegetables -Grains -Milk & milk products	Aim: To investigate the Concordance of Guatemalans diets and eating practices with the recommendations from the dietary guidelines for Americans Main findings: The average number of servings consumed from grains and cereals, vegetables and fruits on average is within the limits recommended by the Guidelines in all four communities.

Author	Study Design, Population Characteristics & Location	Dietary Methods, Chosen nutrients /Foods	Aim & Main Findings
		-Meats and beans -Sugar -Fat -Alcohol	The average number of portions consumed from milk and milk products is relatively low in the whole population due to the lack of availability of these products in the area. The population consumes a large portion of beans. The population consumed 24.5 ± 11.6 portions of the grains, vegetables, and fruits food group contributing on average with $58.8 \pm 9.5\%$ of their daily energy. This varied from a high of $63 \pm 8.1\%$ in Potrerios, the most remote and underdeveloped hamlet community to a low of 56.8% in El Naranjo, another hamlet. The population consumes a very poorly varied diet. Most of the Santa Rosan diets recorded a fat contribution to energy of 30%. The sugar consumption in the population is 2.4 ± 1.9 portions per day, equivalent to $6.1 \pm 4.8\%$ of energy which ranged from $4.2 \pm 3.1\%$ of energy in Potrerios to $7.4 \pm 5.4\%$ in Santa Cruz.
Valdes-Ramos et al Asia Pac J Clin Nutr (2006)	Cross- sectional study Santa Rosa Province Republic of Guatemala (Eastern highlands) n= 256 (55 men; 214 women) 16- 86 years	Food frequency questionnaires (Willett FFQ) 24- hour recalls -Fruits & Vegetables - Starchy or protein rich foods -Refined sugar -Alcohol -Red meat - Dietary fat -Ca, phosphorous, iron, thiamine, vit B2, niacine, vit A & C	Aim: Concordance of Guatemalans diets and eating practices with the World Cancer Research Fund recommendations Main findings: 14.3% individuals for calcium, 13.2% for riboflavin, 10.5% for niacin (in men), 24.1% for iron (in women) had low intakes cumulatively ($>10\%$ of individuals). 75% of the daily energy was composed of grains, legumes, vegetables & fruits. 25% of the daily energy is derived from fats and oils, sugar, animal products. Red meat consumption is very low in rural Guatemala with average of $<5\%$ of energy from meat ($2.5 \pm 2.4\%$ of energy from red meat food group.). Average consumption of fat to the dietary energy was $24.5 \pm 14.9\%$ with 10% of population consuming less than 15% of energy from fats. 3.8 ± 4.2 percentage of energy is consumed from fats and oils food group. Percentage of energy consumed from fruits & vegetables is 15.8 ± 12.3. Intake of sugar is low with 6.1 ± 4.8 percentage of energy.
Bermudez et al Food Nutr Bull. (2008)	Comparative study Guatemala Data from INCAP publications	24- hour dietary recalls and Food Frequency Questionnaires	Aim: To compare the consumption of foods, beverages, intakes of macro and micronutrients over six decades Main findings: The estimated energy intake has increased from 2,000 kcal in INCAP studies to 2,790 kcal in CRONOS

Author	Study Design, Population Characteristics & Location	Dietary Methods, Chosen nutrients /Foods	Aim & Main Findings
	(old foods), CRONOS Studies (CRONOS Metro and Maya) (contemporary or new foods) were compared	Old to New foods transition -Energy -CHO -Fat -Protein -Calcium -Iron -Thiamine -Riboflavin -Niacin -Vitamin C	Metro study to 3,200 kcal in the CRONOS Maya study. The contribution of CHO was decreased from 79% in the earlier studies to 61% in CRONOS Maya and 67% in CRONOS Metro study. The contribution of fat was only 9% in the old study but has increased more than two folds in the recent studies with highest value (27%) in the CRONOS Maya group. Protein consumption was similar in all the three studies at 12% to 13% of the total energy intake. Calcium intake was highest in the INCAP studies. (1.6g/day) Calcium intakes were 145% in the Metro study and 118% in the Maya study of the INCAP study levels. Vit C intake was increased by four folds from INCAP studies to both the CRONOS studies. Vit C consumption among subjects in both CRONOS studies represented more than 400% of the consumption by participants in the INCAP studies. Subjects in the CRONOS Maya study consumed higher amounts of iron, thiamine, riboflavin, and niacin than those of the INCAP studies. Niacin was ingested relatively 200%, thiamin by 180% higher than the INCAP participants. Iron intakes in the CRONOS studies had the lowest relative consumption of any micronutrient. (101% in Metro and 125% in the Maya studies)
Gregory et al. Br J Nutr. (2009)	Cross sectional study Guatemala n = 1220 (469 men and 751 women) Mean Age = 32.7 ± 5.8 year	FFQ 24-hour recalls Food variety score (FVS) Recommended food score (RFS) Not recommended food score (NRFS) and Dietary quality index- international (DQI-I) was assessed. -Traditional foods -Western foods.	Aim: To investigate the FVS, RFS, NRFS and DQI-I of the Guatemalan menu associated with the cardio-metabolic risk. Main findings: CHO contributed 66% of the energy, fat contributed 23% with saturated fat as the primary contributor. Tortillas provided one-third of total energy, followed by fruits and vegetables, sweets, and artificial powdered drinks/sodas. Western foods including hamburgers, hot dogs, pizza, and chips, made up only 2% of the energy intake. Alcohol consumption was very low, particularly among women and accounted for <1% of the energy intake. The NRFS scores were strongly correlated with both the FVS and RFS, but not with the DQI-I. All the 4 scores were correlated with total energy intake ($r = 0.23 - 0.49$; $P < 0.01$). The DQI-I was positively associated with BMI.

Author	Study Design, Population Characteristics & Location	Dietary Methods, Chosen nutrients /Foods	Aim & Main Findings
			Living in the rural environment was associated with higher NRFS and DQI-I scores in both the genders but with lower RFS among women. DQI-I was positively associated with waist circumference. The diet in this sample was dominated by the traditional foods (tortillas and beans). A large portion of energy consumption was from sweets and sugar-sweetened beverages.
Soto-Mendez et al Salud Publica Mex (2011)	Comparative study Republic of Guatemala n= 40 (20 Urban & 20 Rural) 19– 56 years	24- hr dietary Recall for 2 days Dietary variety and dietary diversity scores - Food-groups - Top foods consumed in rural and urban samples	Aim: Comparisons of the diet variety, diversity patterns and dietary characteristics of the Guatemalan women Main findings: Diet variety of the population: The Urban women had significantly more variety of items than the rural women. ($p=0.002$) Maize tortillas, scrambled eggs and boiled white rice was commonly consumed across the two groups. Most of the energy for the rural sample was derived from tortillas and table sugar, whereas urban sample's energy was mostly from beef and boiled white rice with tortillas in the 4th place. Plant based and non-fortified food items contribution is higher than the animal-based, non-processed and fortified foods in both rural and urban population. Animal- to plant-based foods was 1:7 ratio in the rural sample and 1:4 in the urban sample Dietary diversity score: Significant results between the urban and rural were found using the Guatemalan “cooking pot” food group system which includes grains, cereals & potatoes; Herbs & vegetables; Fruits; Meat; Dairy products; Sugars & Fats for the day 2 intake. On individual basis there is no statistically significant difference ($p=0.555$) between the edible items consumed by the rural women & urban women. Mean daily estimated energy consumption is significantly higher in the rural women ($p=0.035$). About six-folds more energy was consumed from the traditional foods by the rural than the urban

Table 2.4- Evidence of dietary adequacy among Guatemalan Pregnant and Lactating Women

Author	Study Design, Population Characteristics & Location	Dietary Methods, Chosen nutrients /Foods	Aim & Main Findings
Fitzgerald et al (1992) Ecol Food Nutr.	Cross-sectional study, Guajitos, Guatemala City n= 52 <u>Pregnant women (3rd trimester)</u>	24-hour food recalls (9-14 in number) Beans, Bread, Tortillas, Meat, Dairy	Aim: Food consumption patters in terms of dietary diversity & energy and protein intakes Main findings: Total of 254 different food and beverages were listed. No. of items reported ranged from 38 to 80 with mean and S.D of 54.2 ± 10.3. Coffee, tortillas, sugar, French and sweet bread, tomatoes, and black beans were consumed daily. Vegetables, fruits, beef, and poultry were consumed once per week. Cheese and milk were eaten more often than pork or fish. Tortillas accounted 25% of daily energy & protein. DD correlated significantly with meat & dairy consumption but not with protein intakes.
Fitzgerald et al (1993) Am J Clin Nutr.	Cross-sectional study, Guajitos, Guatemala City n= 52 <u>Pregnant women (3rd trimester)</u>	24-hour food recalls (9-14 in number) Energy, protein, calcium, zinc, copper, manganese, phytate	Aim: Intakes of energy, protein, calcium, zinc, copper, and Manganese consumption among pregnant Guatemalan Women Main findings: 94% had zinc intakes below recommendations Tortillas were major source of all the nutrients assessed. 19% zinc from flesh foods. The best sources of zinc are cheese, black beans, and meat products (ham, tripe, chow mein, tamales, and sausages: salchinas, chorizo and longanizas). The zinc content of tortillas ranged from 14.3 to 17.4 µg/g edible portion. Copper was found in higher concentrations in black beans and to a lesser extent in chorizo and pacaya. Pacay, black beans and nightshade leaves contained high concentrations of Manganese. Cheese (6650-7870 µg/g edible portion) and pacaya, a green vegetable are good sources of calcium.
Lee, S. E., et al. Public Health Nutr. (2013)	Review Developing countries. (Africa, Asia and the Caribbean and	Diet information from the published papers Mean/median intakes of	Aim: Dietary intakes of women during pregnancy in low- and middle-income countries Main findings: Meal pattern of pregnant women from LAC consisted of grains, accompanied by beans, vegetables, and animal products. The cereal/ grain group contributed 50–60 % of energy intake in the studies from Peru, Guatemala, and Ecuador.

	Central/South America. 62 articles Africa (n=15) Asia (n = 31) LAC (n=16) LAC- Mexico, the Caribbean and Central/South America <u>Pregnant women</u>	-Energy -Protein -Fat -CHO -Vit-A -Vit- C -Riboflavin -Folate -Calcium -Zinc -Iron	Tortillas were the main staple in the studies from Mexico and Guatemala and a primary source of energy, protein, Zn and Ca, but contributed to phytate intakes as well. Beans were also widely consumed by women, but their contributions to Fe, Zn and Ca intakes were not as high as animal products in most studies. In Peru and Ecuador, milk and dairy products were frequently consumed, and were a primary source of riboflavin and Ca.
FANTA (2014)	Cross sectional study Guatemala n=547 <u>Pregnant and lactating women</u>	24- hour recalls Energy, protein, iron, folate, zinc, vit-B12, vit-C, calcium, Vit A	Aim: Describes how the FBRs for the target population groups were developed using the Optifood program Main findings: Iron, folate, and zinc were problem nutrients for pregnant women For zinc, were below 70% of the zinc RDA. For iron, they remained below 100% of the RDA. Common foods consumed by pregnant women: Table salt, maize products, sugar fortified with vitamin A and iron, raw onions, raw red tomatoes, coffee grains, beans products, potatoes, dry broth cubes (chicken or beef), oil.
Lander, R. L. Nutrients (2019)	Cross sectional study. (Guatemala, India, Pakistan, Democratic Republic of the Congo (DRC) n= 240 Pregnant women/site (total = 966)	24-hour dietary recalls -Energy -Protein -fat -CHO 12 micronutrients: Ca, Fe, Zn, vitamin A, thiamine, riboflavin, B6, folate, B12, vit C, anti nutrient phytate.	Aim: Dietary diversity of the women's diets, estimate the usual group energy and nutrient intakes of 1st trimester pregnant women participating in the trial and determine the estimated prevalence of the study population 'at risk' of inadequate nutrient intakes during 1st trimester Dietary diversity of the women's diets, estimate the usual group energy and nutrient intakes of 1st trimester pregnant women participating in the trial and determine the estimated prevalence of the study population 'at risk' of inadequate nutrient intakes during 1st trimester Main findings: Only 20% of the Pakistani women consumed a diet with adequate DD. Only a quarter of women in DRC reported adequate DD. Red palm oil was a main ingredient in most DRC recipes.

	<u>Pregnant women</u>		About 50% of the Guatemalan women consumed a varied diet and had adequate DD. Like DRC and Pakistan, 70% of Guatemalan women who reported a adequate DD had consumed animal-source foods. Unlike other sites, approximately 20% of the energy intake in the Guatemalan diet came from sugar and refined processed foods
Schutz, 1980 Am J Clin Nutr.	Cross sectional Guatemala n=18 18-38 years >6 months lactation <u>Lactating mothers</u>	4- 24-hour recalls Energy	Aim: To estimate the total energy intakes and expenditures of lactating mothers compared to non-lactating women Main findings: No significant differences between lactating and non-lactating groups in terms of daily energy intake, daily energy expenditure, the energy cost of specific activities, or the pattern of activities throughout the day.

Table 2.5- Healthy Diets Improves the Pregnancy Outcomes In LMIC

Author	Study Design	Maternal Dietary Patterns	Main Pregnancy Outcomes
Chia, et al (2019) N=35 - 72,072 21-33 yrs	Meta-analysis 24-hr recalls	Healthy Diet: vegetables, fruits, wholegrains, low-fat dairy, & lean protein Unhealthy diet: refined grains, processed meat, & high in saturated fat or sugar	Healthy diet lowered risk of: • PTB • SGA • LBW
Chia, et al (2016) N= 1247 8-50 yrs	Observational 24-hr recalls & 3-d food dairy	VFR: Vegetables, Fruits, Rice plus • whole grains, • fish, • nuts & seeds	VFR improved: • PTB • Head circumference & body fat at birth
Abu-Saad., et al (2010)	Review Mixed methods	Dairy products: >6 glasses of milk per day Protein intake: 1 gm increase per kg body weight Fatty fish intake: >60g/day	Birth weight increased with : • Dairy products from 6–18 wks • Protein intake PTB reduced with fish intake

Table 2.6- Evidence for Different Relationship of Maternal age With Birth Outcomes: Effect Modification

Author (year)	Study Design	Infant Birth Outcomes Assessed & Main Findings
Chen et al (2007)	Retrospective cohort study	Adolescent mother groups were associated with increased risks for PTB, LBW and neonatal mortality
Gibbs et al (2012)	Systematic Review	Maternal age ≤ 16 years • LBW & PTB- higher in adolescent mother
Ganchimeg et al (2013)	Secondary analysis	Compared with 20-24 years aged mothers, adolescents (16-19 years) had significantly lower risk of caesarean section Adolescent mothers had higher risks of LBW and PTB.
Ganchimeg et al (2014)	Comparative study 29 countries	Maternal age ≤ 20 yrs • LBW & PTB- higher in adolescent mothers
Yu et al (2016)	Cross-sectional 18 countries	Maternal age: 10-19 yrs & 20-24 yrs • LAZ- lower in adolescent mothers
Akseer et al (2022)	20 RCTs of micronutrient supplements Africa & Asia	Adolescent mothers (10-14 yrs) had: • 23% increased risk of PTB • 60-63% increased risk of infant mortality • 28% increased risk of LBW • 22% increased risk of SGA

CHAPTER 3. METHODS

Context

The study was conducted in the 8 rural *Mam* communities located within ~15 km of San Juan Ostuncalco and ~25 km of the city of Quetzaltenango (Chomat et al., 2015). Maternal health care was provided by a full-time community health worker (CHWs), a nurse who visited the community health post every 7 to 14 days, and traditional midwives (comadronas) (Chomat et al., 2015). To ensure that the research was culturally sensitive, respectful, and relevant of the local values, the principles of participatory action research (PAR) were followed (Cargo & Mercer, 2008) (Dadich, Moore, & Eapen, 2019). This included the identification of the most important concerns regarding maternal and child health as identified by 21 *Mam* women that included the research coordinator, two traditional midwives, and 12 community health workers, all of whom provided input into the design, recruitment, questionnaire development, and home visits (Chomat et al., 2015). From June 2012 to March 2013, with the help of 20 community staff (CHWs and comadronas) women were recruited for this study. Recruitment involved different methods (home visits, loudspeaker announcements, and word-of-mouth invitations) to attend meetings at the community health post. At the community center meeting, the study was explained, questions were answered, and women were invited to provide informed written consent (thumbprint if unable to sign) if they wished to participate in the study (Chomat et al., 2015).

Ethics

The institutional review boards of the Centre for Studies of Sensory Impairment, Aging, and Metabolism in Guatemala and McGill University approved the study, and permission was also obtained from the community leaders and the local authorities of the Ministry of Health. All the participants were referred to the public health system whenever medically indicated (Chomat et al., 2015). The current research is a secondary data analysis using the dietary data from the study named "Quantitative Methodologies Reveal a Diversity of Nutrition, Infection/Illness, and Psychosocial Stressors During Pregnancy and Lactation in Rural *Mam*-Mayan Mother-Infant Dyads From the Western Highlands of Guatemala" (Chomat et al., 2015). The objectives of the parent study were "to describe quantitative methodologies; assess construct validity of

questionnaires; report variability in sociodemographic, obstetric, nutritional, infectious, and psychosocial characteristics; and compare characteristics between pregnancy and lactation and between study cohorts of *Mam*-Mayan mother-infant dyads” (Chomat et al., 2015).

Questionnaire

A structured questionnaire in Spanish or *Mam* was administered by a community staff about household, and social factors, obstetric and medical history (20-45 minutes), mothers and infant’s anthropometry (15 minutes) were measured by staff nutritionists and collected a previous-day dietary recall at each sampling period i.e., at pregnancy, early postpartum (1-6 weeks after childbirth) and at late postpartum (4-6 months after childbirth) (Chomat et al., 2015). Community staff performed a follow-up home visit and collected a previous day activity recall (15 minutes) and a second previous-day dietary recall (15 minutes) (Chomat et al., 2015). For our study we have included 449 dietary records (average of 2-24-hr recalls) from mothers and 336 anthropometric measurements from infants (Chomat et al., 2015). Maternal dietary records and infant sample sizes are shown in Fig 3.1 and Fig 3.2, respectively.

Maternal cohort

Mother’s date of birth or self reported age for an unknown date was used to determine the maternal age (Chomat et al., 2015). Age at delivery was categorized as adolescent mothers (≤ 19 years) and adult mothers (> 19 years). Parity, defined as the number of live births, was categorized as 1, 2, 3 and ≥ 4 . Maternal height was measured using a wall stadiometer, shoeless and with the gaze in the Frankfort plane (Chomat et al., 2015). Weight was measured using a digital scale (SECA 803, Hamburg, Germany) (Chomat et al., 2015). Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) and classified as underweight (< 18.5), normal weight (%) (18.5-24.9) and overweight (%) (25.0-29.9) (Organization, 1995).

Maternal dietary records sample size

We subdivided the dietary records into two categories i.e., adolescent, and adult mothers based on age at delivery and into three timepoints i.e., pregnancy, early postpartum (1-6 weeks

after childbirth) and late postpartum (4-6 months after childbirth) shown in Fig 3.1. For our study we have included a total of 449 dietary records (average of 2-24-hr recalls) from mothers. Of these 449 dietary records, 125 dietary records were from adolescent mothers (≤ 19 years) and 324 dietary records were from adult mothers (>19 years). There were 38 pregnant, 46 early PP (postpartum) and 41 late PP dietary records from adolescent mothers (≤ 19 years) and 79 pregnant, 118 early PP and 127 late PP dietary records from adult mothers (>19 years). For our analysis we included the dietary records in early and late PP from adolescent and adult mothers.

Infant cohort

Infant age, sex, height, weight, and head circumference was collected by the staff nutritionist at early PP and late PP. Infant weight (in kgs) was measured using a digital infant scale to the nearest 100 grams. Infant height (in cms) or the recumbent supine length (stature taken while lying down) was measured thrice according to the standardized procedures using an infantometer (SECA 210) and recorded to the nearest 0.5 cm, and the final value was the mean (Chomat et al., 2015). Head circumference of the infants (in cm) was measured thrice using a head circumference baby band (Chomat et al., 2015).

Infant sample size

We included a total of 336 infants in our analysis shown in Fig.2. Of these 336 infants, 87 infants were born to adolescent mothers and 246 infants were born to adult mothers. We also subdivided the infants into early and late PP. We had 46 infants in early PP and 41 infants in late PP born to adolescent mothers and 119 infants in early PP and 127 infants in late PP born to adult mothers.

Exposures of Interest

Maternal Nutrient Intakes

Maternal nutrient intake is the primary exposure of interest for this study. After the dietary data was collected, the foods reported were subdivided into macronutrients (water, energy, carbohydrates, protein, fiber, sugar), lipids (saturated fatty acids, monounsaturated fatty

acids, polyunsaturated fatty acids, cholesterol), fat-soluble vitamins (vitamin A, D, E, K; water-soluble vitamins- B-complex vitamins, vitamin C); minerals (calcium, magnesium, phosphorous, potassium, sodium); and trace elements (iron, zinc, copper, selenium, manganese); antioxidants (lycopene, retinol, lutein & zeaxanthin, beta-cryptoxanthin).

Dietary assessment

The diet data were converted into nutrients for analysis. We analysed the nutrient intakes of all the mothers pooled together and calculated the mean nutrient intakes of the mothers based on their age (adolescent mothers vs adult mothers) at each time point (early PP & late PP). The mother's nutrient adequacy was assessed using the dietary reference standards; Institute of Medicine (IOM) Daily recommended intakes (IOM, 2005) and Institute of Nutrition of Central American and Panama (INCAP) Recommended Daily dosage (RDD) (INCAP, 2012).

Although both DRI and RDD are the standard nutrient requirements, the recommendations for a few nutrients differ from each other. We compared the nutrient requirement by INCAP RDD to the IOM DRI for lactating mothers shown in **Table 3-1**. The table describes the different requirements of INCAP RDD and IOM DRI for adolescent and adult mothers. There is water requirement by IOM DRI but not for INCAP RDD. There is increased requirements of energy, protein, sugar, iron, and zinc for both adolescent and adult mothers by INCAP RDD compared to IOM RDD whereas, IOM DRI had higher requirements for carbohydrates, fiber, calcium, magnesium, phosphorous, copper, selenium, vitamin-C, thiamine, riboflavin, niacin, vitamin-B6, vitamin-B12, folate-total, and vitamin K.

Outcomes of interest

Infant growth outcomes

The anthropometric data of the infants included the weight, height, and head circumference of the infant and the Z-scores for length-for-age z-score (LAZ), weight (WAZ), and head circumference-for-age z-score (HCAZ) were the outcomes of interest for this study. LAZ, WAZ, and HCAZ's were calculated as the indicators of the infant nutritional status using WHO Anthro software (Van den Broeck, Willie, & Younger, 2009). Stunting was defined as when a child fails to achieve a height appropriate for their age, that is, when child's length-for-age (for

children younger than two years) or height-for-age (for children older than two years to age) is below two standard units (<-2 SD z-scores) from the reference. Underweight was defined as when a child's weight-for-age z-score is below minus two standard deviations (-2 SD) from the median of the reference population is considered underweight or acutely undernourished ("WHO Child Growth Standards based on length/height, weight, and age," 2006). Low cranial head circumference was defined as z-scores < -1.0 SD below the respective WHO reference median ("WHO Child Growth Standards based on length/height, weight, and age," 2006) (Chomat et al., 2015).

Effect modifiers

We examined the role of maternal age in modifying the relationships between maternal nutrient intakes and infant growth outcomes (maternal age as an effect modifier). The dietary records in this study were divided into adolescents (≤ 19 years of age) and adults (>19 years of age) based on their age at delivery.

Statistical analysis

All the statistical analysis were performed using the STATA statistical software package 16 (Stata Corporation). For an overview of the dietary intakes of the mothers, we analyzed the means or the averages of the nutrient intakes across the sample by age category (adolescents and adult mothers) to assess the dietary differences among the mothers. To test the nutrient differences between adolescent and adult mothers, we performed variance test (sdtesti) followed by Students T-test (ttesti). P-value $p < 0.05$ was considered significant.

To assess the association between the maternal nutrient intake, on infant growth outcomes (LAZ, WAZ, and HCAZ), we performed multiple linear regressions by timepoints (early PP & late PP) controlling for mothers' height, weight, BMI, and sex of the infant ($p < 0.05$). To assess the impact of maternal nutrients on infant stunting, underweight, and low cranial growth we used multiple logistic regressions controlling for mothers' height, weight, parity, and infant sex ($p < 0.05$). To avoid creating artificially small standard errors (and increasing the chances that

our findings were significant), we analysed time-points separately so repeated assessments of mother/child dyads were not included.

To assess the differential impact of age on the association between maternal nutrient intakes and infant growth outcomes, we used multiple linear regressions and multiple logistic regressions with maternal age (adolescents and adults) as an effect modifier controlling for mothers' height, weight, parity, and infant sex ($p < 0.05$). To determine whether stratified analysis for adolescents and adults were significant, we tested for effect modification by examining the significance of effect modification terms in both crude and adjusted models ($p < 0.05$).

A sensitivity analysis was performed to account for the multiple tests given that we had available 46 nutrients in our dataset. For this, we calculated Bonferonni and Benjamini Hochberg adjusted p-values (Benjamini & Hochberg, 1995). We present the results of p-values controlling for maternal height, weight, parity, and infant sex (least conservative), Bonferonni adjusted p-values (very conservative), and Benjamini Hochberg (moderately conservative) to give added depth to the interpretation of our findings.

Table 3.1-Comparison of maternal nutrient intakes between adolescents & adults during lactation based on RDD & DRI requirements

Nutrient	Adolescent RDD	Adolescent DRI	Adult RDD	Adult DRI
Water, g	- ¹	3800	-	3800
Energy, Kcal	2550	2200	2650	2400
Macronutrients:				
Carbohydrates, g	160	210	160	210
Protein, g	84	71	87	71
Lipids, g	81	-	81	-
Fiber, g	25	29	25	29
Sugar, g	66	58	66	58
SFA, g	29	-	29	-
PUFA, g	25	-	25	-
Cholesterol, mg	300	-	300	-
Vitamins				
Fat-soluble vitamins:				
Vit-A, RAE	-	1200	-	1300
Vit- D, µg	5	-	5	-
Vit-D, IU	-	600	-	600
Vit-E, mg	19	19	19	19
Vit-K, µg	55	75	55	90
Water-soluble vitamins:				
Thiamin, mg	1.1	1.4	1.1	1.4
Riboflavin, mg	1.3	1.6	1.3	1.6
Niacin, mg	13	17	13	17
Pantothenic Acid, mg	7	7	7	7
Vit- B6, mg	1.7	2	1.7	2
Folate-Total, µg	450	500	450	500
Folate, DFE, µg	-	500	-	500
Choline, mg	-	550	-	550
Vit- B12, µg	2.4	2.8	2.4	2.8
Vit-C, mg	100	115	100	120
Macro-minerals:				
Calcium, mg	1000	1300	1000	1000
Magnesium, mg	230	360	230	360
Phosphorus, mg	580	1250	580	700
Potassium, mg	5100	5100	5100	5100
Sodium, mg	1500	1500	1500	1500
Trace elements:				
Iron, mg	15.6	10	15.6	9.0
Selenium, µg	54	70	54	70
Copper, mg	1.0	1.3	1.0	1.3
Manganese, mg	2.6	2.6	2.6	2.6
Zinc, mg	22.6	13	22.6	12

¹ Dash (-) No recommended value for RDD & DRI requirements

Figure 3-1: Sample size of maternal dietary records collected during pregnancy and early lactation

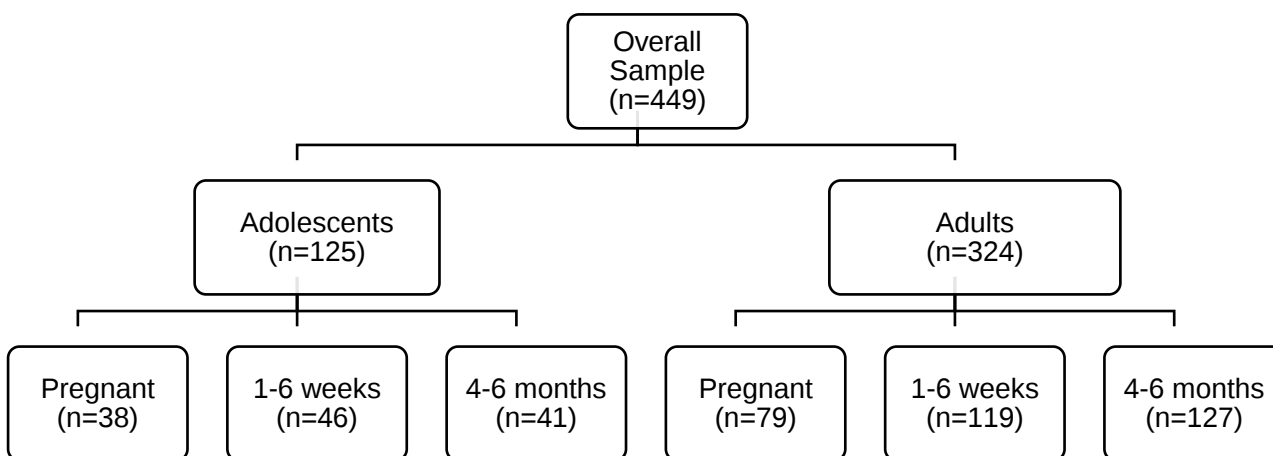
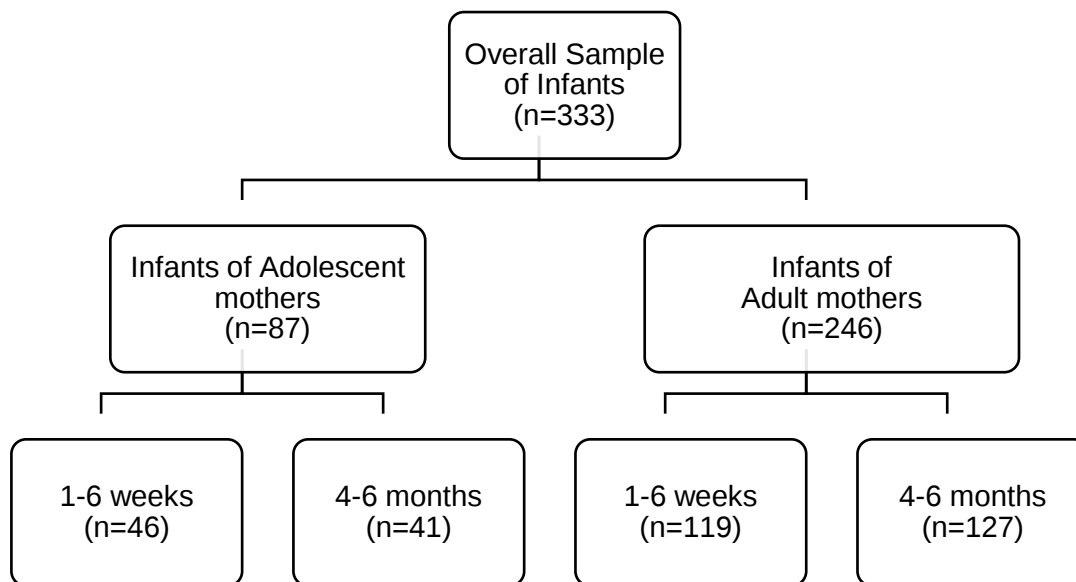


Figure 3.2- Sample sizes of the mother-infant dyads used in the study



CHAPTER 4. RESULTS

Maternal and Infant Characteristics

The study population characteristics of mothers are shown in **Table 4-1**: Of the 449 mothers, 28% (n=125) were adolescent (≤ 19 years of age) with an average age of 16.9 ± 1.3 years (min-13.9, max-19) and 72% (n=324) were adults (>19 years of age) with an average age of 26.7 ± 5.9 years (min-19.1-max-46.2). Not unexpectedly, maternal weight (52.0 kg for adult; 47.8kgfor adolescent mothers), length (146.9 cm for adult; 145.9for adolescent mothers) and BMI (24.0 kg/m² for adult; 22.4 for adolescent mothers) significantly differed between adolescent and adult mothers. Also, the percentages of underweight, normal weight, and overweight mothers differed; 79.3% of adolescent mothers were normal weight, 4.6% were underweight and 16.1% were overweight whereas 65% of adult mothers were normal weight, 2.8% underweight and 32.1% were overweight. Parity averaged 2.7 ± 2.2 children and as expected, parity was lower for adolescents compared to adults.

With regards to maternal diet for the study population (**Supplementary Tables 4-S1.A & 4-S1.B**), most mothers (N=449) had adequate intakes of a few nutrients (carbohydrates, fiber, sugar, magnesium, phosphorous, and sodium) based on RDD and DRI recommendations, but there were also widespread nutrient inadequacies. Mothers consumed inadequate amounts of water, macronutrients [energy, protein, lipids], water-soluble vitamins [vitamin C, thiamine, riboflavin, pantothenic acid, vitamin B6, total folate, vitamin B12], fat-soluble vitamins [A (RAE), E , D] and minerals [calcium, potassium, zinc, manganese]. Despite these nutrient inadequacies in the *Mam*-Mayan communities, the prevalence of protein inadequacy (adolescent - 92%; adults - 98.4%); calcium inadequacy (adolescent - 100%; adult - 94.4%) and potassium inadequacy (adolescent - 89.6%; adult - 97.2%) differed between adolescent and adult mothers. Moreover, the prevalence of inadequate intakes of protein and potassium differed using more stringent Bonferroni ($p < 0.002$) and Benjamini Hochberg ($p = 0.02$) statistical corrections for multiple comparisons between adolescents and adults.

Infant characteristics are also shown in **Table 4-1**. Of the 333 infants with anthropometric measurements, 26.1% (n=87) were born to adolescent mothers and 73.9% (n=246) to adult

mothers. Of these 53.9% (n=181) were males and 46.1% (n=155) were females. This distribution did not differ between adolescent and adult mothers. Average infant length (55.1 cm), weight (4.9 kg) and head circumference (38.1 cm) did not differ by maternal age categories, and neither did infant length-for-age-z-scores (LAZ), but 25.9% (n=87) of infants were stunted and 14% (n=47) infants were severely stunted. The prevalence of stunting did not differ between adolescent and adult mothers. In contrast, infant WAZ differed between adolescent (-1.2 ± 1.2) and adult (-0.8 ± 1.1) mothers ($p=0.0035$), with lower infant WAZ's observed for adolescent mothers. Additionally, HCAZ differed with infants of adolescent mothers having lower head circumference for age compared with infants of adult mothers (-1.06 ± 1.4 vs -0.5 ± 1.4 , $p=0.0034$). Overall, 22.6% of infants had low cranial head circumference ($HCAZ < -1SD$).

Comparison of nutrient intakes between adolescents and adult mothers during lactation

A comparison of mean nutrient intakes between lactating adolescent and adult mothers (N=333) are shown in **Table 4.2**. Nutrient intakes of protein, lipids, choline, retinol, vitamin E, polyunsaturated fatty acids (PUFA), and cholesterol significantly differed between adolescent mothers and adult mothers. Protein intake of adolescent mothers was higher ($42 \pm 17g$) compared to that of adult mothers (38 ± 14) ($p=0.0217$). Lipid intake of adolescent mothers was higher ($21 \pm 8g$) compared to adult mothers ($19 \pm 8g$) ($p=0.0299$). Choline intake of adolescent mothers ($174 \pm 100mg$) was higher compared to adult mothers' intake ($151 \pm 85mg$) ($p=0.0239$). Retinol intake of adolescent mothers ($476 \pm 209\mu g$) was higher compared to adult mothers ($424 \pm 267\mu g$) ($p=0.0312$). Vitamin E of adolescent mothers $3 \pm 1.4mg$ was higher compared to adult mothers' intake $2 \pm 1.5mg$ ($p=0.0152$). Polyunsaturated fatty acids intake of adolescent mothers ($7 \pm 3g$) were higher compared to adult mothers' intake was $7 \pm 2g$ ($p=0.0211$). Cholesterol intakes of adolescent mothers $97 \pm 115g$ were higher compared to adult mothers $69 \pm 86g$ ($p=0.0155$). Overall, adolescent mothers' nutrient intake was higher for the majority of nutrients compared to adult mothers. No significant p-values emerged following Bonferroni and Benjamini Hochberg analyses for multiple comparison tests (**Table 4.2**).

Infant anthropometry

Infant Weight (WAZ)

Impact of maternal nutrient intakes on infant WAZ using multiple linear regressions and adjusting for maternal age, length, weight, parity & infant sex are described in **Tables 4.3.A & 4.3.B**. The maternal intakes of energy, carbohydrates, fiber, calcium, magnesium, selenium, folate DFE (dietary folate equivalents), vit-A RAE (retinol activity equivalents), and polyunsaturated fatty acids during the early postpartum (1-6 weeks after childbirth) were significantly associated with WAZ ($p < 0.05$). WAZ increased with maternal intakes of the following nutrients: polyunsaturated fatty acids (1.014/10g), for macro minerals [calcium (0.129/100mg) and magnesium (0.178/100mg)] and for one trace element selenium (0.257/100µg) during the first six weeks of breastfeeding. As well, increased intakes and folate [(0.192/1000µg of DFE (dietary folate equivalents))] and vitamin A [0.125/100 RAE (retinol activity equivalent)] were associated with increased WAZ. However, the direction of the impact differed between early and late postpartum for retinol. In early postpartum, retinol intake was positively associated with WAZ (0.179/100 µg) but negatively associated with WAZ in late postpartum (-0.197/100µg). Along with retinol, lycopene intake was negatively associated with WAZ in late postpartum (-0.209/100µg).

In **Tables 4.4.A & 4.4.B** we used multiple logistic regressions to describe associations of maternal nutrient intakes with infants classified as underweight ($WAZ < -2SD$) at early (6 wks) and at late (4-6 months) lactation. This multiple logistic regression model, which adjusted for maternal age, length, weight, parity & infant sex, showed lower odds of underweight with higher maternal intakes of folate DFE ($OR = 0.996$, $p = 0.026$) and polyunsaturated fatty acids ($OR = 0.7511$, $p = 0.016$) in early postpartum and with higher intakes of maternal dietary cholesterol ($OR = 0.989$, $p = 0.03$) in late postpartum.

In **Tables 4.5.A & 4.5.B**, we tested for effect modification of maternal age on the association of maternal nutrient intakes with WAZ in our multiple linear regression models. We identified five nutrients whose impacts on WAZ significantly varied between adolescents and adults; these included sugar ($p = 0.046$), selenium ($p = 0.031$), vitamin A ($p = 0.049$) and retinol

($p=0.014$) in early postpartum and lycopene ($p=0.004$) and retinol ($p=0.050$) in late postpartum. These specific associations were as follows. Although the effect modification was significant, the individual associations between sugar and WAZ scores for adolescent and adult mothers were not significant. . For every 10 micrograms increase of selenium, WAZ of infants of adolescent mothers significantly increased by 0.25 ($p=0.005$), while infants of adult mothers did not change ($p=0.52$). For every 100 micrograms increase in vitamin A (RAE), WAZ of infants of adolescent mothers significantly increased by 0.13 ($p=0.033$), while infants of adult mothers did not change ($p=0.81$). For every 100 micrograms increase in retinol, WAZ of infants of adolescent mothers significantly increased by 0.22 ($p=0.011$), while infants of adult mothers did not change ($p=0.17$). In late postpartum (**Table 4.5.B**) we also observed stronger associations of lycopene ($p=0.004$) for adolescent mothers compared to adult mothers in late postpartum. For every 100 micrograms in lycopene, WAZ of infants of adolescent mothers significantly decreased by 0.23 ($p=0.019$), while infants of adult mothers did not change ($p=0.23$). Maternal lycopene intake was also significant at the Bonferonni adjusted significance level ($p\text{-value}<0.025$) ($p\text{-value}$ not shown in table) and Benjamini Hochberg level ($p=0.008$).

In **Tables 4.6.A & 4.6.B**, we also tested for effect modification of maternal age on the association of maternal nutrient intakes with infant underweight in our multiple logistic models. In early postpartum, we identified two nutrients, choline ($p=0.025$) and cholesterol ($p=0.022$), whose impacts on infant underweight ($WAZ<-2SD$) significantly varied between adolescents and adult mothers. When we examined the stratified results among adolescent and adult mothers, there were differences in odds ratios for these nutrients, however adjusted OR for either adolescents or adults were not statistically significant. Of significance, the effect modification terms for maternal choline (adj $p=0.025$) and cholesterol (adj $p=0.025$) were significant when we used the Benjamini Hochberg test. Effect modification by age for choline was also significant at Bonferroni adjusted significance level ($p<0.025$).

Infant Length (LAZ)

Impact of maternal nutrients on infant LAZ using multiple linear regressions and adjusting for maternal age, length, weight, parity & infant sex described in **Tables 4.3.A & 4.3.B**. Maternal intakes of selenium, retinol, lycopene, vitamin E, monounsaturated, polyunsaturated fatty acids

and cholesterol were significantly associated with infant length-for-age-z-scores (LAZ) after adjusting for mothers age, weight, length, parity, and infant sex. LAZ significantly increased for selenium (0.229/10 μ g), vitamin E (4.119/10mg), monounsaturated fatty acids (1.639/10g), and polyunsaturated fatty acids (1.004/10g); in early postpartum. Increased intakes of cholesterol (0.030/10mg) were significantly associated with infant LAZ in late postpartum. The strength and direction of association changed for retinol and lycopene in late postpartum compared to early postpartum. Higher intakes of retinol (-0.233/100 μ g) and lycopene (-0.263/100 μ g) significantly decreased infant LAZ in late postpartum, but were not associated in early postpartum.

Tables 4.4.A & 4.4.B revealed that for our multiple logistic regression models for LAZ<-2SD that adjusted for maternal age, length, weight, parity & infant sex, no maternal nutrient was significantly associated with reducing or increasing the odds of infant stunting in either early or late postpartum.

In **Tables 4.5.A & 4.5.B**, we tested in our multiple linear regression models for effect modification of maternal age on the association of maternal nutrient intakes with LAZ. We identified several nutrients whose impacts on LAZ differed between adolescent and adult mothers in early postpartum: these included selenium (p=0.037), vitamin E (p=0.027), saturated fatty acids (p=0.014) and monounsaturated fatty acids (p=0.018) and in late postpartum retinol (p=0.019), lycopene (p=0.002), and lutein & zeaxanthin (p=0.018). Further, based on Benjamini Hochberg adjusted p-values, there was a significant modification by maternal age group for selenium (p=0.046), vitamin E (p=0.045), SFA (p=0.045), and MUFA (p=0.045).

Specific responses to increased maternal dietary intakes in early postpartum (**Table 4.5.A**) were as follows. We observed that for every 10 micrograms increase in selenium, infant LAZ of adolescent mothers significantly increased by 0.20 (p=0.045), while infant LAZ of adult mothers was unchanged (p=0.65). For every 10 milligrams increase in vitamin E, infant LAZ of adolescent mothers significantly increased by 3.11 (p=0.032), while infant LAZ of adult mothers was unchanged (p=0.79). For every 10 grams increase in saturated fatty acids, infant LAZ of

adolescent mothers significantly increased by 1.41 ($p=0.046$), while infant LAZ of adult mothers was unchanged ($p=0.18$). For every 10 grams increase in monounsaturated fatty acids, infants of adolescent mothers significantly increased by 1.47 ($p=0.017$), while infant LAZ of adult mothers was unchanged ($p=0.63$).

In late postpartum (**Table 4.5.B**), we also identified select nutrients whose impacts on infant stunting significantly varied between adolescent and adult mothers. These included retinol ($p=0.019$), lycopene ($p=0.002$) and lutein & zeaxanthin ($p=0.018$). Although the effect modification was significant, the individual associations between retinol, and lutein & zeaxanthin and LAZ scores for adolescent and adult mothers were not significant. Higher adolescent intake of lycopene significantly decreased ($-0.29/100 \mu\text{g}$) infant LAZ while there was no significant association for adults ($p=0.23$). After Benjamini Hochberg adjustments, there remained a significant effect by maternal age for retinol ($p=0.011$), lycopene ($p=0.006$), and lutein & zeaxanthin ($p=0.011$).

In **Tables 4.6.A & 4.6.B** we report the effect modification of age on the association between maternal nutrient intakes during lactation LAZ. We identified five nutrients whose impact on infant stunting significantly differed by maternal age. Adolescents had reduced odds of infant stunting compared to adults with higher intakes of lipids ($p=0.032$), vitamin B12 ($p=0.034$), vitamin E ($p=0.008$), saturated fatty acids ($p=0.022$), and monounsaturated fatty acids ($p=0.019$). Increased lipid intakes were associated with reduced the odds of stunting for infants of adolescent mothers ($\text{OR}=0.853$, $p=0.018$), but not for adult mothers ($\text{OR}=1.016$, $p=0.57$). Though there was evidence supporting effect modification by maternal age of vitamin B12 and the odds of stunting, we found no significant associations when we analyzed adolescent and adult mothers separately. For adolescent mothers, increased intakes of vitamin E, significantly decreased odds of infant stunting by 80% ($\text{OR}=0.200$, $p=0.015$) for adolescent mothers, whereas there was no significant association ($\text{OR}=1.171$, $p=0.28$) for infants of adult mothers. Increased intakes of saturated fatty acids significantly reduced odds of stunting by 39% for infants of adolescent mothers ($\text{OR}=0.618$, $p=0.029$), but there was no significant association for infants of

adult mothers (OR=1.113, $p=0.28$). Increased intakes of monounsaturated fatty acids reduced odds of stunting by 35% for infants of adolescent mothers (OR=0.652, $p=0.018$), but there was no significant association for infants of adult mothers (OR=1.059, $p=0.44$). Maternal lipids (adj $p=0.034$), vitamin B12 (adj $p=0.034$), vitamin E (adj $p=0.034$), SFA (adj $p=0.034$), and MUFA (adj $p=0.034$) intakes had significant association with infant stunting when we used the Benjamini Hochberg test (shown as FDR adjusted p -value in table). After testing for effect modification by age in our multiple logistic models as described in Tables 4.6.A & 4.6.B, late postpartum showed no effect modification associations between nutrient intakes by mothers with odds of infant stunting.

Infant Head-circumference (HCAZ)

Impact of maternal nutrients on infant HCAZ using multiple linear regressions adjusted for maternal age, length, weight, parity, and infant sex as described in **Table 4-3**: Maternal intakes of energy, carbohydrate, fibre, vitamin B6, folate, and polyunsaturated fatty acids were significantly associated with infant head circumference-for-age-z-scores in early postpartum and lutein & zeaxanthin in late postpartum after adjusting for maternal age, length, weight, parity, and infant sex. HCAZ increased for select macronutrients in early postpartum; this included energy (0.080/100Kcal), carbohydrates (0.378/100g), fibre (0.334/10g) and polyunsaturated fatty acids (1.054/10g); for vitamin B6 (5.828/10mg) and folate (dietary folate equivalents) (0.201/100 μ g) whereas higher intakes of maternal lutein & zeaxanthin (-0.095/1000 μ g) significantly decreased infant HCAZ in late postpartum.

Our multiple logistic models for low cranial head circumference (HCAZ<-1) adjusted for maternal age, length, weight, parity, and infant sex are described in **Tables 4.4.A & 4.4.B**. In early postpartum, we observed lower odds of low cranial growth with higher intakes of fiber (OR=0.9330, $p=0.011$), total folate (OR=0.9945, $p=0.020$), food folate (OR=0.9941, $p=0.015$), folate (dietary folate equivalents) (OR=0.9956, $p=0.003$) and polyunsaturated fatty acids (OR=0.8442, $p=0.039$). No significant associations were found in late postpartum.

In **Tables 4.5A & 4.5.B**, our effect modification of age on the association of maternal nutrient intakes with infant HCAZ did not identify any significant nutrient intakes in either early or late lactation that were associated with HCAZ (Tables 4.5.A & 4.5.B – no data shown).

In **Tables 4.6.A & 4.6.B**, we report the effect modification of age on the association between maternal nutrient intakes during lactation on infant low cranial HC. No significant effect modification was observed in late postpartum, but we identified five nutrients whose impact on infant cranial growth significantly varied between adolescent and adult mothers in early postpartum. These nutrients included calcium ($p=0.044$), iron ($p=0.021$), thiamine ($p=0.024$), zinc (crude association $p=0.047$) and folate DFE ($p=0.030$). Increased calcium intakes were associated with <1% decreased odds of low cranial growth in infants of adult mothers ($OR=0.998$, $p=0.04$) but no association for adolescent mothers ($OR=1.003$, $p=0.21$) was observed. Although the effect modification between zinc and thiamine by age were significant, the stratified analysis for adolescent and adult mothers were not significant. Increased iron intakes were associated with 14% decreased odds of low cranial growth in infants of adult mothers ($OR=0.862$, $p=0.046$), but no association among adolescent mothers ($OR=1.015$, $p=0.60$) was observed. Increased folate, DFE intakes were associated with <1% decreased odds of low cranial growth in infants of adult mothers ($OR=0.994$, $p=0.002$), but no association for adolescent mothers ($OR=1.001$, $p=0.82$) was observed. No significant p-values emerged following Bonferroni and Benjamini Hochberg analyses for multiple comparison tests.

Table-1: Maternal and Infant characteristics of overall study population and comparisons between adolescents & adults subgroups

	Overall Sample (n=449)	Adolescents (n=125)	Adults (n=324)	
Maternal characteristics	Means (SD) or n(%)	Means (SD) or n (%)	Means (SD) or n (%)	p-value
Age, yrs	24.0 (6.7)	16.9 (1.3)	26.7 (5.9)	$<0.0001^2$
Height, cm	146.7 (5.2)	145.9 (5.7)	146.9 (5.1)	0.13 ¹
Weight, kgs	50.9 (8.2)	47.8 (7.0)	52.0 (8.3)	0.001 ¹
BMI	23.6 (3.4)	22.4 (2.9)	24.0 (3.4)	0.0001 ¹
Underweight (<18.5)	11 (2.2%)	4 (4.6%)	7 (2.8%)	0.015
Normal weight (%) (18.5-24.9)	230 (68.7%)	69 (79.3%)	160 (65.0%)	0.015
Overweight (%) (25.0-29.9)	94 (28.1%)	14 (16.1%)	79 (32.1%)	0.015
Parity	2.7 (2.2)	1.1 (0.4)	3.3 (2.3)	0.0001 ¹
1	141 (42.3%)	75 (86.2%)	65 (26.6%)	0.001
2	57 (17.1 %)	11 (12.6%)	46 (18.9%)	0.001
3	42 (12.6 %)	1 (1.2%)	41 (16.8%)	0.001
≥4	93 (27.9%)	-	92 (37.7%)	0.001
Infant Characteristics	Overall Sample (n=333)	Infants of Adolescent mothers (n=87)	Infants of Adult mothers (n=246)	p-value
Sex				
Male	181 (53.9%)	45 (51.7%)	135 (54.7%)	0.64
Female	155 (46.1%)	42 (48.3)	112 (45.3%)	0.64
Age, days	84.3 (65.7)	79.6 (65.7)	86.1 (65.8)	0.43 ¹
Height, cm	55.1 (6.3)	54.2 (6.0)	55.3 (6.4)	0.16 ¹
Length-for-age-z-scores	-1.79 (1.4)	-1.73 (1.3)	-1.06 (1.4)	0.17 ¹
Stunting (LAZ<-2)	87 (25.9%)	27 (31.0%)	60 (23.3%)	0.29
Severely Stunting (LAZ<-3)	47 (14.0%)	14 (16.1%)	33 (13.4%)	0.29
Weight,	4.9 (1.6)	4.7 (1.6)	5.1 (1.6)	0.07 ¹
Weight-for-age-z-score	-0.9 (1.1)	-1.2 (1.2)	-0.8 (1.1)	0.0035 ¹
Underweight (WAZ<-2)	31 (9.2%)	8 (9.2%)	23 (9.3%)	0.81
Severely underweight (WAZ<-3)	16 (4.8%)	8 (9.2%)	8 (3.2%)	0.81
Head circumference, cm	38.1 (3.3)	37.6 (3.3)	38.3 (3.4)	0.06 ¹
HCAZ	-0.67 (1.5)	-1.06 (1.4)	-0.5 (1.4)	0.0034 ¹
(HCAZ <-1 S.D)	75 (22.6%)	19 (21.8%)	55 (22.6%)	0.031
(HCAZ <-2 S.D)	29 (8.7%)	13 (14.9%)	16 (6.6%)	0.031
(HCAZ <-3 S.D)	24 (7.2%)	10 (11.5%)	14 (5.8%)	0.031

¹t test using equal variance; ²t test using unequal variances.

Table 4-2- Comparison of maternal nutrient intakes between adolescents (n=87) & adults (n=245) during lactation based on RDD & DRI requirements for study population¹

Nutrient	Overall Sample	Adolescent RDD	Adult RDD	Adolescent DRI	Adult DRI	Adolescents (n=87)	Adults (n=245)	p-value ² <0.05 ³ BF<0.001	⁴ FDR Adj P-value
Water, g	1470 (467)	-	-	3800	3800	1488 (405)	1457 (465)	0.51	0.71
Energy, Kcal	1317(349)	2550	2650	2200	2400	1356(307)	1305 (356)	0.16	0.38
Protein, g	40 (15)	84	87	71	71	42(17)	38 (14)	0.0217 ²	0.98
Lipids, g	20 (8)	81	81	-	-	21 (8)	19 (8)	0.0299 ²	0.98
Ash, g	11.7 (5.88)	-	-	-	-	11.7 (4.7)	11.75(6.3)	0.98	0.98
Carbohydrates, g	263 (69)	160	160	210	210	266(58)	261 (71)	0.46	0.70
Fiber, g	27 (8)	25	25	29	29	27 (7)	27 (8)	0.94	0.98
Sugar, g	64 (25)	66	66	58	58	67 (21)	63 (27)	0.23	0.47
Calcium, mg	67 (201)	1000	1000	1300	1000	679 (171)	670 (204)	0.66	0.50
Iron, mg	11.5 (9.0)	15.6	15.6	10	9.0	12.6 (14.5)	11 (7)	0.28	0.50
Iron2, mg	11.5 (9.0)	-	-	-	-	12.6 (14.5)	11.1 (7.1)	0.28	0.50
Magnesium, mg	403 (116)	230	230	360	360	408 (97)	403 (118)	0.68	0.50
Phosphorus, mg	2440 (841)	580	580	1250	700	2459 (705)	2442(859)	0.83	0.50
Potassium, mg	1703 (607)	5100	5100	5100	5100	1749 (624)	1683 (588)	0.29	0.33
Sodium, mg	2685 (1782)	1500	1500	1500	1500	2651(1367)	2731 (1985)	0.63	0.33
Zinc, mg	7.9 (12.5)	22.6	22.6	13	12	8.0 (4.5)	7 (2)	0.06	0.33
Copper, mg	1.2 (0.4)	1.0	1.0	1.3	1.3	1.2 (0.3)	1.2 (0.4)	0.30	0.50
Manganese, mg	1.4 (0.6)	2.6	2.6	2.6	2.6	1.5 (0.5)	1.4 (0.6)	0.23	0.47
Selenium, µg	53 (21)	54	54	70	70	56 (22)	52 (20)	0.07	0.33
Vit-C, mg	46 (36)	100	100	115	120	45 (34)	46 (35)	0.81	0.47
Thiamin, mg	0.8 (0.5)	1.1	1.1	1.4	1.4	0.8 (0.6)	0.8 (0.4)	0.24	0.47

Nutrient	Overall Sample	Adolescent RDD	Adult RDD	Adolescent DRI	Adult DRI	Adolescents (n=87)	Adults (n=245)	p-value ² <0.05 ³ BF<0.001	⁴ FDR Adj P-value
Riboflavin, mg	0.6 (0.2)	1.3	1.3	1.6	1.6	0.6 (0.2)	0.6 (0.2)	0.16	0.38
Niacin, mg	13 (5)	13	13	17	17	14.4 (4.9)	13.6 (4.5)	0.13	0.38
Pantothenic Acid, mg	2 (1)	7	7	7	7	2 (1)	2 (0.9)	0.13	0.38
Vit- B6, mg	1 (1)	1.7	1.7	2	2	1 (0.4)	1 (0.43)	0.50	0.71
Folate-Total, µg	145 (98)	450	450	500	500	148 (89)	143 (97)	0.62	0.38
Folic Acid, µg	15.5 (22.5)	-	-	-	-	17.3 (21.6)	14.2 (20.8)	0.16	0.38
Food Folate, µg	129.2 (94.9)	-	-	-	-	130.6 (89.5)	128.8 (94.7)	0.85	0.95
Folate, DFE, µg	459 (157)	-	-	500	500	460 (135)	458 (157)	0.93	0.98
Choline, mg	155 (88)	-	-	550	550	174 (100)	151 (85)	0.0239 ²	0.38
Vit- B12, µg	0.5 (0.7)	2.4	2.4	2.8	2.8	0.6 (0.7)	0.5 (0.6)	0.09	0.38
Vit- A, IU	5009 (4678)	-	-	-	-	5011(4559)	4987 (4495)	0.96	0.98
Vit-A, RAE	759 (367)	-	-	1200	1300	792(302)	750 (392)	0.22	0.47
Retinol, µg	435 (244)	-	-	-	-	476 (209)	424 (267)	0.0312 ²	0.21
Alpha Carotene, (µg)	462 (798)	-	-	-	-	476.9 (818.4)	444.4 (725.1)	0.68	0.82
Beta Carotene, (µg)	1792(2347)	-	-	-	-	1769.5 (2333.5)	1777.6 (2236.4)	0.97	0.98
Beta Cryptoxanthin, (µg)	111(422)	-	-	-	-	95.2 (372.1)	130.4 (483.2)	0.41	0.64
Lycopene, µg	308.4 (1254.1)	-	-	-	-	548.5 (2447.5)	236.01 (518.35)	0.16	0.38
Lutein + Zeaxanthin, (µg)	1685.6 (2214.51)	-	-	-	-	1564.8 (2054.04)	1724.5 (2298.9)	0.49	0.71
Vit-E, mg	2 (1)	19	19	19	19	3 (1.4)	2(1.5)	0.0152 ²	0.38
Vit- D, µg	0.4 (1.0)	5	5	-	-	0.48 (0.8)	0.37 (0.64)	0.13	0.38
Vit-D, IU	16 (31)	-	-	600	600	19 (31)	15 (26)	0.14	0.38

Nutrient	Overall Sample	Adolescent RDD	Adult RDD	Adolescent DRI	Adult DRI	Adolescents (n=87)	Adults (n=245)	p-value ² <0.05 ³ BF<0.001	⁴ FDR Adj P-value
Vit-K, µg	68 (87)	55	55	75	90	63 (72)	71 (96)	0.40	0.33
SFA, g	3 (2)	29	29	-	-	4 (2.6)	4 (2.11)	0.06	0.33
MUFA, g	6 (3)	-	-	-	-	6 (3)	5.9(3)	0.12	0.38
PUFA, g	7 (2)	25	25	-	-	7 (3)	7 (2)	0.0211 ²	0.21
Cholesterol, mg	74 (92)	300	300	-	-	97 (115)	69 (86)	0.0155 ²	0.21

¹ - No recommended value for RDD & DRI;

FDR adj p-value- Benjamini Hochberg multiple comparison test

²Significant at p<0.05 (unadjusted);

³Significant at Bonferroni level p<0.001;

⁴Significant at Benjamini Hochberg level p<0.05

Table 4.3.A- Multiple linear regressions associating maternal nutrient intakes with Infant Growth Outcomes (WAZ, LAZ, HCAZ) in early postpartum (PP)¹

Nutrient	EARLY PP								
	WAZ			LAZ			HCAZ		
	WAZ β Coef.	Unadj. p- value ²	Adj. P- val ^{3,4}	LAZ β Coef.	Unadj. p- value ²	Adj. P-val ^{3,4}	HCAZ β Coef.	Unadj. p- value ²	Adj. P- val ^{3,4}
Energy, Kcal*100	0.074	0.010	0.38	0.0467	0.18	0.62	0.080	0.020 ²	0.25
CHO, g* 100	0.341	0.017	0.13	0.2288	0.19	0.62	0.378	0.029 ²	0.25
Fiber, g *10	0.293	0.022	0.14	0.1913	0.22	0.62	0.334	0.030 ²	0.25
Calcium, mg* 100	0.129	0.010	0.12	0.0944	0.12	0.60	0.1557	0.055	0.31
Magnesium, mg* 100	0.178	0.037	0.36	0.1609	0.12	0.60	0.1908	0.06	0.31
Sodium, mg * 1000	0.089	0.10	0.98	5.53e- 07	0.99	0.99	-0.0633	0.34	0.82
Selenium,µg*10	0.257	0.005	0.83	0.229	0.038 ²	0.46	0.1078	0.05	0.31
Vit-B6,mg *10	4.009	0.06	0.25	2.0243	0.43	0.81	5.828	0.023 ²	0.25
Folate, DFE, µg *100	0.192	0.005	0.12	0.1339	0.11	0.60	0.201	0.015 ²	0.25
Vit-A, RAE *100	0.125	0.045	0.21	-0.0085	0.76	0.91	0.0139	0.62	0.84
Retinol, µg *100	0.179	0.037	0.19	0.101	0.33	0.76	-0.0226	0.61	0.84
Lycopene, µg*100	-0.006	0.59	0.73	0.002	0.87	0.95	0.0071	0.56	0.84
Lut+Zea, µg *1000	0.015	0.72	0.81	0.2375	0.23	0.62	0.031	0.55	0.84
Vit-E, mg *10	1.047	0.10	0.31	4.119	0.007 ²	0.32	1.3444	0.08	0.37
MUFA,g *10	0.438	0.18	0.39	1.639	0.016 ²	0.37	0.5017	0.20	0.61
PUFA,g *10	1.014	0.013	0.12	1.004	0.043 ²	0.46	1.054	0.033 ²	0.25
Cholesterol, mg *10	0.007	0.43	0.62	-0.009	0.39	0.81	-0.0006	0.95	0.96

¹Models adjusted for maternal height, weight, parity, and infant sex² Significant at p<0.05 unadjusted for multiple comparisons³ Significant using Bonferroni adjustment for multiple comparisons (WAZ- p<0.025; LAZ- p< 0.01; HCAZ- p< 0.01)⁴Significant using FDR Benjamini Hochberg (p<0.05) adjustment for multiple comparisons

Table 4.3.B- Multiple linear regressions associating maternal nutrient intakes with Infant Growth Outcomes (WAZ, LAZ, HCAZ) in late postpartum (PP)¹

Nutrient	LATE PP								
	WAZ			LAZ			HCAZ		
	WAZ β Coef.	p-value	FDR Adj P-val	LAZ β Coef.	p-value	FDR Adj P-val	HCAZ β Coef.	p-value	FDR Adj P-val
Energy, Kcal*100	-0.002	0.94	1.03	0.3299	0.26	0.60	-0.001	0.96	0.97
CHO, g*100	-0.014	0.91	1.13	0.1428	0.33	0.60	0.005	0.97	0.97
Fiber, g*10	0.080	0.43	2.20	0.1359	0.25	0.60	-0.057	0.68	0.97
Calcium, mg*100	0.005	0.89	1.20	0.0498	0.30	0.60	-0.0454	0.42	0.97
Magnesium, mg*100	0.007	0.92	1.11	0.0899	0.29	0.60	-0.0407	0.68	0.97
Sodium, mg*1000	0.028	0.55	1.49	0.0472	0.39	0.60	-4.65e-0	0.94	0.97
Selenium, µg*10	0.004	0.96	1.03	0.093	0.30	0.60	-0.0089	0.88	0.97
Vit-B6, mg*10	0.7890	0.70	1.24	2.0632	0.40	0.60	-0.420	0.88	0.97
Folate, DFE, µg*100	0.043	0.40	2.30	0.0711	0.24	0.60	-0.044	0.53	0.97
Vit-A, RAE*100	-0.060	0.29	2.22	0.0174	0.54	0.62	0.0263	0.42	0.97
Retinol, µg*100	-0.197	0.039 ²	0.90	-0.233	0.038 ²	0.58	0.0683	0.15	0.97
Lycopene, µg*100	-0.209	0.008 ²	0.37	-0.263	0.005 ²	0.23	0.0142	0.63	0.97
Lut+Zea, µg*1000	-0.0164	0.61	1.34	-0.0977	0.11	0.60	-0.095	0.031 ²	0.97
Vit-E, mg*10	-0.4297	0.49	1.88	-0.168	0.32	0.60	-0.1583	0.85	0.97
MUFA, g*10	0.0495	0.87	1.25	1.294	0.18	0.60	0.1824	0.69	0.97
PUFA, g*10	-0.014	0.96	1.00	0.176	0.65	0.70	0.026	0.95	0.97
Cholesterol, mg*10	0.014	0.22	2.02	0.030	0.029 ²	0.58	0.0122	0.44	0.97

¹Models adjusted for maternal height, weight, parity, and infant sex²Significant at p<0.05 unadjusted for multiple comparisons³Significant using Bonferroni adjustment for multiple comparisons (WAZ- p<0.025; LAZ- p< 0.01; HCAZ- p< 0.01)⁴Significant using FDR Benjamini Hochberg (p<0.05) adjustment for multiple comparisons

Table 4.4.A- Multiple logistic regressions associating maternal nutrient intakes with Infant Growth Outcomes (underweight, stunting, low cranial growth) in early postpartum (PP)¹

	EARLY PP								
	Underweight (WAZ<-2)			Stunting (LAZ<-2)			Low cranial HC (HCAZ < -1)		
Nutrient	OR	p-value	FDR adj p-value	OR	p-value	FDR adj p-value	OR	p-value	adj p-value
Fiber, g	0.957	0.21	0.88	0.9717	0.25	0.98	0.9330	0.011 ²	0.88
Folate total, µg	0.998	0.54	0.96	0.9985	0.49	0.98	0.9945	0.020 ²	0.23
Food Folate, µg	0.9989	0.70	0.97	0.9994	0.81	0.98	0.9941	0.015 ²	0.23
Folate, DFE, µg	0.9957	0.026 ²	0.60	0.9974	0.06	0.98	0.9956	0.003 ²	0.95
PUFA, g	0.7511	0.016 ²	0.60	0.8899	0.8899	0.98	0.8442	0.039 ²	0.32
Cholesterol, mg	0.9981	0.41	0.88	1.0011	0.49	0.98	0.9998	0.93	0.95

¹ Models adjusted for maternal height, weight, parity, and infant sex² Significant at p<0.05 unadjusted for multiple comparisons³ Significant using Bonferroni adjustment for multiple comparisons (WAZ- p<0.025; LAZ- p< 0.01; HCAZ- p< 0.01)⁴ Significant using FDR Benjamini Hochberg (p<0.05) adjustment for multiple comparisons

Table 4.4.B-Multiple logistic regressions associating maternal nutrient intakes with Infant Growth Outcomes (underweight, stunting, low cranial growth) in late postpartum (PP)¹

	LATE PP								
	Underweight (WAZ<-2)			Stunting (LAZ<-2)			Low cranial HC (HCAZ<-1)		
Nutrient	OR	p-value	adj p-value	OR	p-value	FDR adj p-value	OR	p-value	FDR adj p-value
Fiber, g	0.9983	0.95	0.99	0.9957	0.84	0.97	1.0021	0.91	0.97
Folate total, µg	1.0001	0.96	0.99	1.0021	0.23	0.97	1.0010	0.52	0.97
Food Folate, µg	1.0001	0.95	0.99	1.0017	0.33	0.97	1.0009	0.53	0.97
Folate, DFE, µg	0.9996	0.79	0.99	1.0000	0.99	0.99	1.0001	0.91	0.97
PUFA, g	0.9967	0.98	0.99	0.9601	0.53	0.97	1.0063	0.92	0.97
Cholesterol, mg	0.9895	0.030 ²	0.89	0.9971	0.22	0.97	0.9993	0.76	0.97

¹ Models adjusted for maternal height, weight, parity, and infant sex² Significant at p<0.05 unadjusted for multiple comparisons³ Significant using Bonferroni adjustment for multiple comparisons (WAZ- p<0.025; LAZ- p< 0.01; HCAZ- p< 0.01)⁴ Significant using FDR Benjamini Hochberg (p<0.05) adjustment for multiple comparisons

Table 4.5.A- Age group modifies the association between maternal nutrients and WAZ & LAZ in early postpartum

	Early PP								
	Unadjusted Effect Modification ¹		Adjusted Effect Modification ²			Stratified by maternal age			
						Adolescents ²		Adults ²	
Nutrients	β Coef.	Unadj. p-value ³	β Coef.	Unadj. p-value ³	Adj. p-value ^{4,5}	β Coef. ²	Unadj. p-value ³	β Coef. ²	Unadj. p-value ³
Weight-for-age-z-scores									
Sugar, g*10	-0.21	0.020	-0.02	0.046	0.050	0.15	0.083	-0.04	0.30
Selenium, μ g*10	-0.23	0.035	-0.02	0.031	0.050	0.25	0.005	0.04	0.52
Vit-A, RAE*100	-0.13	0.049	0.00	0.049	0.050	0.13	0.033	-0.01	0.81
Retinol, μ g*100	-0.24	0.013	0.002	0.014	0.050	0.22	0.011	-0.06	0.17
Length-for-age-z-scores									
Sugar, g*10	0.26	0.020	-0.0215	0.05	0.050	0.1201	0.23	-0.0647	0.15
Selenium, μ g*10	-0.27	0.035	-0.0267	0.037	0.0463 ⁴	0.1992	0.045	-0.0296	0.65
Vit-E, mg*10	-3.97	0.023	-0.39	0.027	0.045 ⁴	3.11	0.032	0.24	0.79
SFA, g*10	-2.28	0.024	-0.25	0.014	0.045 ⁴	1.41	0.046	-0.87	0.18
MUFA, g*10	-1.87	0.025	-0.19	0.018	0.045 ⁴	1.47	0.017	-0.23	0.63

¹Unadjusted model²Model adjusted for maternal height, weight, parity, and infant sex³Significant at p<0.05 unadjusted for multiple comparisons⁴Significant using FDR Benjamini Hochberg (p<0.05) adjustment for multiple comparisons⁵Significant using Bonferroni adjustment for multiple comparisons (p<0.001)

Table 4.5.B- Age group modifies the association between maternal nutrients and WAZ & LAZ in late postpartum

Nutrients	Late PP								
	Unadjusted Effect Modification ¹		Adjusted Effect Modification ²			Stratified by maternal age			
						Adolescents ²		Adults ²	
	β Coef.	Unadj. p-value ³	β Coef.	Unadj. p-value ³	Adj. p-value ^{4,5}	β Coef. ²	Unadj. p-value ³	β Coef. ²	Unadj. p-value ³
Weight-for-age-z-scores									
Retinol, μg^*100	0.20	0.048	0.00	0.050	0.05	-0.22	0.07	0.00	0.92
Lycopene, μg^*100	0.23	0.005	0.002	0.004	0.008 ⁴	-0.23	0.019	0.02	0.23
Length-for-age-z-scores									
Retinol, μg^*100	0.30	0.013	0.003	0.019	0.011 ⁴	-0.25	0.07	0.05	0.28
Lycopene, μg^*100	0.29	0.003	0.003	0.002	0.006 ⁴	-0.29	0.008	0.03	0.23
Lut+Zea, μg^*1000	0.20	0.025	0.000	0.018	0.011 ⁴	-0.12	0.10	0.09	0.06

¹Unadjusted model²Model adjusted for maternal height, weight, parity, and infant sex³Significant at $p < 0.05$ unadjusted for multiple comparisons⁴Significant using FDR Benjamini Hochberg ($p < 0.05$) adjustment for multiple comparisons⁵Significant using Bonferroni adjustment for multiple comparisons ($p < 0.001$)

Table 4.6.A- Age group modifies the association between maternal nutrients and infant growth outcomes (underweight (**WAZ<-2SD**), stunting (**LAZ<-2SD**), low cranial growth (**HCAZ<-1SD**)) in early PP in multiple logistic regressions¹

	EARLY PP								
	Unadjusted Effect Modification ¹		Adj. Effect Modification ²			Stratified by maternal age			
						Adolescents ²		Adults ²	
Nutrients	OR	Unadj. p-value ³	OR	Unadj. p-value ³	Adj. p-value ^{4,5}	OR	Unadj. p-value ³	OR	Unadj. p-value ³
Underweight (WAZ < -2SD)									
Choline	1.014	0.030	1.014	0.025 ²	0.025 ⁴	0.989	0.06	1.003	0.35
Cholesterol	1.015	0.030	1.01	0.022 ^{2,3}	0.025 ⁴	0.988	0.06	1.003	0.27
Stunting (LAZ < -2SD)									
Lipids	1.125	0.040	1.133	0.032 ²	0.034 ⁴	0.8534	0.018	1.016	0.57
Vit B12	3.322	0.036	3.675	0.034 ²	0.034 ⁴	0.274	0.06	1.812	0.09
Vit E	2.624	0.012	2.809	0.008 ²	0.034 ⁴	0.200	0.015	1.171	0.28
SFA	1.532	0.026	1.581	0.022 ²	0.034 ⁴	0.618	0.029	1.113	0.28
MUFA	1.445	0.019	1.452	0.019 ²	0.034 ⁴	0.652	0.018	1.059	0.44
Low Cranial HC (HCAZ < -1SD)									
Calcium	0.995	0.038	0.995	0.044 ²	0.06	1.003	0.21	0.998	0.041
Iron	0.825	0.012	0.836	0.021 ²	0.05	1.015	0.60	0.862	0.046
Zinc	0.798	0.047	0.817	0.07	0.07	1.034	0.62	0.877	0.15
Thiamine	0.074	0.016	0.084	0.024 ²	0.05	1.290	0.62	0.166	0.08
Folate, DFE	0.993	0.024	0.993	0.030 ²	0.05	1.001	0.82	0.994	0.002

¹Unadjusted model

²Model adjusted for maternal height, weight, parity, and infant sex

³Significant at p<0.05 unadjusted for multiple comparisons

⁴Significant using FDR Benjamini Hochberg (p<0.05) adjustment for multiple comparisons

⁵Significant using Bonferroni adjustment for multiple comparisons (WAZ- p<0.025; LAZ- p< 0.01; HCAZ- p< 0.01)

Table 4.6.B- Age group modifies the association between maternal nutrients and infant growth outcomes (underweight (**WAZ<-2SD**), stunting (**LAZ<-2SD**), low cranial growth (**HCAZ<-1SD**)) in late PP in multiple logistic regressions¹

	LATE PP								
	Crude Effect Modification		Adj. Effect Modification		Benjamini Hochberg	Adolescents		Adults	
Nutrients	Adj OR	p-value	Adj OR	p-value ² <0.05 ³ BF adj	⁴ FDR Adj p-value	Adj OR	p-value	OR	p-value
	Underweight (WAZ < -2SD)								
Choline	1.020	0.08	1.017	0.15	0.22	0.980	0.12	1.000	0.98
Cholesterol	1.007	0.59	1.007	0.60	0.22	0.984	0.22	0.992	0.13
	Stunting (LAZ < -2SD)								
Lipids	1.050	0.38	1.050	0.42	0.4	0.940	0.35	0.990	0.72
Vit B12	0.739	0.63	1.257	0.75	0.4	0.379	0.34	0.838	0.65
Vit E	0.803	0.48	0.857	0.65	0.4	1.431	0.40	1.070	0.63
SFA	1.080	0.74	1.202	0.48	0.4	0.721	0.36	0.977	0.81
MUFA	1.126	0.47	1.169	0.38	0.4	0.820	0.36	1.000	0.99
	Low Cranial HC (HCAZ < -1SD)								
Calcium	1.001	0.64	1.001	0.67	0.76	0.999	0.76	1.000	0.62
Iron	1.040	0.69	1.050	0.64	0.76	0.959	0.66	1.034	0.55
Zinc	0.966	0.80	1.009	0.95	0.76	1.061	0.65	1.085	0.38
Thiamine	1.945	0.61	2.299	0.54	0.76	0.617	0.69	2.268	0.27
Folate, DFE	1.001	0.58	1.001	0.49	0.76	0.998	0.44	1.001	0.49

¹Unadjusted model

²Model adjusted for maternal height, weight, parity, and infant sex

³Significant at p<0.05 (unadjusted for multiple comparisons)

⁴Significant using FDR Benjamini Hochberg (p<0.05) adjustment for multiple comparisons

⁵Significant using Bonferroni adjustment for multiple comparisons (WAZ- p<0.025; LAZ- p< 0.01; HCAZ- p< 0.01)

Supplementary Table 4-S.1.A- Prevalence of nutrient inadequacy by age category by comparing the maternal nutrient intakes to the INCAP RDD

% of population inadequate based on RDD (n)					
Nutrients	RDD Adolescent/ Adults	Adolescents (n=125)	Adults (n=320)	p-value	Adjusted p-value ¹<0.05 ²<0.002
Energy, Kcal	2550/2650	100 (125)	99.7	0.53	0.83
Protein, g	84/87	97.6 (122)	99.1 (317)	0.23	1.00
Lipids, g	81/81	100 (125)	100 (320)	NC	0.83
Carbohydrates, g	160/160	3.2 (4)	6.2 (20)	0.20	0.990
Fiber, g	25/25	40 (50)	45 (144)	0.34	0.83
Sugar, g	66/66	48.8 (61)	57.8 (185)	0.09	1.00
Calcium, mg	1000/1000	94.4 (118)	94.4 (302)	0.99	0.98
Iron, mg	15.6/15.6	92.8 (116)	91.25 (292)	0.59	0.83
Magnesium, mg	230/230	3.2 (4)	7.19 (23)	0.11	0.97
Phosphorus, mg	580/580	0.8 (1)	0.3 (1)	0.49	1.00
Potassium, mg	5100/5100	100 (125)	100 (320)	NC	0.97
Sodium, mg	1500/1500	24 (30)	26.9 (86)	0.53	0.97
Zinc, mg	22.6/22.6	99.2 (124)	99.7 (319)	0.49	0.83
Copper, mg	1/1	20.8 (26)	26.2 (84)	0.23	1.00
Manganese, mg	2.6/2.6	99.2 (124)	95.9 (307)	0.77	0.83
Selenium, µg	54/54	49.6 (62)	55.6 (178)	0.25	1.00
Vit-C, mg	100/100	91.2 (114)	91.9 (294)	0.81	0.87
Thiamin, mg	1.1/1.1	96.8 (121)	94.4 (302)	0.29	0.97
Riboflavin, mg	1.3/1.3	97.6 (122)	98.4 (315)	0.55	1.00
Niacin, mg	13/13	100 (125)	100 (320)	NC	1.00
Pantothenic Acid, mg	7/7	78.4 (98)	78.7 (252)	0.93	0.990
Vit- B6, mg	1.7/1.7	92.8 (116)	95.0 (304)	0.36	0.83
Folate-Total, µg	450/450	100 (125)	98.7 (316)	0.21	1.00
Vitamin B12, µg	2.4/2.4	96 (120)	98.4 (315)	0.12	1.00
Vit-E, mg	19/19	100 (125)	100 (320)	NC	1.00
Vit- D, µg	5/5	100 (125)	100 (320)	NC	1.00
Vit-K, µg	55/55	60.8 (76)	59.1 (189)	0.74	0.97
SFA, g	29/29	100 (125)	100 (320)	NC	0.83
PUFA, g	25/25	100 (125)	100 (320)	NC	1.00
Cholesterol, mg	300/300	96.6 (117)	97.5 (312)	0.047 ¹	0.83

-No value

NC- Non-computable

FDR adj p-value- Benjamini Hochberg multiple comparison test

¹Significant at p<0.05 (unadjusted)²Significant at Bonferroni level p<0.001³Significant after adjusting FDR (p<0.05)

Supplementary Table 4-S.1.B- Prevalence of nutrient inadequacy by age category by comparing the maternal nutrient intakes to the IOM DRI

% of population inadequate based on DRI (n)				
Nutrients	DRI Adolescent/adult	Adolescents (n=125)	Adults (n=320)	p-value ¹ <0.05 ² BF adj (<0.002)
Water, g	3800/3800	100 (125)	100 (320)	NC
Energy, Kcal	2200/2400	100 (125)	99.7 (319)	0.53
Protein, g	71/71	92.0 (115)	98.4 (315)	0.0007 ^{1,2}
Carbohydrates, g	210/210	20 (25)	23.4 (75)	0.43
Fiber, g	29/29	60 (75)	63.7 (204)	0.46
Sugar, g	58/58	38.4 (48)	43.4	0.33
Calcium, mg	1300/1000	100 (125)	94.4 (302)	0.0068 ¹
Iron, mg	10/9	32.8 (41)	34.1 (109)	0.80
Magnesium, mg	360/360	28.8 (36)	38.1 (122)	0.06
Phosphorus, mg	1250/700	1.6 (2)	0.9 (3)	0.55
Potassium, mg	5100/5100	89.6 (112)	97.2 (311)	0.0009 ^{1,2}
Sodium, mg	1500/1500	45.6 (57)	47.8 (153)	0.67
Zinc, mg	13/12	97.6 (122)	97.5 (312)	0.95
Copper, mg	1.3/1.3	55.2 (69)	60.9 (195)	0.27
Manganese, mg	2.6/2.6	99.2 (124)	95.9 (307)	0.8
Selenium, µg	70/70	75.2 (94)	83.7 (268)	0.0374 ¹
Vit-C, mg	115/120	92.8 (116)	95.6 (306)	0.22
Thiamin, mg	1.4/1.4	98.4 (123)	96.6 (309)	0.30
Riboflavin, mg	1.6/1.6	99.2 (124)	99.4 (318)	0.83
Niacin, mg	17/17	78.4 (98)	78.7 (98)	0.93
Pantothenic Acid, mg	7/7	100 (125)	100 (320)	NC
Vit- B6, mg	2/2	97.6 (122)	98.1 (314)	0.72
Folate, DFE, µg	500/500	65.6 (82)	64.4 (206)	0.81
Folate-Total, µg	500/500	100 (125)	99.1 (317)	0.28
Vitamin B12, µg	2.8/2.8	97.6 (122)	98.7 (316)	0.38
Vit-A, RAE	1200/1300	91.2 (114)	90.9 (291)	0.93
Vit-E, mg	19/19	100 (125)	100 (320)	NC
Vit-D, IU	600/600	100 (125)	100 (320)	NC
Vit-K, µg	75/90	70.4 (88)	76.9 (246)	0.16

-No value

NC- Non-computable

FDR adj p-value- Benjamini Hochberg multiple comparison test

¹Significant at p<0.05 (unadjusted)²Significant at Bonferroni level p<0.001³Significant after adjusting FDR (p<0.05)

CHAPTER 5. DISCUSSION

Major findings

The Guatemalan diet has been more extensively studied than any other diet in developing societies (Solomons, 2000), but there are no recent studies that have examined the adequacy of the Guatemalan diet during lactation. To our knowledge, ours is the first study to explore the nutrient adequacy of the Guatemalan traditional diet and this is also the first study in-the *Mam-Mayan* Indigenous population of Guatemala. Our first objective was to describe the nutrient intakes and adequacy of the pregnant and lactating *Mam-Mayan* mothers and compare the adequacy with both RDD's and DRI's. Based on our observations of wide spread of nutrient deficiencies among the population as shown in supplementary Table 4-S1, we hypothesized that *Mam-Mayan* lactating women would have inadequate intakes of selected nutrients. Our second objective was to explore the associations between maternal nutrient intakes and early infant growth outcomes in the first six months of lactation. The hypothesis was that infant growth would be associated with maternal macro and micronutrient intakes during lactation. The third objective was to assess the differential impact of maternal age (adolescent vs adults) on infant growth and our hypothesis was that the association between maternal nutrient intakes and infant growth outcomes will differ between adolescent and adult mothers.

Four major findings emerged. We found that

1. Intakes of carbohydrates, fiber, sugars, magnesium, phosphorous and sodium were adequate when evaluated using IOM DRI and INCAP RDD, but all other nutrients fell below the DRI and RDD. Furthermore, intakes of protein, total lipids, choline, retinol, vitamin E, and cholesterol were lower in adult compared adolescent mothers .
2. In early postpartum:
 - WAZ- Higher intakes of energy and two macro nutrients (carbohydrates, fiber) and seven nutrients (calcium, magnesium, selenium, folate, vitamin A, retinol, PUFA) were associated with increased WAZ. However, only higher intakes of folate, DFE and PUFA's lowered the odds of the infant being underweight.

- LAZ: Higher intakes of lipids (MUFA & PUFA) and two micro-nutrients (selenium, vitamin E) were associated with higher LAZ. Interestingly, no nutrient was significantly associated with reducing the odds of stunting.
 - HCAZ: Higher intakes of energy, three macro nutrients (carbohydrates, fiber, PUFA) and two micro-nutrients (vitamin B6, folate) were associated with increased HCAZ. Three of these nutrients (fiber, folate total, PUFA) were associated with reduced odds of having low cranial head circumference.
3. In contrast to the early postpartum, far fewer nutrients were associated with infant growth in late lactation. Retinol and lycopene were negatively associated with WAZ and LAZ whereas lutein & zeaxanthin were negatively associated with HCAZ. The only nutrient positively associated with infant growth was cholesterol, which was associated with higher LAZ and also the only nutrient which was associated with reduced odds of infant being underweight.
 4. We found that maternal intakes during first six weeks of breastfeeding had a significant impact on infant growth outcomes compared to nutrient intakes during late postpartum.
 5. Finally, maternal age modified the association between maternal nutrients and infant growth parameters. Adolescent mother's nutrient intakes were more often associated with infant growth outcomes compared to adult mother's nutrient intakes.

Adequacy of traditional Guatemalan diet

The traditional Indigenous diet of Guatemala is a “triad” of corn, beans, and squashes and is mostly plant-based (Solomons, 2000). We relate our findings to these staple foods of the Guatemalan diet. Corn , beans, and squashes compliment each other nutritionally when consumed together.

Corn

Corn is ingested in a standard form of tortillas (Bermudez, Hernandez, Mazariegos, & Solomons, 2008; Lee, Talegawkar, Merialdi, & Caulfield, 2013; Solomons, 2000). Tortillas were one of the top ten frequently consumed foods by the pregnant women of Guatemala (Schutz, Lechtig, & Bradfield, 1980). Corn is a rich source of energy, carbohydrates, fiber, good source of magnesium, phosphorous, zinc, and calcium. Our findings for mean energy intake of the lactating

mothers 1317 ± 349 Kcal was less than previously reported (1929 ± 360 Kcal) (Fitzgerald et al., 1993; Lander et al., 2019; Schutz et al., 1980). This difference in our energy intake findings could be related to the time of our data collection. Late November to early March is considered as dry season in Guatemala. Approximately half of our dietary data from our lactating mothers was collected during this dry period. The maternal intake in dry season is comparatively less and this might have shown impact on our energy findings (Chomat et al., 2015). On the other hand, high intakes of corn provide adequate amounts of fiber (Fitzgerald et al., 1993). However, mean fiber intake of our population was 27 ± 8 g which aligns with the previous findings (Fitzgerald et al., 1993).

The Guatemalan diet is mostly plant-based and high intake of corn in the form of tortillas is the main source of carbohydrates (Bermudez et al., 2008; Lander et al., 2017; Lander et al., 2019; Lee et al., 2013; Solomons, 2000). The carbohydrate intakes among our study population were 263 ± 69 g. Our results align with the previous findings (Lander et al., 2019; Solomons, 2000) showing that the population was more than adequate for carbohydrates intake. Since the diet was mostly plant-based and rich in carbohydrates and fiber, this explains the carbohydrate and fiber adequacy of the diet when compared to both IOM DRI and INCAP RDD among the lactating mothers. The plant-based diet was also rich in sugars, and our data also shows that the population is consuming more than required amounts of sugar. Because the Guatemalan diet was largely plant-based, the population consumed less amounts of animal meat (Valdes-Ramos, Cervantes, Mendoza-Perdomo, Anderson, & Solomons, 2006) and the average fat consumption was 20 ± 8 g, where 100% of mothers had inadequate intakes based on RDD. These findings corroborate with the previous findings of fat intakes among the Guatemalan population (Lander et al., 2019; Solomons, 2000; Valdes-Ramos et al., 2006).

Corn (tortillas) is also considered a good source of magnesium, phosphorous, and zinc (Fitzgerald et al., 1993; Krause et al., 1992; Lander et al., 2019; Lee et al., 2013; Solomons, 2000). Our population intakes of magnesium, phosphorous, and iron were adequate when compared to the nutrient standards. These findings aligned with previous reports findings of adequacy of these nutrients due to the high consumption of corn (tortillas) (Fitzgerald et al., 1993; Lander et al.,

2019; Lee et al., 2013; Solomons, 2000). Our data also showed that the diet of lactating mothers was also adequate for magnesium and phosphorous when compared to both IOM DRI and INCAP RDD but iron was adequate when compared to the IOM DRI only. However, our population intakes for calcium and zinc were lower compared to these previous findings (Lander et al., 2019; Schutz et al., 1980; Solomons, 2000; Vossenaar & Solomons, 2012), and were inadequate for calcium and zinc when compared to the RDD and DRI.

Beans

Beans are a rich source of protein, fiber, and iron. Along with corn, beans also provide a source of dietary protein. Despite the consumption of corn and beans, the of the majority population had inadequate protein intake in our analysis. Our results do not align with the earlier findings of Fitzgerald and Lander for pregnant and lactating mothers' protein intakes of (Fitzgerald et al., 1993; Lander et al., 2019). Fitzgerald concluded that the mean protein intake of the pregnant mothers in Guatemala was $63 \pm 13.3\text{g}$ (Fitzgerald et al., 1993), Lander's finding was also similar to Fitzgerald findings, and both were higher than our population of lactating mothers (adolescent $42 \pm 17\text{g}$; adult $38 \pm 14\text{g}$). Furthermore, these protein intakes significantly differed between adolescent and adult mothers in our analysis. In general, the prevalence of protein inadequacy among adolescent mothers was 97.6% based on the RDD and 92% based on the DRI; whereas for adult mothers it was 99.1% based on the RDD and 98.4% based on the DRI (Table 4-S1). Regardless of the nutrient standard used, protein intakes were highly inadequate for both adolescents and adult mothers.

Squashes

Squashes are a rich source of carotenoids, and a good source of vitamin C. Our findings showed that lactating mothers were deficient for vitamin C intakes. These finding do not align with the previous findings of vitamin C (Lander et al., 2019; Lee et al., 2013). Although the populations diet contains vitamin C rich food sources, they are deficit for vitamin C. This shows that the lactating mothers were not consuming enough of the available foods that provide vitamin C, potentially due to the lower access of these foods during the dry season.

Comparative Adequacy of Nutrient Intake

This is the first set to uncover the significant gap in the literature of the maternal intakes of adolescent and adult mothers during lactation among Guatemalan Mam-Mayan women. We couldn't compare our findings with that of other findings as there are no studies that looked at the maternal nutrient intakes of adolescent and adult mothers.

Water-soluble vitamins

We calculated the water-soluble vitamin (thiamine, riboflavin, niacin, choline, pantothenic acid, vitamin B6, vitamin B12, folate) intakes of the lactating mothers and compared their intake to the RDD and DRI. Our findings do not corroborate previous findings about the water-soluble vitamin's inadequacy among the Guatemalan mothers (Lander et al., 2019; Solomons, 2000; Valdes-Ramos et al., 2006). We found that except folate (DFE), the mothers were inadequate for all the other vitamins when compared to both RDD and DRI (Lander et al., 2019). Moreover, prevalence of vitamin B12 inadequacy in our study was even higher (Jones, Ramirez-Zea, Zuleta, & Allen, 2007) as the population consumed less animal sources foods and had lower protein intakes than previously reported (Jones et al., 2007).

Fat-soluble vitamins

We calculated the maternal intakes of vitamin A, D, E, and K and compared them to RDD and DRI to know the adequacy of the lactating mothers of Guatemala. We found that the mothers were consuming inadequate amounts of vitamin A, D, and E when compared to both RDD and DRI. Vitamin K was adequate among the mothers when compared to RDD but was inadequate when compared to DRI. Our findings align with the previous findings for vitamin A, and D deficiency (Solomons, 2000) (Bielderman, Vossenaar, Melse-Boonstra, & Solomons, 2016) (Sud et al., 2010). Vitamin E intakes were considered to be normal to high in the Guatemalan population (Solomons, 2000), but were deficient in our study population. This observation for vitamin E would suggest a decline in vitamin E source foods such as (list) since the earlier reports where low intakes had not been reported (Solomons, 2000).

Minerals

Our study population was adequate for sodium intake when compared to RDD and DRI. In fact, mothers' sodium consumption surpassed the nutrient recommendations for both RDD and DRI. However, our finding does not corroborate previous findings by Solomons. He concluded that the sodium intake in the Guatemalan population was low (Solomons, 2000).

Trace elements

We calculated the iron, zinc, copper, selenium, and manganese intakes of the mothers and compared them to RDD and DRI. We found that the majority of mothers were consuming inadequate amounts of manganese when compared to both RDD and DRI but adequate amounts of copper and selenium when compared to DRI only. Our findings about copper align with the previous findings by Fitzgerald but does not align with the manganese findings (Fitzgerald et al., 1993). He found that the Guatemalan mothers manganese intake was $2.8 \pm 0.6\text{mg}$ but our population manganese intake was $1.4 \pm 0.6\text{mg}$.

Association of maternal nutrient intakes on infant growth outcomes

We investigated the linear association (Tables 4.3.A & 4.3.B) of maternal nutrient intakes on infant WAZ, LAZ & HCAZ and the odds of maternal nutrient intakes on infant underweight, stunting, and low cranial head circumference during early and late lactation and after adjustment, we observed that distinct nutrients emerged as the determinants of WAZ, LAZ, HCAZ and infant underweight, stunting, and low cranial HC between early and late lactation. Specific findings included: 1) in our linear regression models, ten nutrients (energy, carbohydrates, fiber, calcium, magnesium, selenium, folate (DFE), vitamin A (RAE), retinol, and PUFA) were associated with infant WAZ, six nutrients (energy, carbohydrates, fiber, vitamin B6, folate (DFE), and PUFA) were associated with infant HCAZ and only four nutrients (selenium, vitamin E, MUFA, and PUFA) were associated with infant LAZ in early lactation, 2) in our logistic regression models, more nutrients (fiber, folate total, food folate, folate (DFE), PUFA) were associated with infant low cranial HC, only two nutrients (folate (DFE), and PUFA) were associated with infant underweight and none with infant stunting in early lactation,

Notably, energy, carbohydrates, fiber, PUFA were associated with infant WAZ and HCAZ but not with infant LAZ (Tables 4.3.A & 4.3.B). Few nutrients were associated with infant anthropometry in late but none of these nutrients in early and late lactation were significant with more conservative post-hoc comparisons using either Bonferroni or Benjamini Hochberg adjustments. From our findings in multiple logistic models, we observed that the odds of infant having low cranial HC was low with higher intakes of larger number of nutrients (fiber, folate, and PUFA) with PUFA having the largest impact on infant low cranial HC and infant underweight but none of these nutrients were significant with more conservative post-hoc comparisons using either Bonferroni or Benjamini Hochberg adjustments. Based on the findings by Rao et al., maternal energy and protein intakes were not associated with birth size of the infants, but higher fat intakes were associated with neonatal length, and birth weight (Rao et al., 2001). But our findings showed that higher energy and carbohydrate intakes were associated with increased infant WAZ and HCAZ, but the LAZ remained unaffected. Our findings do align with these previous findings showing higher fat intake was associated with infant LAZ, but in our population, energy was also associated with higher infant WAZ and HCAZ. Ramakrishnan et al., in his observational study suggested that the preconceptional intake of vitamin and mineral supplements were associated with reduced risk of low birth weight (Ramakrishnan, Grant, Goldenberg, Zongrone, & Martorell, 2012). Our findings extend this observation to the first six months of lactation. Our results showed that the increased maternal micronutrient intakes, significantly increased infant LAZ.

Colón-Ramos et al., found that mothers in Southern US who consumed processed-South dietary pattern which had the highest energy content, total fat%, total sugar, iron, zinc, sodium, and meats and low whole grains content and mothers who consumed processed dietary patterns with highest trans fat, total grains and potato servings were negatively associated with infant WAZ and HCAZ and positively associated with infant LAZ (Colon-Ramos et al., 2015). Our findings do not align with these previous findings of Colon-Ramos et al., for infant WAZ and HCAZ but align with the findings of LAZ (Colon-Ramos et al., 2015). Our results showed that increased maternal intakes of energy and fats have significantly increased infant WAZ and HCAZ (Tables 4.3.A & 4.3.B). Our findings in Tables 4.3.A & 4.3.B, also revealed that maternal vitamin A did not

have any significant effect on infant LAZ. Our findings do not align with the previous findings of Prawirohartono et al., and conclusions of prenatal vitamin A intakes effect on infant LAZ among children up to 2 years in rural Java, Indonesian . They also concluded that maternal zinc intakes do not have any significant impact on infant LAZ and WAZ (Prawirohartono et al., 2011). Our findings align with the previous findings about the non-significant effect of zinc on infant WAZ and LAZ.

We tested for effect modification of maternal age on the association of maternal nutrient intakes with infant WAZ, LAZ, and HCAZ. In our multiple linear regression models (Tables 4.5.A & 4.5.B), significant effect modification by maternal age was observed for maternal intakes of selenium, vitamin A, and retinol on infant WAZ during early lactation. However, infant WAZ was significantly lowered for infants of adolescent mothers with higher lycopene intakes in late lactation which was significant following both the Bonferroni and Benjamini Hochberg adjustments but not for infants of adult mothers. In contrast, different nutrients were associated with infant LAZ compared to nutrients that impacted infant WAZ. Significant effect modification by maternal age was observed for maternal intakes of selenium, vitamin E, SFA, and MUFA in early lactation. In this case, adolescent mothers who had higher intakes of vitamin E, SFA, and MUFA had higher LAZ in the first six months of lactation whereas infants of adult mothers showed no improvement. In late lactation, maternal lycopene intakes were associated with significant reduction of infant LAZ. These nutrients were significant at the Benjamini Hochberg adjustment for multiple comparisons.

We also tested for effect modification of maternal age on the association of maternal nutrient intakes with infant underweight, stunting, and low cranial HC in our multiple logistic models (Tables 4.6.A & 4.6.B). We had four nutrients (lipids, vitamin E, SFA, MUFA) that were associated with lower odds of infant stunting for only adolescent mothers in early lactation, whereas in adult mothers, three nutrients (calcium, iron, and folate) that were associated with reduced odds of infant low cranial HC in early lactation. We could not compare our results for effect modification with previous literature because there are no studies that have investigated this relationship.

Findings by Roth et al., revealed that maternal supplementation of vitamin D from mid-pregnancy until 6 months postpartum did not improve infant growth outcomes (infant LAZ, WAZ, HCAZ) (Roth et al., 2018). Our findings also observed that maternal intakes of vitamin D had no significant impact on infant growth outcomes (WAZ & HCAZ) but our findings align with the previous findings of no association of vitamin D on infant growth outcomes (Roth et al., 2018). However, Prawirohartono et al., from their randomized controlled trial discovered that prenatal vitamin A supplementation had small but significant effect on infant LAZ until 18 months of age (Prawirohartono, Nyström, Ivarsson, Stenlund, & Lind, 2011). In our study we discovered mixed relationship between vitamin A and infant growth outcomes.

Roberfroid et al., from their randomized controlled trial observed that infants of mothers who received prenatal multiple micronutrient supplementation containing one RDA of 15 micronutrients (vitamin A, D, E, B6, B12, C, thiamine, riboflavin, niacin, folic acid, zinc, iron, copper, selenium, iodine) had significantly higher LAZ, WAZ and HCAZ by the end of first year of life (Roberfroid et al., 2012). Our findings revealed that maternal consumption of selenium, vitamin B6, folate, and vitamin E increased infant growth outcomes (WAZ, LAZ & HCAZ). Our findings align with the previous findings of Roberfroid et al., about the significant impact of selenium, vitamin B6, folate, and vitamin E on infant growth outcomes (WAZ, LAZ & HCAZ) (Roberfroid et al., 2012).

Impact of maternal age on infant growth outcomes

From our results we can conclude that age varied the relationship between the select nutrient intakes of mothers and infant WAZ (Tables 4.5.A & 4.5.B). In early postpartum, higher intakes of selenium, vitamin A, and retinol significantly increased infant WAZ. Higher intakes of selenium, vitamin E, SFA, and MUFA significantly increased infant LAZ in early postpartum. Therefore, our findings indicate that, we need to focus on improving the nutrition for adolescent mothers in early postpartum for better infant WAZ.

In Tables 4.6.A & 4.6.B, our logistic models showed that adolescent mothers nutrients intake had increased the odds of infant underweight and stunting. Our findings align with the previous findings of increased risk of SGA and stunting among the infants of adolescent mothers

(Johnson & Moore, 2016). Akseer et al., in a recent review article compared the birth outcomes for adolescent mothers (≤ 19 years) to adult mothers (>19 years) in low and middle-income countries (Akseer et al., 2022). They obtained the data from 20 randomized controlled trials. Infants of mothers aged 10-14 years had a 23% increased risk of preterm birth, 60% increased risk of perinatal mortality, 63% increased risk of neonatal mortality, 28% increased risk of LBW and 22% increased risk of SGA when compared to infants of mothers aged 20-29 years (Akseer et al., 2022). Our findings align with the previous findings, where the infants of the adolescent mothers had adverse growth compared to infants of adult mothers.

Public policy recommendations

Intakes of carbohydrates, fiber, sugars, magnesium, phosphorous and sodium were adequate when evaluated using IOM DRI and INCAP RDD, but all other nutrients fell below the DRI and RDD. Furthermore, intakes of protein, total lipids, choline, retinol, vitamin E, and cholesterol were lower in adult compared adolescent mothers

Strengths

Our study is the first to investigate the association of maternal diet with infant growth in *Mam-Mayan* Indigenous population of Guatemala. We are also first to examine the effect modification of maternal age on the association between maternal nutrient intakes and infant growth outcomes. We have used the largest and most comprehensive dietary data set of *Mam-Mayan* Guatemala. We also adjusted p-values using the Bonferroni and Benjamini Hochberg analysis, since we conducted multiple tests.

Limitations

Our study design also had few limitations. First, dietary recalls and infant growth measurements were assessed at the same time point, and we therefore did not assess maternal intakes before growth measurements. Our study design is dependent on 24-hour recalls. Recall bias during the dietary data collection might have affected the results to a small extent. We could not capture the other reasons for impaired infant growth for example infectious diseases and limited access to health care. We did not report/analyze the traditional food diet but connected our nutrient

intake and inadequacy findings to the nutritional characteristics of the traditional diet. Future research can examine the dietary characteristics of the Guatemalan traditional diet and how it relates to nutrient inadequacy, however our findings provide some insight into the opportunities for supporting healthy intakes including traditional foods. Though we were able to do some exploratory linear regression that these findings are exploratory only because of the outcome variables that the nutrient intakes were not normally distributed and so we emphasize the logistic regressions.

CHAPTER 6. CONCLUSION

Our findings add largely to the scientific literature because there is a huge knowledge gap in understanding the nutrient adequacy of the Indigenous *Mam*-Mayan women living in Guatemala. Guatemala has the sixth-highest rate of chronic undernutrition in the world and the third-highest adolescent birthrate in Central America (WHO, 2011) (Guatemala, 2017). It is necessary to evaluate the nutrient adequacy of a growing nation like Guatemala to help prevent the adverse health and birth outcomes of infants. No study prior to ours was investigated the association of maternal nutrients and maternal age on infant growth outcomes.

Our novel findings reported in this thesis include 1) evidence of the nutrient adequacy of *Mam*-Mayan pregnant and lactating women living in Guatemala (based on RDD and DRI); 2) evidence of the association of infant growth with maternal macro and micronutrient intakes during lactation; 3) evidence of the differential impact of maternal age and maternal nutrient intakes with infant growth outcomes. From our findings we concluded that maternal intakes of carbohydrates, fiber, sugars, magnesium, phosphorous & sodium were adequate using IOM DRI and INCAP RDD. Maternal intakes of protein, total lipids, choline, retinol, vitamin E, & cholesterol were lower in adult mothers compared adolescent mothers. We also concluded In early postpartum that (1) for WAZ higher intakes of energy and two macro nutrients & seven nutrients Increased WAZ and that higher intakes of folate, DFE and PUFA's lowered the odds of the infant being underweight; (2) for LAZ, higher intakes of lipids (PUFA & PUFA) and two micro-nutrients (selenium, vitamin E) increased LAZ but no nutrient was associated with lowering the odds of stunting and (3) for HCAZ, higher intakes of energy, three macro nutrients & two micro-nutrients increased HCAZ and 3 of these nutrients (fiber, folate total, PUFA) reduced odds of having low cranial growth. Different associations emerge in late postpartum period with as fewer nutrients were associated with infant growth. Only retinol and lycopene were negatively associated with WAZ and LAZ whereas lutein & zeaxanthin were negatively associated with HCAZ. In contrast cholesterol was positively associated with LAZ & reduced odds of infants being underweight. Thus, maternal intakes of more nutrients during first six weeks of lactation had a significant impact on infant growth than maternal intakes during late postpartum highlighting the importance of adequate maternal nutrient intakes in early lactation. Finally, it appears that the

diet of adolescent mothers is important for ensuring optimal neonatal growth during early lactation.

Policy Implications of Research Findings

Community and Role of traditional foods

Corn, beans, and squashes are traditional foods of Guatemala with some fruits and vegetables like *busnay*, tomatoes, green leafy vegetables, potatoes, root crops, onions, carrots, cucumber, cabbage, and fruits including mango, apple, orange, and watermelon. Nutritionally the traditional foods possess all the nutrients and when the traditional foods are consumed adequately on a daily basis they are sufficient to prevent deficiencies. By educating the population with the nutritional benefits of traditional foods and by implementing a policy of traditional foods awareness programs and celebrating traditional food weeks can help people in understanding and consuming adequate amounts of these foods.

Though the traditional foods of Guatemala include beans which is a high protein source, the adolescents were deficient for protein. Adolescent mothers consumed only 50% of the protein recommended. This might be due to the low consumption of the quality protein rich beans in their daily diet. By increasing the proportion of the given food recommendation of eating two tablespoons of beans to four tablespoons of beans per tortilla can help combat not only the protein deficiency among the mothers but also iron and zinc. Vitamin C consumption was low among the mothers. The Guatemalan diet usually have abundance of vitamin C due to the presence of squashes. Consuming more amounts of the readily available traditional foods like squashes, tomatoes, green leafy vegetables can help prevent vitamin C deficiency among mothers. Folate during pregnancy is a vital nutrient. Adolescent and adult mothers during pregnancy can consume already available green leafy vegetables, beans, fresh fruits to increase their folate content for a healthy infant.

Mothers

From our study, we discovered that the mothers in Guatemala are deficient for most of the vital nutrients which include water, energy, protein, lipids (SFA, MUFA, PUFA, cholesterol), calcium,

phosphorous, zinc, vitamin C, thiamine, riboflavin, pantothenic acid, folate, vitamin B12, choline, vitamin E, and vitamin D.

The mother's vitamin A status is partially adequate. According to Solomons ([Solomons, 2000](#)) the Guatemalan population's vitamin A is an intrinsic problem due to the low consumption of animal protein sources. To combat vitamin A deficiency in Guatemalan population, sugar fortification with vitamin A was started in 1975. But all the food fortification programs were terminated in 1979 but reinitiated in 1988 ([Fiedler & Helleranta, 2010](#)) Considering the success of vitamin A fortification and its positive effect on the nutritional status of the population, there is a scope for food fortification of other nutrients to help in increasing the nutrient efficiency among the population.

Child Health Promotion Policies

Guatemala has one of the highest rates of stunting in the world among children under 5 years of age. Our results also showed that adolescent mothers' nutrient intakes were more often associated with infant growth outcomes. Thus, focusing on the nutritional status of adolescents might be useful in developing healthy babies. We also discovered that the first six weeks postpartum had a significant association on the infant growth outcomes.

Protein, vitamin, and mineral deficiencies can be reduced by consuming animal products like meat, chicken, liver, fish, or eggs at least twice in a week. By implementing a food policy of animal product consumption at least twice in a week would be beneficial in mitigating micronutrient deficiencies during pregnancy and lactation. But, because of the SES of the Guatemalan population, they might not afford high quality animal products. By implementing a policy which includes providing nutrient rich foods like eggs, during pregnancy and in early postpartum, there might be a positive impact on the infant's growth. Vitamin D plays an important role in the bone growth and might help in reducing stunting of children. Vitamin D status was low among this population, and this might also be one of the reasons for high rates of stunting. To increase the vitamin D status, vitamin D supplementation may be required. Zinc status is also compromised among the population due to phytic acid inhibitors that are present in corn. Administering zinc through supplementation or via food fortification might be beneficial to the population.

In conclusion, the government should also start campaigns explaining the importance of foods and nutrients during pregnancy and lactation to adolescent and adult mothers. By educating them and teaching them the techniques of how to choose and utilize the foods around them can help them make a better food choice.

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