PLACING A LENS ON THE FIRST 1000 DAYS OF LIFE: PRENATAL INTAKE, INFANT FEEDING, THE MICROBIOME AND CHILD GROWTH

by

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To my Dad, my Mom, and my siblings, Mohamad, Rami, & Reem

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ABSTRACT

The first 1000 days of life, from conception until the child's second birthday, constitute a critical window for child growth and development. During infancy and early childhood, significant and rapid physical changes occur, including increases in weight, height, and brain size and organ development accompanied by cognitive and psychomotor development. Adequate infant feeding, including breastfeeding and complementary feeding, that meets the infants' energy and nutrient requirements can help protect against growth faltering, infant and child morbidity and mortality, and delayed mental and motor development. Adequate nutrition during this critical period can also protect against adverse health outcomes and chronic diseases later in life according to the hypothesis of developmental origins of health and disease.

A web of factors that are country- and culture- specific influence infant feeding practices and child growth. Further, the microbiome has been suggested as a strong potential player in the association between infant nutrition and child growth. Therefore, the overarching theme of the current dissertation is to investigate hypotheses that can provide evidence to inform the paradigm linking socio-demographic, maternal, and child determinants including prenatal intake to infant feeding, the breast milk and infant gut microbiome, and child growth within the first 1000 days of life. Specifically, aims one and two examine the socio-demographic, maternal, and child determinants of child growth and breastfeeding in a cross-sectional survey of mother-child dyads in Lebanon, a middle-income country undergoing nutrition transition in the Middle East. The third aim focuses on the CHILD cohort study, a multi-center longitudinal prospective birth cohort study, to examine the associations between prenatal diet and supplement intake and the breast milk microbiome. Finally, the fourth aim is to review the evidence for the potential of the infant gut microbiome as a promising target linking complementary feeding to child undernutrition in low- and middle-income countries (LMIC) with the highest burden of undernutrition.

The results for aim one revealed sex-specific determinants of child growth in Lebanon. The determinants examined through a hierarchical conceptual framework included: maternal and paternal education among boys and crowding index among girls at the distal sociodemographic level, and maternal obesity among girls at the intermediate maternal level. The proximal child

determinants included birth length, number of children in the household and breastfeeding duration among girls, birthweight among boys and child's age among boys and girls.

In the analysis for aim two, breastfeeding practices were suboptimal in Lebanon as less than half (41.5%) of the infants were exclusively breastfed during the 40-day rest period and 12.3% were exclusively breastfed during the 6-month duration recommended by the World Health Organization. Higher socioeconomic status, as reflected by a larger number of cars owned, and C-section delivery were consistently inversely associated with lower odds of exclusive breastfeeding for 40 days and 6 months. Belonging to a family with more children was associated with higher odds of exclusive breastfeeding for 40 days; while maternal overweight and obesity were associated with lower odds of exclusive breastfeeding for 6 months.

Findings from aim three suggested that prenatal supplement use, but not prenatal dietary quality and patterns, modulate the breast milk microbiota composition in the CHILD cohort in Canada. This project was exploratory and utilized one of the largest birth cohort studies with available breast milk microbiome data. Specifically, use of vitamin C and D supplements plus multivitamins during any trimester in pregnancy was consistently associated with milk microbial diversity and genus composition before and after adjustment for socio-demographic, maternal, and child covariates. Use of other supplements such as fish oil, folate, and calcium was less consistently associated with the breast milk microbiome.

The fourth aim of the review chapter focused on the infant gut microbiome. The effects of complementary feeding on the infant gut microbiome are less commonly studied than those of breastfeeding, with most research conducted in high-income countries but not LMIC. In contrast, associations between inadequate complementary feeding and undernutrition have been examined in LMIC where undernutrition is most prevalent. Further, a disrupted gut microbiota has been associated with child undernutrition. Indeed, animal studies have suggested a causal association although the direction of the causality is not clear and is potentially bi-directional depending on genetic and environmental conditions. In light of the current state of knowledge described in our review supporting the potential of the gut microbiota as a key player in the relation between complementary feeding and undernutrition, the development of microbiota-directed interventions during the complementary period offers a promising route for undernutrition management.

Findings from the studies presented in this dissertation highlight several culture-specific determinants of child growth and breastfeeding in Lebanon. The findings also highlight the need for future research using longitudinal prospective cohorts, intervention trails and animal models to provide evidence for the proposed links to enhance the understanding of the paradigm. Such a holistic view of the determinants of and pathways between infant feeding and child growth are of great public health significance to improve the health of children throughout their lives.

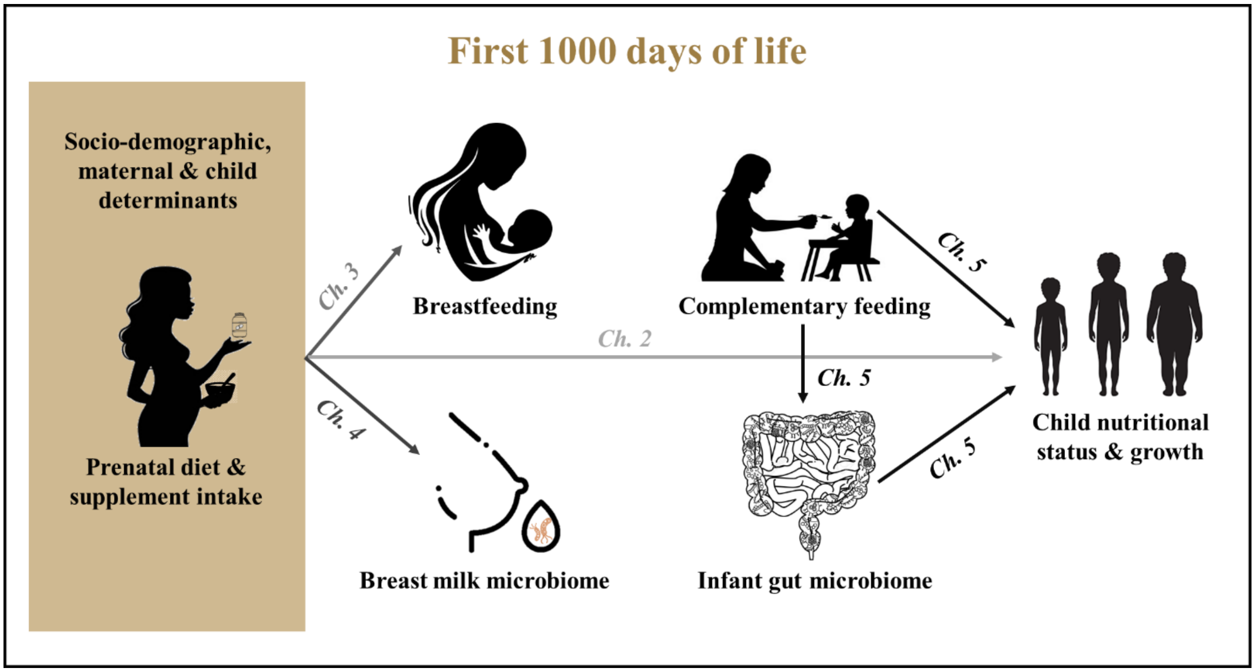
CHAPTER 1. INTRODUCTION: THE FIRST 1000 DAYS OF LIFE ARE A CRITICAL WINDOW FOR CHILD GROWTH

1.1 The first 1000 days of life are a critical window of opportunity

The first 1000 days of life, from conception until the child's second birthday, constitute a critical window for child growth and development. During pregnancy, fetal organ and commensurate systems are formed, with maternal nutrition playing a critical role during this prenatal period, as well as in the postnatal period during lactation (1). At birth, the newborn experiences major physiological shifts that necessitate adaptive responses during the first years of life to ensure the establishment of physical and mental abilities (2). The period after birth can be divided into infancy and early childhood. Infancy covers the first year of life characterized by rapid nutrition-dependent phase of growth; and early childhood covers the following years until the age of 5, which are characterized as a 'more stable, slowly decelerating growth hormone-dependent phase' (2).

During infancy and early childhood, significant and rapid physical changes occur, including increases in weight, height and brain size and organ development accompanied by cognitive and psychomotor development. The energy requirements per body size are highest during infancy to support rapid growth (3). Poor nutrition that does not meet the infants' energy and nutrient requirements increases the risk of growth faltering and deficiencies of certain micronutrients which can in turn increase the infants' risk of morbidity and mortality and delayed mental and motor development (4). Poor nutrition during this critical period is also associated with adverse health outcomes and chronic diseases later in life as suggested by the hypothesis of developmental origins of health and disease (DOHaD) (5). The pathways and mechanisms underlying DOHaD are under investigation, with the microbiome being a strong potential player in the association between infant nutrition and child growth.

In this dissertation, I aim to put a lens on the first 1000 days of life to examine the associations and pathways linking environmental exposures such as socio-demographic characteristics and prenatal diet with infant feeding practices, the microbiome and child growth. The explored links in each of the dissertation chapters are highlighted in the paradigm in Figure 1.1 that guided the analysis.



Grey arrows represent links in the paradigm that were explored in the current dissertation. Ch.: Chapter.

Figure 1.1 Placing a lens on the first 1000 days of life: prenatal intake, infant feeding, the microbiome and child growth

1.2 Nutritional status is closely related to child growth

The nutritional status of infants and children reveals valuable information about their growth patterns and trajectories (6). A combination of nutritional assessments including anthropometric, biochemical, clinical (physical examination), and diet assessments help determine if a child is malnourished or well-nourished and are thus important for promoting health and preventing and treating diseases (6).

1.2.1 Anthropometric measures as indicators of child nutritional status

Anthropometric measurements are widely used for the assessment of nutritional status as they are a simple and inexpensive means to assess adequacy of diet and growth patterns in children (7). The two most common anthropometric measurements are weight and length or height as they provide essential information about the different body components and the changes they undergo during the growth process (2). Measurements of recumbent length are recommended for children younger than 2 years of age while those of standing height are recommended for children aged 2 years and older and adults.

Weight and length measurements are more interpretable if they are related to each other or to age and compared to a reference population by calculating Z-scores such as weight-for-length, weight-for-age and length-for-age Z-scores (8). The most commonly used reference data worldwide are based on the Multicenter Growth Reference Study (MGRS) by the World Health Organization (WHO) (9). The MGRS was implemented between 1997 and 2003 and collected data from a diverse group of children from six countries (Brazil, Ghana, India, Norway, Oman and USA) to generate charts for assessing the growth and development of children worldwide.

1.2.2 Malnutrition is the most common 'disease' among children

'Malnutrition refers to deficiencies, excesses, or imbalances in the intake of energy and/or nutrients' (10). The two main forms of malnutrition are undernutrition and overnutrition (Table 1.1).

Table 1.1 Malnutrition forms and features

Forms of malnutrition		Severity of malnutrition	Anthropometric indicator	Z-score classification	Features
Under-nutrition	Wasting	Moderate	Weight-for-length	-2 to -3 SD	*Indicates acute and severe weight loss
		Severe		<-3 SD	*Encompasses two forms of SAM: Marasmus and Kwashiorkor
	Stunting	Moderate	Length-for-age	-2 to -3 SD	*Results from chronic or recurrent undernutrition
		Severe		<-3 SD	*Is a commonly used indicator for growth impairments and undernutrition
	Underweight	-	Weight-for-age	<-2 SD	* Can reflect acute or chronic undernutrition or both
Over-nutrition	Overweight	Moderate	Weight-for-length or Weight-for-age	2 to 3 SD	*Associated with abnormal of excessive fat accumulation
	Obesity	Severe		>3 SD	*Increases risk of NCDs

Source: World Health Organization (10)
SD: standard deviations from the median of the reference population; SAM: severe acute malnutrition; NCDs: non-communicable diseases

A total of 149.2 million (22.0%) children under 5 years of age suffered from stunting in 2020, 45.4 million (6.7%) suffered from wasting, while 38.9 million (5.7%) were overweight, rendering malnutrition the most common ‘disease’ among children (11). These prevalence rates are expected to increase significantly due to limited access to healthy and nutritious diets and essential nutrition services during the COVID-19 pandemic, with the effects on stunting potentially taking years to manifest (11).

Child malnutrition is disproportionately prevalent. Low- and middle- income countries (LMIC), namely those in South Asia and Sub-Saharan Africa, bear the greatest disease burden of child undernutrition (11), which is associated with 45% of child deaths (12). Poor infant feeding, including breastfeeding and complementary feeding, in addition to the wide prevalence of infectious diseases, are the main proximal causes of undernutrition during the first 2 years of life in LMIC (4). On the other hand, overweight and obesity are highly and increasingly prevalent in high-income countries (HIC) (13). It is worth noting that overweight and obesity rates are similarly increasing in LMIC (14).

The coexistence of both forms of malnutrition, known as the double burden of malnutrition, in the same country and in the same household is a hallmark of the nutrition transition happening in LMIC and complicates the planning and implementation of interventions and policies (15). The latter are further complicated by the web of factors influencing nutritional status that exert their effects at multiple distal, intermediate and proximal levels. In chapter 2, I examine the multilevel determinants of nutritional status during the first 1000 days of life by child’s sex in Lebanon, a middle-income country in the Middle East undergoing nutrition transition with increasing rates of childhood overweight and obesity (16).

1.3 Adequate infant feeding is crucial for optimal child growth

Infant feeding practices during the first 2 years of age can be divided into two parts according to the WHO recommendations (12): (1) exclusive breastfeeding for the first 6 months of life followed by (2) complementary feeding with continued breastfeeding between 6 months and 2 years of age. Updated indicators by the WHO can help assess the adequacy of infant and young child feeding practices and adherence to the recommendations (17).

1.3.1 Breastfeeding

Breastfeeding is the optimal form of infant nutrition, with well-established benefits to the mother and child (18). The WHO (19) and American Academy of Pediatrics (AAP) (20) recommend exclusive breastfeeding for the first 6 months of life with continued breastfeeding for 1-2 years.

The benefits of breastfeeding on infants and children differ by the country's income level and the examined breastfeeding indicator (breastfeeding initiation, exclusivity, or duration) (18). In LMIC, exclusive breastfeeding reduced the risk of death by 88% compared to never breastfeeding (21). Any breastfeeding also had a protective effect against mortality compared to never breastfeeding in children aged 6-23 months old. Breastfeeding also decreased mortality risk in HIC, albeit to a lesser extent, where ever breastfeeding was associated with 36% reduction in sudden infant death compared to never breastfeeding (22). In terms of morbidity, breastfeeding can reduce the risk of diarrhea and respiratory infections by half in LMIC, whereas in HIC, breastfeeding was protective against otitis media (23) and asthma with inconclusive evidence on protection against allergic disorders (24). Associations between breastfeeding and child growth were inconsistent in LMIC, whereas in HIC, longer exclusive and partial breastfeeding were associated with slower growth rates during infancy (25) and 26% lower odds of overweight or obesity (26).

Research on the long-term benefits of breastfeeding on non-communicable disease risk is mostly conducted in HIC. Breastfeeding for longer duration was associated with 35% reduction in incidence of type 2 diabetes, with no evidence of protective effect on blood pressure or cholesterol levels (26). Further, breastfeeding was positively associated with performance in intelligence tests (27) and attained schooling (28) both of which can enhance human capital but might be confounded by the higher socioeconomic status of the mothers who choose to breastfeed.

Despite the well-established benefits of breastfeeding, current practices worldwide do not meet the recommendations with only 41% of infants under 6 months of age being exclusively breastfed and 45% being breastfed for 2 years of age (29). Such global mean breastfeeding rates mask dramatic disparities across countries, with breastfeeding rates being usually higher in LMIC compared to HIC (18). Poorer families in LMIC are more likely to breastfeed for longer periods than their richer counterparts. On the contrary, in HIC, richer women with higher education tend to breastfeed more commonly than their poorer, less educated counterparts (18).

Breastfeeding practices are influenced by numerous historical, socioeconomic, cultural, and individual factors (30). Understanding the culture-specific facilitators of and barriers to breastfeeding in each country is of immense public health significance to better inform interventions aiming to scale up breastfeeding. In chapter 3, I report the prevalence of exclusive breastfeeding during the 40-day rest period, a cultural practice in Lebanon with religious underpinnings, and the recommended 6 months. I also examine the sociodemographic, maternal, and child factors associated with exclusive breastfeeding in a nationally representative sample of mother-child dyads in Lebanon.

1.3.2 Complementary feeding

Around the age of 6 months, the infant's energy and nutrient requirements exceed the levels provided in breast milk (12). Further, the infant at that age is developmentally ready for other foods. This transition is referred to as complementary feeding when the infant is introduced to liquids other than breast milk and to semi-solid and solid foods at 6 months with the aim of continued breastfeeding until 2 years (12).

Unlike the single recommendation of exclusive breastfeeding for the first 6 months, the recommendations for complementary feeding between 6 months-2 years are more complex and encompass multiple dimensions (4). The guiding principles for adequate complementary feeding include (1) introduction of complementary foods at 6 months (2) maintenance of frequent on-demand breastfeeding for 2 years, (3) responsive feeding to hunger and satiety cues, (4) safe preparation and storage of complementary foods under hygienic conditions, (5) sufficient amounts of complementary foods, (6) adequate food consistency adapted to the infant's requirements and ability, (7) appropriate meal frequency and energy density such that the infant receives complementary foods 2–3 times a day between 6–8 months and increase to 3–4 times daily between 9 months-2 years with additional nutritious snacks 1–2 times per day for ages 1-2 years as desired (12) and (8) variety of nutrient dense foods (4).

Adequate complementary feeding practices that meet the aforementioned guiding principles are critical for ensuring optimal child growth and development in LMIC and HIC (31). Interventions targeting complementary feeding practices are often successful in improving complementary feeding practices but fall short of creating (long-lasting) effects on child growth and health outcomes (32, 33). This might be due to the web of factors associated with child growth and to the numerous biological and environmental exposures during the period of the first 1000 days of life. To better inform such interventions, it is critical to understand the pathway(s) associating complementary feeding to child nutritional status, one potential pathway of which is related to the microbiome (34).

1.4 The microbiome is a critical player during the first 1000 days

The complex interplay between the microbiome, maternal immune constituents and infant gut colonization during the first 1000 days is of great importance to the development of the human microbiome (35), which refers to the ecological community of all the microorganisms living in and/or on the human body (36). While some of these microorganisms are pathogenic, the majority are commensal and even essential to human health (36). For example, microorganisms synthesize vitamins such as vitamin K that cannot be produced by the human body, extract nutrients and produce others such as short chain fatty acids, and guide the maturing immune system especially in infants. A growing body of literature has supported the pivotal role of the microbiome in various health outcomes, raising the possibility that manipulation of the microbial communities could be used to prevent and/or treat disease (36).

The breast milk microbiome is among the most recently discovered human microbiomes, negating previous dogmas that it is sterile. In fact, the infant is estimated to ingest 10^5-10^7 bacteria daily per 800 ml/day of breast milk (37). These bacteria are thought to seed the infant gut microbiome and guide the maturing immune system, both functions of which have critical implications on the child’s nutritional status and health later in life (38).

1.4.1 The breast milk microbiome

As discussed previously, breastfeeding confers numerous health benefits including protections against infections and atopic diseases in children (39). These benefits have been associated with the unique composition of the breast milk including nutrients and bioactive components such as lactoferrin, growth factors, cytokines, human milk oligosaccharides (HMOs) (40) and the breast milk microbiome. Vertical transfer of bacteria via breastfeeding may help seed the infant gut microbiome (41), with disruptions in this process leading to a dysbiotic microbiota and increasing the risk of chronic diseases such as allergy, asthma and obesity (42).

Different factors shape the breast milk microbiota composition. Among the most commonly studied ones are lactation stage, mode of delivery, gestation age at birth and infant's sex (43, 44). The role of maternal diet and supplement intake in shaping the breast milk microbiome has been scarcely studied despite evidence of its importance. Understanding the effects of a modifiable factor like maternal intake on the composition of breast milk microbiota can help guide recommendations on maternal intake during pregnancy. In chapter 4, I examine the associations between maternal prenatal diet and supplement intake and the breast milk microbiota composition using data from the CHILD cohort study in Canada.

1.4.2 The infant gut microbiome

The colonization of the infant gut microbiome starts in utero, as recent evidence has highlighted the presence of a unique microbiome in the placenta and in the meconium of newborns (45). The birthing canal or maternal skin (depending on the mode of delivery: vaginal or C-section) and breast milk (among breastfed infants) provide the main and first sources of bacteria seeding the infant gut microbiome postnatally (42). Other factors such as infant feeding practices, antibiotic use, pet exposure, etc. shape the infant gut microbiome during the first 1000 days of life (46, 47).

Some studies suggest that the infant gut microbiome is established during the first 2-3 years of life, where by the end of this period, the microbiota profile resembles that of adults (48). Disruptions in the colonization process have been linked to increased risk of malnutrition, including undernutrition in LMIC (49), and non-communicable diseases such as allergy, asthma, and irritable bowel syndrome (42). As suggested earlier, the infant's gut microbiome may be a critical player linking complementary feeding to child nutritional status. In chapter 5, I review the evidence for the relation between complementary feeding and undernutrition and highlight the potential of the gut microbiota to be a promising target in this relation.

1.5 Linking it all together: The role of infant feeding and the microbiome in child growth

Optimal infant feeding during the first 1000 days of life, in terms of breastfeeding and complementary feeding, is critical for child growth and health later in life. The first two studies in this dissertation examine the socio-demographic, maternal and child determinants of child growth and breastfeeding in Lebanon from the Early Life Nutrition and Health in Lebanon (ELNAHL) study (Figure 1.1). Lebanon is a middle-income country in the Middle East undergoing nutrition transition with increasing rates of overweight and obesity and associated non-communicable diseases among children (16). Understanding the country-specific determinants of child growth and breastfeeding can inform interventions and policies aiming to support child growth and scale up breastfeeding.

Identifying the players and deciphering the mechanisms linking infant feeding to child growth are also important. The microbiome is one of the proposed players and is the focus of the second two studies in this dissertation (Figure 1.1). The breast milk microbiome seeds the infant gut microbiome which is in turn associated with health outcomes. Several factors shape the breast milk microbiome. The third study examines the effects of prenatal diet and supplement intake on the breast milk microbiota composition using data from the CHILD cohort study in Canada, which is among the largest birth cohort studies with available breast milk microbiome data. In addition to breastfeeding, complementary feeding can shape the maturing gut microbiome during early childhood with strong implications on nutritional status. The fourth and final study reviews the evidence on the potential of the gut microbiota as a promising target in the relation between complementary feeding and undernutrition in LMIC with the highest burden of child undernutrition.

CHAPTER 2. DETERMINANTS OF NUTRITIONAL STATUS DURING THE FIRST 1000 DAYS OF LIFE IN LEBANON: SEX OF THE CHILD MATTERS

Chehab RF, Nasreddine L, Forman MR. Determinants of nutritional status during the first 1000 days of life in Lebanon: Sex of the child matters. Paediatr Perinat Epidemiol. 2021 Jan 11. doi: 10.1111/ppe.12747. Epub ahead of print. PMID: 33428236.

2.1 Abstract

Background: The first 1000 days of life support child growth and long-term health, but limited research exists in Lebanon.

Objective: To examine the determinants of nutritional status among Lebanese children ≤2 years old by child's sex.

Methods: We analyzed data from a cross-sectional survey of 466 mother-child dyads. We classified socio-economic, maternal, and child characteristics using a hierarchical conceptual framework into distal, intermediate, and proximal levels, respectively. Sex-stratified multiple linear regression using weighted data to reflect a nationally-representative sample was computed to identify the determinants of length-for-age z-scores (LAZ) and weight-for-length z-scores (WLZ).

Results: The mean (standard deviation) of LAZ and WLZ was -0.3 (1.6) and 0.5 (1.5) among boys and -0.1 (1.4) and 0.5 (1.0) among girls, respectively. At the distal level, maternal intermediate or high school education was associated with higher boys' LAZ (β 0.99, 95% CI 0.20, 1.78), and crowding index was inversely associated with girls' LAZ (β 0.81, 95% CI 0.27, 1.35). At the intermediate level, maternal obesity was associated with lower girls' LAZ (β -0.91, 95% CI -1.41, -0.41). At the proximal level, birth length directly (β 0.11, 95% CI 0.03, 0.19) and breast feeding duration inversely (β -0.05, 95% CI -0.09, -0.01) influenced girls' LAZ. For WLZ, paternal attainment of university degree or technical diploma was associated with lower boys' WLZ (β -0.94, 95% CI -1.80, -0.07). Among the proximal determinants, birthweight directly influenced boys' WLZ (β 1.18, 95% CI 0.60, 1.75) while being a third or later child was associated with higher girls' WLZ (β -0.49, 95% CI -0.83, -0.15). Child age was directly associated with WLZ among both sexes (β=0.06, 95% CI 0.02, 0.09 and β=0.05, 95% CI 0.03, 0.07).

Conclusions: Nutritional status determinants differed by child's sex in Lebanon. Research using sex-stratified hierarchical analysis can inform interventions to improve child growth.

Keywords: First 1000 days; growth; sex-specific; hierarchical conceptual framework; Lebanon

2.2 Background

The first 1000 days of life, which span the period between conception and the child's second birthday, constitute a unique window of opportunity to support child growth and long-term health.(5) This period is characterized by the highest plasticity and rapid development of systems including the brain,(50) neural and immune systems as well as the gut microbiome.(51) Environmental factors such as socio-economic status (SES) and diet during the first 1000 days influence the child's nutritional status, growth, and health later in life.(5)

The determinants of nutritional status are multifactorial and exert their effects at multiple —distal, intermediate, and proximal— levels. To account for such complex interrelationships, the use of hierarchical conceptual frameworks has been recommended.(52, 53) The factors at each level of the framework differ by study depending on the outcome of interest, target population, and a plethora of other factors. For example, some studies examine community factors such as hospital accessibility and sanitation practices at the distal level, (54) whereas others focus on household factors including SES and parental education.(55-57) Proximal factors typically highlight child characteristics like birth size, sex, infant feeding, and illness.(55-57)

Many countries in the Eastern Mediterranean Region (EMR) are experiencing a nutrition transition characterized by a shift towards sedentary lifestyle and replacement of traditional Mediterranean diets with Western diets.(58) These changes have been associated with a higher prevalence of non-communicable diseases and alarming rates of childhood overweight and obesity.(58) In the EMR, 6.9% of children under five years are overweight – higher than the global average of 6.2%.(59) At the same time, stunting, wasting, and underweight are persistent in the region's low- and middle-income countries.(60) In Lebanon, a small middle-income country in the EMR, 6.5% of children are overweight, 2.7% are obese, and 26.3% are at risk of overweight,(16) and stunting is prevalent at 7.3%, wasting at 1.1% and underweight at 1.6%.(61)

Sex differences exist in growth patterns and nutritional status of children.(62) Compared to girls, boys are larger at birth.(63) In one of the few studies examining the variations in growth patterns by child's sex in Saudi Arabia, boys were generally taller and heavier than girls until the age of 7-10 years, after which girls were taller and heavier from 10 to 14-15 years.(64) Then, boys again were taller and heavier, with such differences in growth dependent on age of maturation.

Few studies focus on infants and children ≤2 years of age in the EMR(65) and none exist in Lebanon, rather studies of the determinants of nutritional status are among older children (>2 years),(66) adolescents,(16) and adults.(67) Further, the studies do not account for the hierarchical nature among the multilevel determinants of nutritional status, nor stratify the analysis by child's sex. With these considerations in mind, the aims of this study are to examine the multilevel determinants of nutritional status during the first 1000 days in Lebanon and to investigate whether the determinants differ by child's sex. The results are critical to inform interventions aiming to help children achieve their full developmental potential.

2.3 Methods

2.3.1 Study design and sample

The current analysis uses data from the Early Life Nutrition and Health in Lebanon (ELNAHL) cross-sectional study conducted between September 2011 and August 2012. Details of the study population and data collection are described elsewhere.(16) Briefly, a nationally representative sample of mothers and children ≤5 years old was selected using a complex stratified two-stage cluster sampling design. The six Lebanese governorates served as the sampling strata and the clusters were selected further at the district level. Households, which served as the primary sampling units, were selected from districts based on a probability proportional to size approach using systematic sampling, whereby a higher number of households was drawn from more populous districts. Mother-child dyads living in sampled households were eligible to participate if the mother was Lebanese, aged 19-40 years, did not have hypertension or diabetes, and was not taking medications that interfere with eating and breast feeding or affect body weight; and if the child was ≤5 years old, was born at term (gestational age between 37-42 weeks), and had no chronic medical conditions, inborn errors of metabolism, or physical malformations that interfere with feeding patterns and body composition. Of the 1194 eligible mother-child dyads, 1029 dyads participated in the ELNAHL study (response rate 86%). Dyads with a child ≤2 years old (n=472, 45.9% of the dyads in the original ELNAHL study) were included in the current analysis.

Trained nutritionists conducted in-person interviews with mothers and inquired about SES and maternal and child characteristics including infant feeding practices. The nutritionists also collected anthropometric measurements. Length was measured among children ≤2 years old without clothes and diapers using an infantometer (SECA 354, Hamburg, Germany) to the nearest 0.1 cm; weight was measured on an electronic pediatric scale (SECA 210) to the nearest 0.1 kg. Maternal height was measured in light clothes without shoes using a portable stadiometer (SECA 213) to the nearest 0.5 cm; weight was measured using a scale (SECA 770, Germany) to the nearest 0.1 kg. All measurements were taken twice and repeated a third time if the first two measurements differed by more than 0.5 cm for height and 0.3 kg for weight. The average of the measurements was calculated and used for the analysis.

2.3.2 Ethics approval

The study was approved by the Institutional Review Boards at the American University of Beirut (NUT.LN.13) and Purdue University (Protocol number: 1902021663). Mothers provided written informed consent prior to study participation.

2.3.3 The hierarchical conceptual framework of exposure and outcome variables

The exposure variables were classified according to the hierarchical conceptual framework in Figure 2.1, which was adapted from similar studies.(55, 57) Distal factors included SES variables such as crowding index, paternal and maternal education and employment. Crowding index was defined as the average number of individuals per room, excluding the kitchen and bathroom.(68) Data on other variables such as family income, house and car ownership, and presence of a paid helper at home were collected; however, crowding index was correlated with SES in Lebanon and used as an objective indicator of the family's SES.(68) At the intermediate level, maternal characteristics such as age, height, BMI, and smoking during pregnancy were included. Only five mothers had a BMI <18.5 kg/m2; therefore, BMI categories of underweight and normal weight were merged. Pregnant mothers (n=31) were excluded from the current analysis. Child characteristics including birth order, mode of delivery, birthweight and birth length, child's age, whether breast fed at the time of the study, and breast feeding duration were included at the proximal level. Birthweight and birth length were abstracted from a copy of the medical booklet unique for each child born in Lebanon which the mother keeps at home.(69)

The outcome variables were continuous values of length-for-age z-scores (LAZ) and weight-for-length z-scores (WLZ). Z-scores were calculated according to the WHO sex- and age-specific child growth standards using the WHO AnthroPlus software.(70)

2.3.4 Statistical analysis

Normal distributions of LAZ and WLZ were confirmed visually using histograms and QQ plots. The associations of LAZ and WLZ with the exposure variables by child's sex were examined using multiple linear regression analysis. Sex interactions were evaluated for each of the exposure variables with the outcomes of interest and underscored the need for sex-stratified models. Three regression models for LAZ and WLZ were created, one for each level of the hierarchical conceptual framework in Figure 2.1. Potential confounders that were adjusted for in the models were chosen based on the results of the unadjusted analysis and on the literature.(71, 72) Adjusted βs and 95% CIs for each exposure variable were calculated at the level (distal, intermediate, or proximal) at which they were initially entered, regardless of their performance when variables from subsequent levels were added to the model. Such an approach helped reduce attenuation of the association between the distal or intermediate factors and the outcome variable when proximal factors were added.(52, 53) Further, this approach helped protect against over adjustment of an intermediate factor on the causal pathway between distal factors and the outcome of interest as though it were a confounder.(73)

Statistical assumptions were checked for each model. Multicollinearity among exposure variables was checked using variance inflation factors (VIF). Normal distribution and homoscedasticity of the residuals were checked using QQ plots and scatterplots, respectively. All analyses were weighted by the sample weights to account for the stratified two-stage cluster sampling design and were conducted using Stata (StataCorp. 2019. Stata: Release 16. Statistical Software. College Station, TX: StataCorp LLC).

2.3.5 Missing data

A total of six observations were missing sampling weights and three were missing length measurements. Specific LAZ (n=3) and WLZ (n=8) were biologically implausible according to the WHO definition and deleted.(70)

2.4 Results

Among the sample of 466 children ≤2 years, 231 (49.6%) were boys. LAZ and WLZ of boys and girls were approximately normally distributed. The weighted mean (standard deviation (SD)) for LAZ was -0.3 (1.6) for boys and -0.1 (1.4) for girls, and for WLZ was 0.5 (1.5) for boys and 0.5 (1.0) for girls (data not shown). Rates of stunting and wasting among boys were 10.9% and 6.1%, respectively and among girls were 7.9% and 3.1%, respectively. The prevalence of overweight and obesity was 14.0% and 2.6% among boys and 5.1% and 0.6% among girls, respectively (data not shown).

Table 2.1 presents the socio-economic, maternal, and child characteristics of the sample of mother-child dyads in Lebanon by child's sex. The majority of children lived in households with higher crowding index (>1 individual/ room) and had fathers and mothers with intermediate or high school education. Most of the children had fathers who worked in the private sector or had a self-owned business and mothers who were housewives. Mothers had a mean age of 32 years and mean height of 159.7 cm with the majority being overweight or obese and not smoking during pregnancy. The majority of the children were the second or later child and delivered vaginally, while one-third were still breast feeding at the time of the study. The children had a mean birthweight of 3.2 kg, mean birth length of 50.2 cm, aged 11.6 months at the time of the study and were breast fed for a mean duration of 5.0 months.

Table 2.2 presents the regression models of the sex-specific determinants of LAZ. Among boys at the distal level, maternal intermediate or high school education compared to primary education was associated with higher LAZ. At the intermediate and proximal levels, no factors were associated with boys' LAZ in the adjusted models. Among girls at the distal level, a crowding index ≤1 individual/room compared to >1 individual/room was associated with higher LAZ. At the intermediate level, after adjusting for crowding index, maternal obesity was associated with lower LAZ. At the proximal level after additional adjustment for maternal height and BMI, birth length was positively associated with LAZ, while breast feeding duration was negatively associated with LAZ.

In the sex-specific models of WLZ in Table 2.2, paternal attainment of university degree or technical diploma compared to primary or lower education was associated with lower WLZ among boys. There were no intermediate determinants of boys' WLZ. At the proximal level after adjustment for paternal education and employment, birthweight and child's age were positively associated with boys' WLZ. Among girls, no distal or intermediate factors were associated with WLZ. At the proximal level, being the third or later child was associated with lower WLZ while child's age was positively associated WLZ.

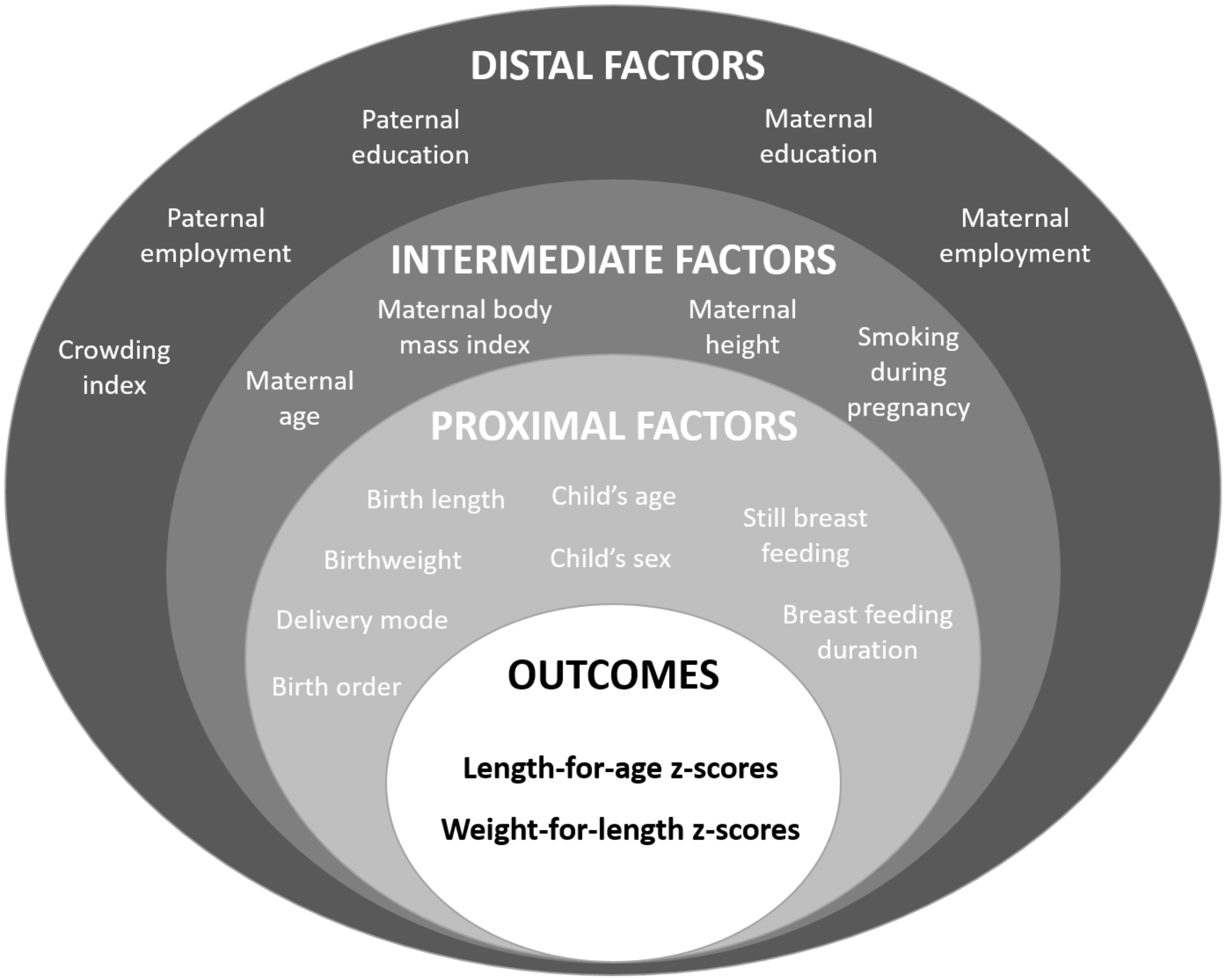


Figure 2.1 Hierarchical conceptual framework of socio-economic, maternal, and child characteristics influencing nutritional status during the first 1000 days of life in Lebanon.

Table 2.1 Socio-economic, maternal and child characteristics of the sample of mother-child (≤2 years) dyads by child's sex in Lebanon

Variables[1]	Total (N=466) n (%) or mean (SD)[3]	Boys (n=231) n (%) or mean (SD)[3]	Girls (n=235) n (%) or mean (SD)[3]
Distal socio-economic characteristics			
Crowding index (individual/ room)			
>1	300 (67.6)	151 (69.4)	149 (65.6)
≤1	166 (32.4)	80 (30.6)	86 (34.4)
Paternal education			
Primary or lower	82 (20.2)	48 (22.3)	34 (17.9)
Intermediate or high school	258 (53.2)	115 (45.5)	143 (61.7)
University degree or technical diploma	121 (26.6)	66 (32.2)	55 (20.4)
Paternal employment			
Government	116 (23.3)	54 (20.7)	62 (26.1)
Private sector	168 (39.9)	84 (40.8)	84 (38.9)
Self-owned business	162 (36.9)	82 (38.6)	80 (35.0)
Maternal education			
Primary or lower	43 (11.3)	22 (10.1)	21 (12.6)
Intermediate or high school	254 (55.9)	118 (53.3)	136 (58.8)
University degree or technical diploma	169 (32.8)	91 (36.6)	78 (28.6)
Maternal employment			
Housewife	372 (84.3)	183 (85.1)	189 (83.3)
Employed	94 (15.7)	48 (14.9)	46 (16.7)
Intermediate maternal characteristics			
Maternal age (years)[2]	30.2 (6.3)	30.0 (5.8)	30.4 (6.7)
Maternal height (cm)[2]	159.7 (5.9)	159.9 (5.8)	159.4 (5.9)
Maternal BMI			
Normal	187 (41.2)	95 (35.8)	92 (47.4)
Overweight	140 (34.5)	69 (37.0)	71 (31.7)
Obese	108 (24.3)	54 (27.3)	54 (20.9)
Smoking during pregnancy			
No	387 (87.6)	192 (88.0)	195 (87.3)
Yes	71 (12.4)	34 (12.0)	37 (12.7)
Proximal child characteristics			
Birth order			
Only or 1st child	180 (33.1)	89 (33.1)	91 (33.1)
2nd child	115 (22.8)	58 (21.4)	57 (24.2)
3rd or later child	171 (44.2)	84 (45.5)	87 (42.7)
Delivery mode			
Vaginal	251 (59.5)	119 (60.8)	132 (58.1)
Caesarean section	212 (40.5)	111 (39.2)	101 (41.9)
Birthweight (kg)[2]	3.2 (0.5)	3.3 (0.4)	3.1 (0.5)
Birth length (cm)[2]	50.2 (2.7)	50.4 (2.7)	50.0 (2.8)
Child's age (months)[2]	11.6 (7.0)	11.0 (7.4)	12.3 (6.4)
Still breast feeding			
No	320 (67.1)	160 (68.9)	160 (65.0)
Yes	146 (32.9)	71 (31.1)	75 (35.0)
Breast feeding duration (months)[2]	5.0 (5.2)	4.3 (4.9)	5.8 (5.5)

BMI: body mass index; SD: standard deviation.
[1] Data reported as n (%) unless otherwise stated.
[2] Data reported as mean (SD).
[3] n is computed using unweighted data while % and mean (SD) are computed using weighted data.

Table 2.2 Sex-specific determinants of length-for-age and weight-for length z-scores among children ≤2 years in Lebanon

Variables	LAZ (N=460)				WLZ (N=455)			
	Boys (n= 228)		Girls (n= 232)		Boys (n= 223)		Girls (n= 232)	
	Unadjusted β (95% CI)	Adjusted β (95% CI)[1]	Unadjusted β (95% CI)	Adjusted β (95% CI)[2]	Unadjusted β (95% CI)	Adjusted β (95% CI)[3]	Unadjusted β (95% CI)	Adjusted β (95% CI)[4]
Distal socio-economic characteristics								
Crowding index (individual/room)								
>1	0.00 (Reference)	-	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	-	0.00 (Reference)	
≤1	0.03 (-0.87, 0.92)		0.81 (0.27, 1.35)	0.81 (0.27, 1.35)	0.24 (-0.52, 0.99)		0.20 (-0.25, 0.65)	-
Paternal education								
Primary or lower	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)		0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	
Intermediate or high school	0.10 (-0.58, 0.79)	-0.31 (-1.06, 0.44)	-0.12 (-0.72, 0.48)	-	-0.49 (-1.21, 0.22)	-0.39 (-1.14, 0.38)	0.05 (-0.57, 0.66)	-
University degree or technical diploma	1.07 (0.23, 1.91)	0.38 (-0.57, 1.33)	-0.28 (-1.07, 0.51)		-1.08 (-2.09, -0.07)	-0.94 (-1.80, -0.07)	0.03 (-0.67, 0.73)	
Paternal employment								
Government	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)		0.00 (Reference)	0.00 (Reference)	0.00 (Reference)	
Private sector	0.86 (0.09, 1.63)	0.53 (-0.09, 1.15)	-0.29 (-1.19, 0.61)	-	-1.01 (-1.99, -0.03)	-0.61 (-1.33, 0.12)	-0.14 (-0.58, 0.30)	-
Self-owned business	-0.16 (-0.92, 0.61)	-0.30 (-0.93, 0.33)	-0.26 (-1.15, 0.64)		-0.47 (-1.20, 0.25)	-0.42 (-1.07, 0.23)	0.02 (-0.33, 0.37)	
Maternal education								
Primary or lower	0.00 (Reference)	0.00 (Reference)	0.00 (Reference)		0.00 (Reference)		0.00 (Reference)	
Intermediate or high school	0.95 (0.13, 1.78)	0.99 (0.20, 1.78)	0.11 (-0.45, 0.66)	-	-0.68 (-1.89, 0.53)	-	-0.15 (-0.94, 0.64)	-
University degree or technical diploma	0.75 (-0.13, 1.63)	0.81 (-0.05, 1.66)	0.16 (-0.61, 0.93)		-0.28 (-1.25, 0.70)		0.16 (-0.62, 0.94)	
Maternal employment								
Housewife	0.00 (Reference)	-	0.00 (Reference)	-	0.00 (Reference)	-	0.00 (Reference)	-
Employed	0.06 (-0.62, 0.74)		0.22 (-0.32, 0.76)		-0.02 (-0.76, 0.72)		0.17 (-0.22, 0.57)	
Intermediate maternal characteristics								
Maternal age (years)	-0.05 (-0.11, 0.02)	-	-0.03 (-0.06, 0.01)	-	0.05 (-0.00, 0.11)	-	0.00 (-0.02, 0.02)	-
Maternal height (cm)	0.08 (0.01, 0.15)	0.06 (-0.02, 0.13)	0.06 (0.03, 0.10)	0.02 (-0.01, 0.05)	0.00 (-0.05, 0.05)	-	0.01 (-0.02, 0.04)	-
Maternal BMI								
Normal	0.00 (Reference)		0.00 (Reference)	0.00 (Reference)	0.00 (Reference)		0.00 (Reference)	
Overweight	0.35 (-0.52, 1.21)	-	-0.20 (-0.72, 0.33)	-0.22 (-0.71, 0.28)	-0.28 (-1.46, 0.89)	-	0.28 (-0.11, 0.68)	-
Obese	-0.59 (-1.48, 0.31)		-1.17 (-1.68, -0.65)	-0.91 (-1.41, -0.41)	-0.09 (-0.80, 0.62)		0.28 (-0.17, 0.72)	
Smoking during pregnancy								
No	0.00 (Reference)	-	0.00 (Reference)	-	0.00 (Reference)	-	0.00 (Reference)	-
Yes	0.57 (-0.28, 1.42)		0.19 (-0.43, 0.81)		0.50 (-0.17, 1.16)		0.36 (-0.03, 0.76)	
Proximal child characteristics								
Birth order								
Only or 1st child	0.00 (Reference)		0.00 (Reference)		0.00 (Reference)		0.00 (Reference)	0.00 (Reference)
2nd child	0.06 (-0.37, 0.50)	-	-0.41 (-1.07, 0.25)	-	-0.05 (-0.83, 0.74)	-	0.03 (-0.40, 0.46)	-0.23 (-0.67, 0.20)
3rd or later child	-0.48 (-1.45, 0.49)		-0.50 (-1.08, 0.08)		-0.15 (-1.14, 0.84)		-0.39 (-0.73, -0.05)	-0.49 (-0.83, -0.15)
Delivery mode								
Vaginal	0.00 (Reference)	-	0.00 (Reference)	-	0.00 (Reference)	-	0.00 (Reference)	-
Caesarean section	-0.08 (-0.82, 0.66)		0.13 (-0.38, 0.64)		0.19 (-0.59, 0.98)		0.03 (-0.48, 0.53)	

Table 2.2 continued

Birthweight (kg)	0.50 (-0.18, 1.18)	-	0.28 (-0.41, 0.96)	-	1.19 (0.39, 1.98)	1.18 (0.60, 1.75)	0.52 (-0.06, 1.10)	-
Birth length (cm)	0.08 (0.00, 0.15)	0.02 (-0.06, 0.10)	0.15 (0.04, 0.25)	0.11 (0.03, 0.19)	0.13 (0.06, 0.20)	0.07 (-0.02, 0.15)	0.05 (-0.03, 0.13)	-
Child's age (month)	-0.03 (-0.09, 0.02)	-	-0.04 (-0.08, 0.00)	-	0.08 (0.04, 0.13)	0.06 (0.02, 0.09)	0.05 (0.02, 0.07)	0.05 (0.03, 0.07)
Still breast feeding								
No	0.00 (Reference)	-	0.00 (Reference)	-	0.00 (Reference)	-	0.00 (Reference)	-
Yes	-0.82 (-1.65, 0.01)		-0.32 (-0.94, 0.31)		-0.20 (-1.11, 0.71)		-0.36 (-0.74, 0.01)	
Breast feeding duration (months)	-0.05 (-0.11, 0.01)	-	-0.07 (-0.11, -0.03)	-0.05 (-0.09, -0.01)	0.04 (-0.02, 0.10)	-	0.00 (-0.03, 0.04)	-

BMI: body mass index; CI: Confidence interval; LAZ: length-for-age z-scores; WLZ: weight-for-length z-scores.

[1]The complete LAZ model for boys adjusted for paternal education and employment, maternal education, maternal height, and birth length, with an adjusted R^2= 24.8%.

[2]The complete LAZ model for girls adjusted for crowding index, maternal height and BMI, birth length, and breast feeding duration, with an adjusted R^2= 24.6%.

[3]The complete WLZ model for boys adjusted for paternal education and employment, birthweight, birth length, and child's age, with an adjusted R^2= 36.1%.

[4]The complete WLZ model for girls adjusted for birth order and child's age, with an adjusted R^2= 12.5%.

2.5 Comment

2.5.1 Principal findings

The current study reports the determinants of LAZ and WLZ of Lebanese children aged <2 years using sex-stratified linear regression analysis based on a hierarchical conceptual framework. Distal socio-economic determinants of LAZ included maternal education among boys and crowding index among girls, whereas those of WLZ included paternal education among boys. Intermediate maternal determinants of LAZ included maternal BMI among girls. Among the proximal child determinants, birth length and breast feeding duration were associated with girls' LAZ. Birthweight was associated with boys' WLZ while birth order was associated with girls' WLZ, and child's age was a proximal determinant of WLZ among both sexes.

2.5.2 Strengths of the study

Our study has a number of strengths. First, the data were derived from a nationally representative sample of mother-child dyads in Lebanon. Second, our results fill a gap in the literature on the determinants of nutritional status and growth during the first 1000 days of life in a country of the EMR. Third, we examine the determinants of linear growth and weight as continuous variables rather than those of stunting, wasting, and/or overweight and obesity. Although the latter is important, Lebanon as a middle-income country has lower rates of undernutrition than lower-income countries of the EMR and therefore fewer observations for comparison with the normal. Further international child growth standards use the cut-off of LAZ less than 2 SD below the median for stunting, which might misclassify children with LAZ close to -2 SD as normal rather than stunted. Finally, the approach of hierarchical modelling offers insights into the multi-level determinants of nutritional status and helps avoid underestimating the effects of distal determinants.

2.5.3 Limitations of the data

The results of this study should be interpreted with certain limitations. Our cross-sectional design describes associations and point prevalence estimates, not temporal or causal relations. Conducting prospective longitudinal studies of pregnant women through their delivery and children's early years would reduce variability from a one point in time estimate and improve the understanding of how environmental and lifestyle factors exert their effects on growth. Follow-up studies can examine whether the sex-specific differences in the distributions of LAZ and WLZ remain over time and investigate the effect of such differences on health later in life. Another limitation is that maternal weight and height, and hence BMI, were measured at the time of study not before or during pregnancy. Additionally, mothers reported infant feeding practices including breast feeding duration of their children, with potential for recall bias. Nevertheless, data on infant feeding collected retrospectively by maternal recall of events that took place less than 18 months ago have shown to be accurate.(74)

2.5.4 Interpretation

Most prior research on the nutritional status of children has been conducted among children ≤5 years and did not stratify by the child's sex. Further, prior research focused on the determinants of stunting, wasting, and/or overweight and obesity rather than linear growth and weight as continuous variables. Therefore, few studies among children ≤2 years and stratified by child's sex were available for comparison.

In the current analysis, boys had lower mean LAZ than girls, but there were no sex differences in mean WLZ. Other studies among children in the EMR showed that mean length of boys ≤2 years old in Iran was higher than that of girls,(75) while mean length of boys and girls in Qatar did not differ.(76)

At the distal level, SES characteristics including higher maternal education and lower crowding index were associated with higher LAZ, while higher paternal education was associated with lower WLZ. In a pooled analysis across 39 low- and middle-income countries, secondary or higher maternal and paternal education, compared to primary education, were independently associated with higher height-for-age z-scores among 36-59-month-old children, with the associations being stronger for maternal than paternal education.(77) In Lebanon, household crowding was inversely associated with the family's SES as defined by education and employment status of the parents.(68) Similarly in our study, crowding index was inversely associated with maternal and paternal education (data not shown). A number of studies using similar hierarchical analysis among 10-15 month old children in the Brazilian Amazon(55) and of 6-23 month old children in Ethiopia(56) noted that lower household SES was associated with poorer linear growth. As for WLZ, a study examining the risk factors of overweight and obesity among Portuguese children reported that paternal secondary school and university education, compared to primary school education, were associated with decreased odds of childhood obesity.(78)

At the intermediate level, maternal obesity was associated with lower LAZ. Maternal overweight and obesity were prevalent at 34.5% and 24.3% in our study, respectively. Interventions focused on improving maternal nutritional status and combating overweight and obesity are crucial given their health benefits not only for women, but also for their children and the next generation.(79)

At the proximal level, birth length was positively associated with LAZ and birthweight and child's age were positively associated with WLZ. On the other hand, being a third or later child was associated with lower WLZ. In a pooled analysis of 28 twin cohorts in 17 countries, birthweight and birth length were positively associated with length at one and two years of age.(80) Further, birth weight was positively associated with BMI-for-age z-score within the first year of life the aforementioned Brazilian child study.(55)

Longer breast feeding duration was among the proximal factors associated with lower LAZ. This inverse association has been previously reported in low- not middle-income countries like Lebanon, but results have been inconsistent with some studies reporting positive or null associations.(81, 82) Also, the potential for reverse causality where the child's nutritional status dictates whether the mother continues to breastfeed should be considered.(83) Given the well-established benefits of breast feeding for the mother and child, the current WHO recommendation of exclusive breast feeding for the first 6 months with continued breast feeding for 2 years of age or beyond should be encouraged.(84) However, similar to other studies in Lebanon,(85-87) we report suboptimal breast feeding practices where less than a third of the children were breast fed at the time of the study and the mean breast feeding duration was 5.1 months.

2.6 Conclusions

Our study reveals sex-specific distal, intermediate, and proximal factors associated with nutritional status in Lebanese children aged <2 years. There is a dire need for studies of nutritional status and growth among infants and children in Lebanon and in the EMR given that the first 1000 days of life are a window of opportunity with long-term effects on health of individuals. Future research should address sex differences in the determinants of child growth using similar hierarchical frameworks. Results from such research will help inform culture-specific interventions and policies to improve child growth and health later in life.

2.6.1 Acknowledgments

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2.6.2 Funding

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CHAPTER 3. EXCLUSIVE BREASTFEEDING DURING THE 40-DAY REST PERIOD AND AT SIX MONTHS IN LEBANON: A CROSS-SECTIONAL STUDY

Chehab RF, Nasreddine L, Zgheib R, Forman MR. Exclusive breastfeeding during the 40-day rest period and at six months in Lebanon: a cross-sectional study. Int Breastfeed J. 2020 May 19;15(1):45. doi: 10.1186/s13006-020-00289-6. PMID: 32430076; PMCID: PMC7236524.

3.1 Abstract

Background: Exclusive breastfeeding is recommended for the first 6 months of life with well-established benefits to the mother and child. The traditional practice of the 40-day rest period helps establish and maintain exclusive breastfeeding. This study aims to estimate the prevalence and examine the factors associated with exclusive breastfeeding for 40 days and 6 months in Lebanon.

Methods: A cross-sectional survey was conducted in 2011-2012 as part of the "Early Life Nutrition and Health in Lebanon" study. A nationally representative sample of 1005 children aged 5 years or younger and their mothers was drawn from households using a stratified cluster sampling design. Trained nutritionists interviewed eligible mothers about sociodemographic characteristics of the household and maternal and child characteristics including infant feeding practices. Anthropometric measurements of the mother and child were collected. Multinomial logistic regression analysis was conducted to examine the characteristics associated with exclusive breastfeeding.

Results: The prevalence of exclusive breastfeeding was 41.5% at 40 days and 12.3% at 6 months. Children in families with three or more children had higher odds of exclusive breastfeeding for 40 days (Adjusted Odds Ratio (AOR): 1.76, 95% Confidence Interval (CI): 1.19, 2.60). Children in families owning two or more cars had lower odds of exclusive breastfeeding for 40 days (AOR: 0.45, 95% CI: 0.24, 0.83) and 6 months (AOR: 0.32, 95% CI: 0.14, 0.77). Similarly, children delivered via C-section had lower odds of exclusive breastfeeding for 40 days (AOR: 0.49, 95% CI: 0.34, 0.71) and 6 months (AOR: 0.39, 95% CI: 0.24, 0.65). The odds of exclusive breastfeeding for 6 months were lower among children of overweight (AOR: 0.50, 95% CI: 0.26, 0.95) or obese (AOR: 0.56, 95% CI: 0.32, 0.98) mothers.

Conclusions: The association between higher socioeconomic status, as reflected by car ownership, and C-section delivery with lower odds of exclusive breastfeeding persisted across the first 6 months in Lebanon. Future research should investigate the factors associated with exclusive breastfeeding in prospective cohort studies and help to better understand the cultural practice of the 40-day rest period in relation to breastfeeding.

Keywords: Exclusive breastfeeding; 40-day rest period; socioeconomic status; C-section delivery; Lebanon

3.2 Background

Many societies in the Middle East observe a 40-day postpartum period of rest, seclusion, and ritual that helps establish and maintain breastfeeding (88, 89) and protects the mother and newborn from illnesses (90). During this 40-day rest period, the mother often stays at home and receives help with household chores and congratulatory visits from related women and neighbors (90). She is encouraged to eat a special diet rich in meat, poultry, soups, and other foods thought to be good for milk production (90). Other than the cultural aspect, the 40-day rest period has religious underpinnings. For both Muslims and Christians, the 40 days following birth coincide with the period of vaginal discharge resulting from involution of the uterus (91-93). Despite being closely linked to infant feeding practices, few studies have examined the 40-day rest period in relation to total and exclusive breastfeeding (88, 89).

The World Health Organization (WHO) recommends exclusive breastfeeding (EBF) for the first 6 months of life (94). Despite these recommendations and the well-established benefits of breastfeeding, the proportion of mothers who EBF for 6 months in the Middle East is estimated at 20.5% (95% CI: 14.5, 28.2) (95). The prevalence of EBF for 6 months in specific countries of the Middle East ranges from 2% in Kuwait (96) to 56.4% in Iran (97). In Lebanon, a small Middle Eastern country on the Mediterranean sea, the prevalence of EBF for 6 months was 10.1% in a national sample of mother-child dyads recruited from primary health care centers in 2004 (87).

More recently in 2016, the prevalence of EBF for 4-6 months was estimated at 16.5% among mothers of toddlers attending daycare centers (98).

None of the previous studies conducted in Lebanon focused on the 40-day rest period as a cultural practice for breastfeeding. Understanding the context-specific patterns and determinants of breastfeeding practices is essential to ensure successful promotion strategies (99). Therefore, it is timely to place a lens on the 40-day rest period and to examine the factors associated with EBF in Lebanon. The objectives of this paper are to estimate the prevalence of EBF for 40 days and 6 months, and to examine the sociodemographic, maternal, and child factors associated with EBF in a nationally representative sample of mother-child dyads in Lebanon in 2011-2012.

3.3 Methods

The study was designed as a cross-sectional survey as part of the "Early Life Nutrition and Health in Lebanon (ELNAHL)" project (16).

3.3.1 Sampling strategy

A representative sample of children (N=1194) aged 5 years or younger of both sexes was drawn from households using a stratified cluster sampling design. The strata were the six Lebanese governorates and the clusters were selected at the district level. Within each district, households were selected following a probability proportional to size approach, whereby a higher number of participating households was drawn from more populous districts. Housing units constituted the primary sampling unit in the districts of Lebanon.

3.3.2 Eligibility criteria

Mother-child dyads were eligible to participate in the study if the mother was Lebanese, aged 19-40 years, did not have hypertension or diabetes, and was not taking medications that interfere with eating and breastfeeding or affect body weight. Children were eligible if they were 5 years old or younger, were born at term (gestational age between 37-42 weeks), and had no chronic medical conditions, inborn errors of metabolism, or physical malformations that interfere with feeding patterns and body composition.

3.3.3 Data collection

Data were collected between September 2011 and August 2012. Trained nutritionists administered the survey through face-to-face interviews with the mothers. The survey inquired about sociodemographic characteristics of the household, current and future family planning practices of the mother, access to maternal and child health services, and mother's knowledge and practices related to infant feeding. Questions on infant feeding practices focused on the duration of total and exclusive breastfeeding, which was assessed using the life-long approach (100), the age of introduction of formula milk and solid food, and the reasons for breastfeeding, not breastfeeding, and breastfeeding cessation. Anthropometric measurements of the mother and child were collected. This study was approved by the Institutional Review Boards at the American University of Beirut (NUT.LN.13) and Purdue University (Protocol number: 1902021663). Mothers provided written informed consents.

3.3.4 Definitions of infant feeding practices

Infant feeding practices were defined as follows:

1. Exclusive breastfeeding (EBF): The infant received breast milk from his/her mother or expressed breast milk and no other fluids or solids.
2. Mixed feeding: The infant received breast milk with formula milk and/or other fluids and/or solid food.
3. Exclusive bottle feeding (EBOT): The infant received formula milk with or without other fluids.
4. Bottle and solid feeding (BOT+SF): The infant received formula milk and/or other fluids and solid food.

3.3.5 Statistical analysis

Frequencies with percentages (%) and means with standard deviations (SD) were calculated to describe categorical and continuous variables, respectively. Since only 17 mothers (1.8%) of the sample were underweight (Body Mass Index (BMI) <18.5 kg/m2) at the time of the interview, they were merged with the normal weight mothers (BMI= 18.5-24.9 kg/m2) into one BMI category (normal weight (<25 kg/m2)). Chi-squared test and Analysis of Variance (ANOVA) followed by post hoc comparisons (Bonferroni) were calculated, as appropriate, to compare infant feeding practices at 40 days and 6 months by sociodemographic, maternal, and child characteristics. Multinomial logistic regression models were computed to estimate the adjusted odds ratio (AOR) and 95% confidence intervals (CI) of EBF compared to mixed feeding and EBOT at 40 days, as well as the odds of EBF compared to mixed feeding and BOT+SF at 6 months. Variables selected for inclusion in the multinomial analysis had a p-value <0.10 in the bivariate analysis. All statistical analysis was conducted using the Statistical Analysis Package for Social Sciences (SPSS, version 24.0).

3.4 Results

Of the 1194 eligible mother-child dyads that were contacted, 1029 participated in the survey (response rate 86%). Twenty-four surveys with missing data were excluded, leaving 1005 mother-child dyads in the analysis. At the time of the interview, 947 children were 40 days or older and had complete information about feeding practices at 40 days, while 893 children were 6 months or older and had complete information about feeding practices at 6 months and thereby included in the analysis at 40 days and 6 months, respectively.

3.4.1 Prevalence of exclusive breastfeeding and other infant feeding practices at 40 days and 6 months

The prevalence of infant feeding practices at 40 days and 6 months are presented in Figure 3.1. Of the total 1005 children included in the analysis, 89.5% were ever breastfed. Among those, 27.8% were first breastfed within one hour after delivery, 53.1% after one hour but within the first 24 hours, and 19.1% within days after delivery (data not shown). At 40 days, 41.5% (95% CI: 38.4, 44.7) of the 947 children were EBF, 38.1% (95% CI: 34.8, 41.2) were mixed fed, and 20.2% (95% CI: 17.5, 22.7) were EBOT. At 6 months, 12.3% (95% CI: 10.2, 14.6) of the 893 children were EBF, 38.4% (95% CI: 35.3, 41.7) were mixed fed, and 40.1% (95% CI: 36.7, 43.3) were BOT+SF.

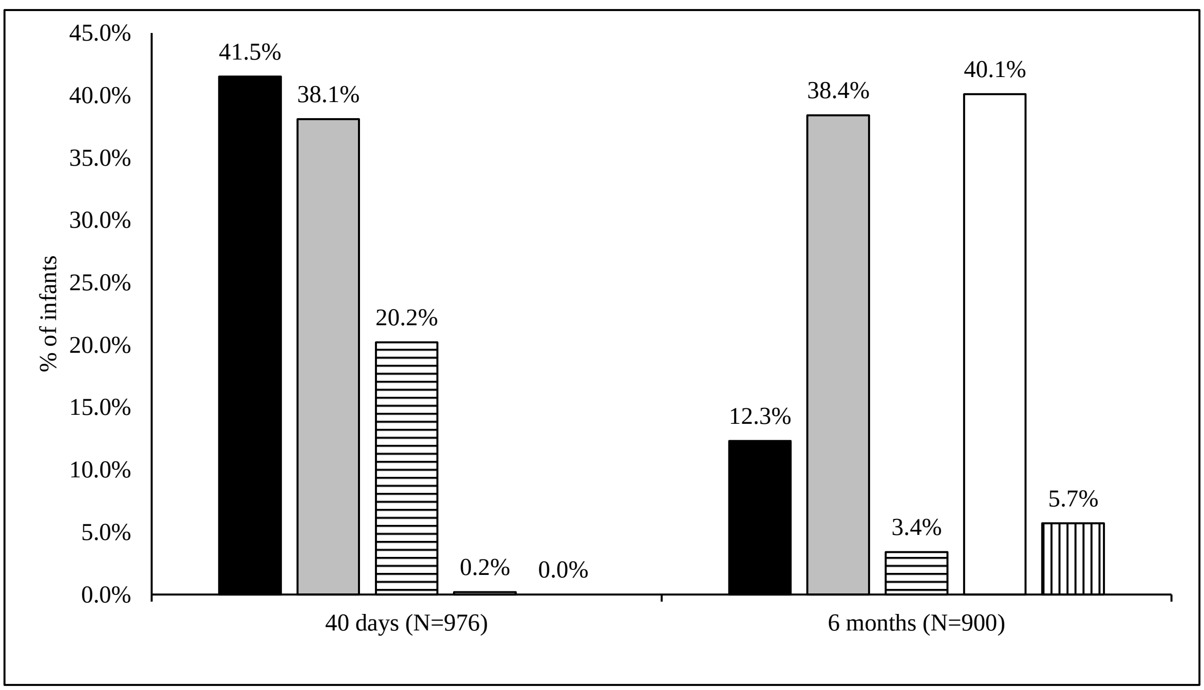
3.4.2 Sociodemographic, maternal, and child characteristics by infant feeding practice at 40 days and 6 months

Sociodemographic, maternal, and child characteristics by infant feeding practice at 40 days and 6 months are presented in Table 3.1. There were significant differences between the infant feeding groups at 40 days in terms of paternal educational level, number of owned cars, number of children in the family, mode of delivery and child's age, weight and height at the time of the interview (overall p-value <0.05). Specifically, a lower proportion of EBOT children had fathers with an education at an intermediate level or lower compared to mixed fed children (Bonferroni adjusted p-value= 0.008 (data not shown)). A higher proportion of EBOT children were in families that owned two or more cars (p-value= 0.013) and had less than three children (p-value <0.001) compared to EBF children. In addition, a higher proportion of EBOT children was delivered via C-section compared to EBF (p-value <0.001) and mixed fed (p-value= 0.002) children. EBF children were older and taller at the time of the interview than mixed fed (p-value= 0.001 and 0.002, respectively) and EBOT (p-value= 0.002 and 0.016, respectively) children. Moreover, EBF children were heavier at the time of the interview than mixed fed children (p-value= 0.015).

At 6 months, there were significant differences between the infant feeding groups in terms of governorate of residence, paternal and maternal education, monthly income, number of owned cars, and number of children in the family (overall p-value <0.05). The groups also differed in terms of maternal BMI, mode of delivery, and child's age, weight and height at the time of the interview. A lower proportion of BOT+SF children were in families that lived in North Lebanon (Bonferroni adjusted p-value= 0.001), had lower monthly income (p-value <0.001), and had fathers and mothers with an education at an intermediate level or lower (p-value= 0.004 and <0.001, respectively) compared to mixed fed children. In addition, a higher proportion of BOT+SF children were in families that owned two or more cars (p-value <0.001) and had less than three children (p-value <0.001) compared to mixed fed children. A higher proportion of EBF children had mothers with normal weight compared to mixed fed children (p-value= 0.016). A higher proportion of BOT+SF children was delivered via C-section compared to EBF (p-value <0.001) and mixed fed (p-value <0.001) children. EBF children were older than BOT+SF (p-value= 0.002), heavier than mixed fed children (p-value= 0.005), and taller than mixed fed (p-value= 0.013) and BOT+SF (p-value= 0.011) children.

Table 3.2 presents the results of the multinomial logistic regression analysis of the factors associated with EBF for 40 days. Although certain variables had significant overall and/or Bonferroni adjusted p-values in the bivariate analysis, they were not significant after adjusting for all the variables in the model. Children in families owning two or more cars and those delivered via C-section had lower odds of EBF than EBOT at 40 days postpartum (AOR: 0.45, 95% CI: 0.24, 0.83 and AOR: 0.49, 95% CI: 0.34, 0.71, respectively). On the other hand, children in families with three or more children had higher odds of EBF than EBOT at 40 days (AOR: 1.76, 95% CI: 1.19, 2.60).

Table 3.3 presents the results of the multinomial logistic regression analysis at 6 months. The odds of EBF were half those of mixed feeding at 6 months among children of overweight (AOR: 0.50, 95% CI: 0.26, 0.95) or obese (AOR: 0.56, 95% CI: 0.32, 0.98) mothers. Children in families owning two or more cars and those delivered via C-section had lower odds of EBF than of BOT+SF at 6 months (AOR: 0.32, 95% CI: 0.14, 0.77 and AOR: 0.39, 95% CI: 0.24, 0.65, respectively).



EBF: Exclusive breastfeeding; EBOT: Exclusive bottle feeding; BOT+SF: Bottle and solid feeding; ESF: Exclusive solid feeding

Figure 3.1 Prevalence of infant feeding practices at 40 days and 6 months in Lebanon

Table 3.1 Sociodemographic, maternal, and child characteristics by infant feeding practice at 40 days and 6 months in Lebanon

Characteristics [#]	**At 40 days (N=947)**				**At 6 months (N=893)**			
	EBF (n=393)	Mixed fed (n=361)	EBOT (n=191)	*p*-value*	EBF (n=110)	Mixed fed (n=343)	BOT+SF (n=358)	*p*-value*
Sociodemographic characteristics								
Governorate of residence								
Beirut	43 (10.9)	31 (8.6)	15 (7.9)	0.074	14 (12.7) [a,b]	31 (9.0) [a]	33 (9.2) [b]	**0.009**
Mount Lebanon	123 (31.3)	95 (26.3)	67 (35.1)		36 (32.7) [a,b]	83 (24.2) [a]	126 (35.2) [b]	
North Lebanon	151 (38.4)	134 (37.1)	58 (30.4)		37 (33.6) [a,b]	152 (44.3) [a]	107 (29.9) [b]	
South Lebanon & Nabatieh	53 (13.5)	67 (18.6)	34 (17.8)		17 (15.5) [a,b]	52 (15.2) [a]	64 (17.9) [b]	
Bekaa	23 (5.9)	34 (9.4)	17 (8.9)		6 (5.5) [a,b]	25 (7.3) [a]	28 (7.8) [b]	
Family monthly income (USD)								
≤ 400	39 (9.9)	38 (10.6)	10 (5.2)	0.341	6 (5.5) [a,b]	49 (14.4) [a]	17 (4.8) [b]	**<0.001**
400.1 - 1000	180 (45.9)	153 (42.6)	89 (46.6)		56 (50.9) [a,b]	161 (47.2) [a]	157 (44.0) [b]	
1000.1 - 2000	66 (16.8)	65 (18.1)	47 (24.6)		15 (13.6) [a,b]	49 (14.4) [a]	82 (23.0) [b]	
> 2000	36 (9.2)	30 (8.4)	15 (7.9)		13 (11.8) [a,b]	16 (4.7) [a]	40 (11.2) [b]	
Doesn't know	42 (10.7)	42 (11.7)	16 (8.4)		12 (10.9) [a,b]	44 (12.9) [a]	29 (8.1) [b]	
Refused to answer	29 (7.4)	31 (8.6)	14 (7.3)		8 (7.3) [a,b]	22 (6.5) [a]	32 (9.0) [b]	
Paternal educational level								
Intermediate or lower	210 (54.7) [a,b]	205 (57.6) [a]	92 (48.2) [b]	**0.035**	57 (52.3) [a,b]	208 (61.7) [a]	175 (49.2) [b]	**0.023**
Secondary education or technical diploma	113 (29.4) [a,b]	96 (27.0) [a]	76 (39.8) [b]		35 (32.1) [a,b]	87 (25.8) [a]	123 (34.6) [b]	
University degree	61 (15.9) [a,b]	55 (15.4) [a]	23 (12.0) [b]		17 (15.6) [a,b]	42 (12.5) [a]	58 (16.3) [b]	
Paternal employment status								
Government or private sector	229 (58.3)	209 (57.9)	117 (61.3)	0.400	67 (60.9)	196 (57.1)	219 (61.2)	0.414
Self-employed	137 (34.9)	136 (37.7)	67 (35.1)		34 (30.9)	130 (37.9)	122 (34.1)	
Unemployed	27 (6.9)	16 (4.4)	7 (3.7)		9 (8.2)	17 (5.0)	17 (4.7)	
House ownership								
Yes	230 (58.5)	215 (59.6)	122 (63.9)	0.454	60 (54.5)	206 (60.1)	222 (62.0)	0.375
No	163 (41.5)	146 (40.4)	69 (36.1)		50 (45.5)	137 (39.9)	136 (38.0)	
Number of owned cars								
0	90 (22.9) [a]	87 (24.1) [a,b]	36 (18.8) [b]	**0.046**	24 (21.8) [a,b]	96 (28.0) [a]	57 (15.9) [b]	**<0.001**
1	235 (59.8) [a]	204 (56.5) [a,b]	102 (53.4) [b]		66 (60.0) [a,b]	202 (58.9) [a]	198 (55.3) [b]	
≥2	68 (17.3) [a]	70 (19.4) [a,b]	53 (27.7) [b]		20 (18.2) [a,b]	45 (13.1) [a]	103 (28.8) [b]	
Crowding index (individuals/room)								
≥1	349 (88.8)	309 (85.6)	163 (85.3)	0.334	99 (90.0)	303 (88.3)	301 (84.1)	0.138
<1	44 (11.2)	52 (14.4)	28 (14.7)		11 (10.0)	40 (11.7)	57 (15.9)	

Table 3.1 continued

Number of children								
1-2	199 (50.6) [a]	203 (56.2) [a,b]	127 (66.5) [b]	**0.001**	59 (53.6) [a,b]	162 (47.2) [a]	229 (64.0) [b]	**<0.001**
≥3	194 (49.4) [a]	158 (43.8) [a,b]	64 (33.5) [b]		51 (46.4) [a,b]	181 (52.8) [a]	129 (36.0) [b]	
Maternal characteristics								
Age (years) [$]	32.1 ± 5.7	31.2 ± 6.6	31.4 ± 6.6	0.122	32.5 ± 5.9	32.2 ± 6.3	31.3 ± 6.4	0.064
Educational level								
Intermediate or lower	201 (51.1)	180 (49.9)	79 (41.4)	0.067	52 (47.3) [a,b]	193 (56.3) [a]	150 (41.9) [b]	**0.001**
Secondary education or technical diploma	103 (26.2)	106 (29.4)	72 (37.7)		30 (27.3) [a,b]	97 (28.3) [a]	121 (33.8) [b]	
University degree	89 (22.6)	75 (20.8)	40 (20.9)		28 (25.5) [a,b]	53 (15.5) [a]	87 (24.3) [b]	
Employment status								
Employed	65 (16.5)	62 (17.2)	40 (20.9)	0.404	14 (12.7)	47 (13.7)	71 (19.8)	0.050
Housewives	328 (83.5)	299 (82.8)	151 (79.1)		96 (87.3)	296 (86.3)	287 (80.2)	
BMI (kg/m^2)								
Normal weight (<25)	163 (44.4)	137 (42.2)	71 (40.3)	0.903	57 (53.3) [a]	118 (37.7) [b]	148 (45.8) [a,b]	**0.024**
Overweight (25-29.99)	117 (31.9)	108 (33.2)	58 (33.0)		29 (27.1) [a]	103 (32.9) [b]	105 (32.5) [a,b]	
Obese (≥30)	87 (23.7)	80 (24.6)	47 (26.7)		21 (19.6) [a]	92 (29.4) [b]	70 (21.7) [a,b]	
Child characteristics								
Age (months) [$]	29.6 ± 15.6 [a]	25.9 ± 16.1 [b]	25.2 ± 16.4 [b]	**0.001**	32.3 ± 13.7 [a]	28.9 ± 14.7 [a,b]	27.5 ± 15.5 [b]	**0.014**
Gender								
Male	187 (47.6)	192 (53.2)	103 (53.9)	0.204	49 (44.5)	174 (50.7)	195 (54.5)	0.176
Female	206 (52.4)	169 (46.8)	88 (46.1)		61 (55.5)	169 (49.3)	163 (45.5)	
Mode of delivery								
Vaginal	241 (61.5) [a]	197 (55.0) [a]	79 (41.4) [b]	**<0.001**	74 (67.3) [a]	205 (59.9) [a]	157 (44.1) [b]	**<0.001**
C-section	151 (38.5) [a]	161 (45.0) [a]	112 (58.6) [b]		36 (32.7) [a]	137 (40.1) [a]	199 (55.9) [b]	
Birth weight (g) [$]	3218.2 ±500.9	3244.4 ±562.7	3169.7 ±547.0	0.297	3293.5 ±512.0	3232.1 ±540.3	3188.6 ±536.9	0.178
Birth length (cm) [$]	50.3 ± 3.0	50.3 ± 3.0	50.3 ± 3.4	0.986	50.3 ± 3.3	50.3 ± 2.8	50.3 ± 3.1	0.988
Weight (kg) [$]	13.2 ± 3.8 [a]	12.5 ± 4.2 [b]	12.7 ± 3.9 [a,b]	**0.043**	14.0 ± 3.5 [a]	12.9 ± 3.5 [b]	13.2 ± 3.5 [a,b]	**0.018**
Height (cm) [$]	87.9 ± 12.8 [a]	84.8 ± 14.2 [b]	84.9 ± 14.3 [b]	**0.004**	90.6 ± 11.1 [a]	87.4 ± 12.0 [b]	87.4 ± 12.4 [b]	**0.035**

[#] Data are presented as frequency (%) unless otherwise stated.

[$] Data are presented as mean ± SD.

* Overall *p*-values compare the three infant feeding groups together and are calculated using chi-squared test for categorical variables and ANOVA for continuous variables.

[a-b] Infant feeding practices with different superscripts differ significantly after Bonferroni adjustment (*p*-value <0.0167).

Table 3.2 Multinomial logistic regression analysis of factors associated with exclusive breastfeeding for 40 days in Lebanon

Characteristics #	At 40 days (N=947) #	
	EBF vs Mixed feeding [1]	EBF vs EBOT [2]
Sociodemographic characteristics		
Governorate of residence		
Beirut	1.00	1.00
Mount Lebanon	1.04 (0.59, 1.83)	0.89 (0.44, 1.79)
North Lebanon	0.88 (0.51, 1.54)	1.00 (0.49, 2.02)
South Lebanon & Nabatieh	0.60 (0.33, 1.10)	0.64 (0.30, 1.38)
Bekaa	0.54 (0.26, 1.14)	0.58 (0.23, 1.43)
Paternal educational level		
Intermediate or lower	1.00	1.00
Secondary education or technical diploma	1.11 (0.66, 1.86)	1.44 (0.75, 2.76)
University degree	1.25 (0.85, 1.82)	0.86 (0.55, 1.34)
Number of owned cars		
0	1.00	1.00
1	1.10 (0.75, 1.60)	0.93 (0.57, 1.52)
≥2	0.84 (0.50, 1.41)	**0.45 (0.24, 0.83)**
Number of children		
1-2	1.00	1.00
≥3	1.23 (0.90, 1.68)	**1.76 (1.19, 2.60)**
Maternal characteristics		
Educational level		
Intermediate or lower	1.00	1.00
Secondary education or technical diploma	1.09 (0.67, 1.76)	1.39 (0.77, 2.49)
University degree	0.85 (0.58, 1.24)	0.76 (0.48, 1.19)
Child characteristics		
Age (months)	1.00 (0.98, 1.03)	1.03 (0.99, 1.06)
Mode of delivery		
Vaginal	1.00	1.00
C-section	0.78 (0.58, 1.06)	**0.49 (0.34, 0.71)**
Weight (kg)	0.96 (0.87, 1.07)	0.93 (0.83, 1.05)
Height (cm)	1.03 (0.98, 1.07)	1.01 (0.95, 1.06)

Data are presented as adjusted odds ratio (95% confidence interval).

[1] The reference category is Mixed feeding; [2] The reference category is EBOT.

EBF: Exclusive breastfeeding; EBOT: Exclusive bottle feeding

Table 3.3 Multinomial logistic regression analysis of factors associated with exclusive breastfeeding for 6 months in Lebanon

Characteristics	At 6 months (N=893) #	
	EBF vs Mixed feeding [1]	EBF vs BOT+SF [2]
Sociodemographic characteristics		
Governorate of residence		
Beirut	1.00	1.00
Mount Lebanon	1.24 (0.55, 2.80)	0.81 (0.36, 1.82)
North Lebanon	0.73 (0.32, 1.70)	0.74 (0.32, 1.72)
South Lebanon & Nabatieh	1.09 (0.44, 2.74)	0.73 (0.29, 1.81)
Bekaa	0.77 (0.23, 2.59)	0.56 (0.17, 1.85)
Family monthly income (USD)		
≤400	1.00	1.00
400.1- 1000	0.39 (0.11, 1.47)	2.08 (0.51, 8.50)
1000.1- 2000	1.19 (0.47, 3.02)	1.98 (0.80, 4.88)
>2000	0.75 (0.25, 2.23)	0.87 (0.31, 2.45)
Doesn’t know	1.47 (0.44, 4.93)	1.86 (0.60, 5.75)
Refused to answer	1.08 (0.35, 3.27)	2.32 (0.77, 7.00)
Paternal educational level		
Intermediate or lower	1.00	1.00
Secondary education or technical diploma	0.88 (0.37, 2.12)	1.02 (0.43, 2.40)
University degree	1.16 (0.65, 2.09)	0.97 (0.54, 1.74)
Number of owned cars		
0	1.00	1.00
1	0.93 (0.51, 1.68)	0.66 (0.35, 1.23)
≥2	1.01 (0.42, 2.43)	**0.32 (0.14, 0.77)**
Number of children		
1-2	1.00	1.00
≥3	0.85 (0.50, 1.45)	1.18 (0.69, 2.02)
Maternal characteristics		
Age (years)	1.01 (0.97, 1.05)	1.03 (0.98, 1.07)
Educational level		
Intermediate or lower	1.00	1.00
Secondary education or technical diploma	1.46 (0.66, 3.24)	1.73 (0.80, 3.78)
University degree	0.89 (0.48, 1.64)	1.03 (0.56, 1.90)
Employment status		
Employed	1.00	1.00
Housewives	1.70 (0.82, 3.53)	1.63 (0.80, 3.30)
BMI (kg/m^2)		
Normal weight (<25)	1.00	1.00
Overweight (25-29.99)	**0.50 (0.26, 0.95)**	0.70 (0.36, 1.35)
Obese (≥30)	**0.56 (0.32, 0.98)**	0.60 (0.34, 1.05)
Child characteristics		
Age (months)	0.99 (0.95, 1.04)	1.01 (0.96, 1.05)
Mode of delivery		
Vaginal	1.00	1.00
C-section	0.67 (0.40, 1.11)	**0.39 (0.24, 0.65)**
Weight (kg)	1.17 (0.98, 1.39)	1.06 (0.90, 1.26)
Height (cm)	1.00 (0.92, 1.08)	1.00 (0.92, 1.08)

Data are presented as adjusted odds ratio (95% confidence interval).

[1] The reference category is Mixed feeding; [2] The reference category is BOT+SF.

EBF: Exclusive breastfeeding; BOT+SF: Bottle and solid feeding

3.5 Discussion

To our knowledge, this study is the first in the Middle East to examine the prevalence and predictors of EBF during the 40-day rest period. From a nationally representative survey in Lebanon, we report a prevalence of EBF of 41.5% at 40 days and 12.3% at 6 months.
While belonging to a family with more children was positively associated with EBF for 40 days, belonging to a family owning more cars or being born via C-section was associated with lower odds of EBF for 40 days and 6 months. In addition, the odds of EBF for 6 months were lower among children whose mothers were overweight or obese.

The 40-day rest period is practiced in several cultures worldwide including the Amazon (101), Greece (91), China (102), Malaysia (103), Nepal (93), India (92), Burma (104), Turkey (105), Negev Bedouins (88, 90), and Egypt (89). However, few studies have examined the prevalence of total and exclusive breastfeeding during this period (88, 89). In a study of Negev Bedouin Arab women (88), 24% of the women exclusively breastfed their infants for the first 2 months regardless of whether women received help during the first 40 days. In a qualitative study of maternal beliefs about breastfeeding in a poor urban neighborhood in Egypt (89), women reported exclusively breastfeeding their infants for the first 40 days, after which they supplemented the breastmilk with fluids and foods to promote growth and fatness and to decrease the time spent breastfeeding. In Lebanon, the prevalence of EBF for 8-12 weeks was 27.4% among a sample of first-time mothers residing in the capital, Beirut, as part of a randomized trial of postpartum depression (106). The lower rate of EBF in that study compared to ours (41.5%) might be due to the longer duration at which EBF was assessed (56-84 days), the sample of women representing an urban population in which EBF rates have been suggested to be lower (107, 108), and the selective nature of participating in a postpartum depression study.

The prevalence of EBF for 6 months in our study was estimated at 12.3% in 2012. The figure is slightly higher than that estimated by Batal et al. in 2004 (10.1%) among a national sample of women recruited from health centers operated by the Ministry of Social Affairs (87). A more recent study by Mattar et al. in 2016 estimated the prevalence of EBF for 4-6 months at 16.5% among mothers with 12-36-month-old toddlers recruited from a representative sample of licensed daycare centers by the Lebanese Ministry of Public Health (98). Compared to other countries in the Middle

East, our prevalence estimate of EBF for 6 months is closest to that of 12.2% in Saudi Arabia (109). Despite the fact that the Levant countries are in geographic proximity and share similar traditions and population characteristics, the rates of EBF for 6 months in Lebanon were only similar to those in Syria at 12.9% (110), but lower than those in the Gaza Strip in Palestine (24.4%) (111) and higher than those in Jordan (1%) (112).

A higher number of cars owned in a household, an indicator of higher socioeconomic status, was associated with lower odds of EBF for 40 days and 6 months. Indeed, the number of cars was correlated with other socioeconomic variables including mother's and father's educational level, family monthly income, house ownership, and crowding index (data not shown). Unlike in high-income countries where breastfeeding rates are higher among wealthier and more educated women, breastfeeding rates are lower and the duration is shorter among wealthier women in low- and middle-income countries (18). According to two cross-sectional studies conducted in middle-income countries, the first in Nigeria in 2012 (113) and the second in Morocco in 2016 (114), mothers of higher socioeconomic status had a lower likelihood of EBF for 6 months. Lebanon, a middle-income country, followed a similar trend.

Compared to vaginal delivery, C-section delivery was consistently associated with lower odds of EBF for 40 days and 6 months. Other studies from Lebanon yielded mixed results on the association between mode of delivery and exclusive breastfeeding. While Mattar et al. (98) found that C-section delivery was associated with shorter duration of exclusive breastfeeding, Batal et al. (87) did not. However, the latter found differences in hospital practices that support or hinder breastfeeding initiation, which in turn affect breastfeeding exclusivity and duration (115). For example, a smaller proportion of women who delivered via C-section reported that the hospitals discussed the benefits of breastfeeding with them, allowed 24-hour rooming-in, and brought the baby for night feeds compared to women who delivered vaginally. Indeed, 24-hour rooming-in and bringing the baby often to the mother for feeding were associated with higher odds of breastfeeding initiation within few hours after birth. Rates of C-section delivery are high in Lebanon with an estimated prevalence of 49% among a sample of women who delivered between 2000 and 2015 (116). This prevalence greatly exceeds the rate for C-section deliveries of 10-15% suggested by the WHO (117). C-section delivery along with the associated maternal and newborn complications have been reported to hinder skin-to-skin contact after birth and delay breastfeeding initiation which in turn reduce breastfeeding duration (118, 119).

The number of children in the family was associated with EBF for 40 days, where mothers with a larger number of children were more likely to EBF. In a prospective cohort study conducted in six low- and middle-income countries in 2010 (120), nulliparity was associated with lower odds of EBF for the first 42 days postpartum in two of the included countries, Guatemala and Pakistan.

Overweight and obese mothers were less likely to EBF for 6 months. Given the cross-sectional nature of the study, we cannot determine the direction of the association, notably whether mothers who were presumably overweight or obese in pregnancy were less likely to EBF or whether mothers who did not EBF were more likely to be overweight or obese at the time of the interview. Evidence from the literature supports both directions of association between maternal BMI and duration of EBF. In a cross-sectional study in Germany (121), EBF duration was shorter among obese mothers than among normal-weight mothers. In an analysis of CDC Pregnancy Risk Assessment Monitoring System of 19,145 mothers from 2004-2008 (122), overweight and obese mothers, compared to the normal weight, had higher odds of discontinuing breastfeeding before 6 months due to insufficient milk and breastfeeding difficulties. On the other hand, shorter breastfeeding duration has been implicated in maternal overweight and obesity. In a study of 212 women in Finland in 2007 who were surveyed 16-20 years postpartum (123), mothers who breastfed for less than 6 months had higher total body fat mass and fat mass percentage than mothers who breastfed for more than 6 months.

This study has a number of limitations. First, it is a cross-sectional study with unclear chronology of the factors associated with EBF. Second, mothers reported infant feeding practices of their children, with potential for more recall bias among mothers of older children due to a longer time since delivery; however, almost half of the children were younger than 2 years of age.

3.6 Conclusions

The study offers a unique lens into infant feeding practices related to the 40-day rest period and first 6 months of life in Lebanon. The prevalence of EBF for 40 days and 6 months was 41.5% and

12.3%, respectively. The inverse association of higher socioeconomic status, as reflected by the number of cars owned, and C-section delivery with lower odds of EBF persisted across the first 6 months. Future research should investigate the factors associated with EBF in prospective cohort studies and help to better understand the cultural practice of the 40-day rest period in relation to breastfeeding. Such research can guide effective planning for interventions to improve breastfeeding practices and ultimately children's health status.

Abbreviations

EBF: Exclusive breastfeeding; EBOT: Exclusive bottle feeding; BOT+SF: Bottle and solid feeding; ESF: Exclusive solid feeding; ANOVA: Analysis of variance; AOR: Adjusted odds ratio; CI: Confidence interval; BMI: Body mass index

Declarations

Ethical approval and consent to participate

This study was approved by the Institutional Review Boards at the American University of Beirut and Purdue University. Mothers provided written informed consents.

Consent for publication

Not applicable

Availability of data and materials

The dataset analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

R.F.C. conducted the statistical analysis and drafted the manuscript. L.N. was responsible for the conceptualization of the study objectives and methodology and critically reviewed the manuscript. R.Z. contributed to the data collection and critically reviewed the manuscript. M.R.F. provided valuable input for analysis, data interpretation and write-up of the manuscript.

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CHAPTER 4. MATERNAL PRENATAL SUPPLEMENTATION, BUT NOT DIETARY PATTERNS, IS ASSOCIATED WITH THE BREAST MILK MICROBIOTA COMPOSITION IN THE CHILD COHORT STUDY

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4.1 Abstract

Background: The breast milk microbiota may help seed the infant's gut microbiota and guide the maturing immune system, and it composition is affected by several factors.

Objective: To examine the associations between prenatal diet quality and patterns and supplementation and the breast milk microbiota composition.

Methods: The CHILD study is a prospective longitudinal Canadian birth cohort study. Prenatal diet reported using a self-administered food frequency questionnaire was analyzed for dietary quality and patterns. Maternal-reported prenatal supplementation was collected separately for the first two and the third trimester via standardized questionnaires. The milk microbiota was analyzed using 16S rRNA gene sequencing. Among 3455 eligible participants, 794 mothers provided one breast milk sample between 2-6 months postpartum and used multivitamin supplements in the first two and/or the third trimester. Associations between prenatal diet and supplementation and the milk microbiota alpha diversity and taxonomic structure were assessed with adjustment for covariates using multivariate linear regression permutation models.

Results: Prenatal supplementation, but not dietary quality and patterns, was associated with breast milk microbiota composition. Among multivitamin supplement users in the first two and/or the third trimester, additional vitamin D, fish oil and vitamin C supplementation was associated with differential microbiota diversity. Vitamin D, folate, calcium and vitamin C supplement users had differential genus abundance compared to non-users that persisted after covariate adjustment. In a sensitivity analysis of multivitamin supplement users throughout all trimesters (n=721), additional

fish oil and vitamin C supplementation was associated with microbiota diversity; vitamin D and C supplementation was associated with differential genus abundance.
Conclusions: In this first exploratory analysis, prenatal supplementation, especially vitamin C and D, was associated with the breast milk microbiota among multivitamin supplement users. These findings require replication and may inform future hypotheses to elucidate mechanisms linking maternal supplementation to the breast milk microbiota and child health.
Keywords: human milk, microbiome, diet, supplements, pregnancy

4.2 Introduction

Breast milk confers numerous benefits to the infant's health including helping guide the maturing immune system (39, 124). It does so through its diverse constituents including nutrients and bioactive components such as lactoferrin, growth factors, cytokines, and human milk oligosaccharides (HMOs) (40). A complex community of bacteria has been discovered in breast milk, negating previous dogmas that breast milk is sterile and adding to the potential pathways through which breast milk influences infant health (38). Vertical transfer of bacteria via breastfeeding may help seed the infant gut microbiota (41), with disruptions in this process potentially leading to a dysbiotic microbiota and increasing the risk of chronic diseases such as allergy, asthma and obesity (42).

There are two main proposed pathways for the origin of bacteria in breast milk: the entero-mammary pathway from the mother's gut microbiota and retrograde-flux from the infant's oral microbiota (125). Depending on the source of bacteria, different factors could contribute to shaping the milk microbiota. Among the most commonly studied factors are lactation stage (43, 126-129), mode of delivery (43, 44, 127, 128, 130-133), gestation age at birth (43, 44, 129), and infant's sex (44, 129, 134). Some of the less studied factors include maternal body mass index (BMI) (128, 129) breastfeeding mode (direct from the breast vs. indirect pumped milk) (134), geographic location (133), and nutrient content of breast milk (133, 135).

The role of maternal diet in shaping the breast milk microbiota has been scarcely studied, while that of vitamin mineral supplement use has not been studied despite the following evidence of their importance. First, maternal diet has been shown to modulate the breast milk's nutrient composition

(136-138), which in turn influences the microbiota composition (139). Similarly, maternal supplement use, namely that of fat-soluble vitamins, vitamin B1, B2, and vitamin C, influences the breast milk composition of these micronutrients (140). Second, maternal diet influences the gut microbiota of pregnant and lactating women (141). Considering the entero-mammary pathway as a contributing source to the bacteria in breast milk, it is plausible that maternal diet influences the breast milk microbiota through its effect on the maternal gut microbiota. Third, few studies have reported associations between maternal diet and the breast milk microbiota (129, 142-144). These studies however suffered from a limited sample size or did not adjust for covariates known to influence maternal intake and/or the breast milk microbiota. Further, no study has examined the effect of prenatal supplement use on the breast milk microbiota composition, despite the significant contribution of the latter to the micronutrient intake of pregnant women (145).

The aim of this study is to examine the associations between maternal prenatal dietary quality and patterns and supplement use and the breast milk microbiota composition using data from the CHILD Cohort Study in Canada. Given the limited research in this area, we recognize our effort as hypothesis-generative and exploratory.

4.3 Methods

4.3.1 Study design and sample

The CHILD Cohort Study is a longitudinal, prospective, population-based birth cohort study conducted across four centers in Canada: Vancouver, Edmonton, Manitoba, and Toronto (146). The main aim of the study is to examine the developmental origins of pediatric asthma and allergy. Pregnant women were enrolled in the study during the second or third trimester from 2008 through 2012 (n=3621). Women were eligible to participate if they had singleton pregnancies and delivered a healthy infant >35 weeks of gestation (n=3455).

Mother-infant dyads in this study were selected from a representative subset across the cohort (n= 428) (134) and from an additional subset of dyads enriched for maternal and infant health conditions (atopy, asthma, obesity) (n= 766) (147) (Figure 4.1). This study was approved by the

Human Research Ethics Boards at McMaster University and the Universities of Manitoba, Alberta, Toronto, and British Columbia and Purdue University.

4.3.2 Maternal prenatal diet and supplement use

Maternal diet during pregnancy was assessed using a validated semi-quantitative food frequency questionnaire (FFQ) adapted from the Fred Hutchinson Cancer Center tool (148). The FFQ was self-administered and completed between 24-28 weeks of pregnancy. Details on the diet analysis were described elsewhere (149). Briefly, responses to the FFQ were linked to the United States Department of Agriculture (USDA) nutrient composition database and modified for a Canadian setting (150) to estimate total energy intake. Diet quality was analyzed using the Healthy Eating Index 2010 (HEI-2010) score (151) and dietary patterns were identified using principal components analysis (PCA). Three dietary patterns emerged among the CHILD cohort study participants: plant-based (dairy, legumes, vegetables, whole grains, and an aversion to meats), Western (fats, meats, processed foods, and starchy vegetables) and balanced (diverse sources of animal proteins (especially fish), vegetables, fruits, nuts and seeds) (152). The PCA scores for each of the three patterns represented how close the individuals' dietary intakes were to the dietary patterns, with positive scores indicating adherence and negative scores indicating avoidance. Dietary pattern scores were adjusted to the mean energy intake (2500 kcal per day) using the residual method (153).

The mothers completed self-administered questionnaires about prenatal supplement use at two time points: the first questionnaire about supplement use during the first two trimesters of pregnancy was completed during pregnancy, while the second about supplement use during the third trimester was completed soon after birth. Frequency of individual and multivitamin supplement use was reported based on the following options: Never, <1 per month, 1-3 times per month, 1-3 times per week, 4-6 times per week, every day, while dosage was assessed using a range of options applicable for each supplement. In the current analysis, frequency of supplement use was defined as follows: users (reported using the supplement <1 per month, 1-3 times per month, 1-3 times per week, 4-6 times per week, or every day) vs. non-users (reported never using the supplement).

4.3.3 Maternal and infant characteristics

Demographic characteristics of the mother such as age, parity, gestation weight gain, intrapartum antibiotic use and those of the child such as sex, birth weight, gestational age at birth, and mode of delivery (normal vaginal vs. Cesarean section) were documented from hospital records or from standardized questionnaires. Maternal pre-pregnancy BMI in kg/m^2 was calculated from measured height abstracted from medical records and self-reported pre-pregnancy weight from the standardized questionnaires.

Infant feeding practices were reported using standardized questionnaires completed at 3, 6, and 12 months. At the time of breast milk sample collection, breastfeeding status was classified as: exclusive (breast milk only) or partial (breast milk supplemented with infant formula (n=230 of 815, 28.22%), and/or other liquids (water, herbal teas) and/or solid food (n=122 of 815, 14.97%)). Similarly, at the time of breast milk sample collection, the mode of breast milk feeding was classified as directly from the breast only (no feeding of expressed milk) or expressed breast milk in the bottle (fed expressed milk at least once in the two weeks prior to sample collection).

4.3.4 Milk sample collection and microbiota profiling analysis

Details on milk sample collection and analysis are described elsewhere (134). Briefly, mothers provided one sample of milk in a sterile milk container provided by the CHILD study. A mix of foremilk and hindmilk from multiple feeds during a 24-hour period was collected aseptically using hand expression or a pump. Samples were refrigerated at the participants' home for up to 24 hours before transport and processing by study staff. Samples were stored at -80°C until analysis.

Milk microbiota was analyzed at the University of Manitoba using 16S rRNA gene sequencing of the V4 hypervariable region on a MiSeq platform (Illumina, San Diego, CA, USA) (134). Negative controls composed of sterile DNA-free water were used in sequencing library preparation, while positive controls consisted of DNA extracted from 8 species with known theoretical relative abundances (Zymo Research, USA).

Microbiota data pre-processing is previously described (154). Briefly, overlapping paired-end reads were processed with DADA2 pipeline (155) using the open-source software QIIME 2 v.2018.6 (https://qiime2.org) (156). Unique amplicon sequence variants (ASVs) were assigned a taxonomy and aligned to the 2013 release of the Greengenes reference database at 99% sequence similarity (157). The milk microbiota profile was then rarefied to 8,000 sequencing reads per sample, leaving 877 breastmilk samples (73% of total 1,194 samples). To eliminate sequencing artifacts, ASVs with less than 60 reads across each dataset (0.001% of total reads per sample on average) were removed (158), resulting in 1,121 ASVs in breastmilk. The number of sequencing reads per sample was then relativized to a total sum of 8,000 for downstream analyses. Because lactation stage influences the breast milk microbiota composition (128), we limited our analysis to the 815 samples (92% of 877 samples) collected between 2-6 months postpartum.

4.3.5 Statistical analysis

Frequency (%) and mean ±standard deviation (SD) were computed to describe the study sample characteristics. Maternal dietary quality and patterns were examined as categorical and continuous variables. Dietary quality were categorized as below vs. above 50^{th} percentile of 74.82 for HEI-2010 scores, and dietary patterns were categorized as avoidance (negative PCA scores) vs. adherence (positive PCA scores) (152)). ASVs were agglomerated at the genus level, and genus abundances were centered log-ratio (CLR) transformed using the CoDaSeq package (158) after zeros were imputed using a Bayesian-multiplicative replacement method (159).

Associations between maternal diet and supplement use during pregnancy and breast milk microbiota diversity (Shannon index) and clr-transformed abundances of genera with mean relative abundance >0.1% were examined using Wilcoxon signed-rank test and Spearman correlation for categorical and continuous variables, respectively. Associations with clr-transformed genus abundances were further tested using univariate and multivariate linear regression permutation tests (160). Multivariate models adjusted for variables that were identified in the literature as factors influencing the milk microbiota (134, 147, 161) and/or were significant in the univariate analysis. The variables that were tested as covariates for adjustment included: breastfeeding exclusivity, mode of breast milk feeding, and age of the child at the time of milk sample collection as well as maternal parity, pre-pregnancy BMI, gestation weight gain, HEI-2010 scores, infant sex, mode of delivery, gestation age and batch of analysis. The Benjamini-Hochberg's false discovery rate (FDR) procedure was used to correct *P*-values for multiple comparisons for all analysis except that for alpha diversity.

4.4 Results

4.4.1 Characteristics of the study sample of 794 multivitamin supplement users in the first two trimesters and/ or the third trimester of pregnancy

A total of 815 women provided breast milk samples between 2-6 months postpartum that were retained after microbiome pre-processing (Figure 4.1). Among the 815 women, 97.4% (n=794) were multivitamin supplement users in the first two trimesters, the third trimester, or all three trimesters of pregnancy and constitute the primary analytic sample.

Among the 794 women, 79.6% were Caucasian, 57.1% were nulliparous, and 44.8% had a normal pre-pregnancy BMI with a mean ±SD of 24.3 ±5.3 kg/m^2 (Table 4.1). The majority of the infants were delivered vaginally (72.8%) and 55.5% were boys. The mean ±SD age of the infants at breast milk sample collection was 3.7 ±0.8 months with the majority (71.8%) aged 2-3.99 months, 51.1% were exclusively breastfed and 59.3% received expressed milk in the two weeks prior to sample collection. The mean ±SD HEI-2010 score was 74.0 ±7.9 (range: 44.64, 94.64). The mean ±SD energy-adjusted scores for each of the dietary patterns were as follows: plant-based pattern: 0.04 ±1.02 (range: -1.52, 9.19), western pattern: 0.03 ±1.00 (range: -2.13, 5.55) and balanced pattern: 0.03 ±1.01 (range: -4.72, 4.37).

The most common vitamin and mineral supplements were multivitamins, vitamin D, folate, calcium, fish oil, iron and vitamin C; therefore, the current analysis focused on these supplements. In addition to multivitamins, the percentages reported for use of the following individual supplements during the first two and/or the third trimester were: 37.2% for vitamin D, 35.1% for folate, 34.0% for calcium, 29.1% for fish oil, 27.3% for iron, and 16.8% for vitamin C.

4.4.2 Associations with alpha diversity

Mean ±SD alpha diversity (Shannon index) was 1.75 ±0.66. Vitamin D (mean ±SD: 1.71 ±0.64 Shannon index) or fish oil (1.62 ±0.65) supplement users had lower alpha diversity than non-users (1.78 ±0.66, P=0.03 or 1.78 ±0.67, P=0.03 respectively). Vitamin C supplement users had higher alpha diversity (2.00 ±0.58) than non-users (1.72 ±0.65, P=0.001) (Figure 4.2). Diet quality and diet patterns were not associated with alpha diversity (Figure 4.3).

4.4.3 Taxonomic structure of the breast milk microbiota

Firmicutes were the predominant phylum (mean relative abundance ±SD: 59.79% ± 34.85), followed by Proteobacteria (31.41% ±37.18), Actinobacteria (6.03% ±7.50), Bacteroidetes (1.53% ±4.42) and Fusobaceria (0.13% ±0.67) (Figure 4.4). At the genus level, *Streptococcus* (mean relative abundance ±SD: 39.27% ±31.26) and *Staphylococcus* (13.02% ±21.40) were most abundant, followed by *Actinobacter* (9.98% ±20.06) and *Pseudomonas* (6.27% ±17.67). *Streptococcus, Staphylococcus* and *Acinetobacter* were present in ≥90% of the 794 samples.

4.4.4 Associations with the most abundant genera

Vitamin D, calcium and vitamin C supplement use among multivitamin supplement users during the first two and/or third trimester of pregnancy was associated with differential genus abundance in breast milk (Figure 4.5). Compared to vitamin D supplement non-users, users had higher abundance of unclassified *Bacillales* (0.30% ±3.35 vs 0.02% ±0.13, $P=0.003$) and *Aeromonas* (0.31% ±1.43 vs. 0.05% ±0.33, $P=0.01$), but lower abundance of unclassified *Comamonadaceae* (0.17% ±0.85 vs. 0.23% ±1.77, P=0.003), and *Finegoldia* (0.13% ±0.72 vs. 0.13% ±0.93, P=0.02). Calcium supplement users had higher abundance of unclassified *Comamonadaceae* (0.25% ±1.11 vs. 0.19% ±1.66, P=0.02) than non-users. Compared to vitamin C supplement non-users, users had higher abundance of *Pseudomonas* (mean ±SD: 8.16% ±18.97 vs. 5.91% ±17.43, $P=0.04$), unclassified *Caulobacteraceae* (0.61% ±0.97 vs. 0.38% ±0.80, $P=0.04$) and *Enterococcus* (0.56% ±3.90 vs. 0.24% ±2.22, P=0.02), but lower abundance of unclassified *Methylobacteriaceae* (0.06% ±0.46 vs. 0.14% ±1.68, P=0.02), *Pseudoxanthomonas* (0.05% ±0.30 vs. 0.13% ±1.57, P=0.02) and *Janthinobacterium* (0.004% ±0.03 vs. 0.17% ±3.70, P=0.02). Diet quality and pattern scores were not associated with the abundance of genera in milk (Figure 4.6).

In the univariate linear regression analysis, vitamin D, folate, calcium and vitamin C supplement use during the first two and/or third trimester of pregnancy was associated with specific genera. In addition, maternal parity as well as child age, breastfeeding exclusivity and mode of breast milk feeding at the time of sample collection were associated with the most abundant genera. Although diet quality and patterns were not significant in the univariate models, we adjusted for diet quality (HEI-2010 scores) because it was associated with supplement use (data not shown).

In the multivariate models that adjusted for batch of analysis, maternal parity and HEI-2010 scores, as well as child age, breastfeeding exclusivity and mode of breast milk feeding at the time of sample collection, use of each of the four aforementioned supplements remained associated with the breast milk microbiota composition (Figure 4.7). Vitamin D supplement users had higher abundance of unclassified *Bacillales* and unclassified *Comamonadaceae* but lower abundance of *Enhydrobacter* than non-users. Folate supplement users had lower abundance of *Bifidobacterium*, *Rothia*, *Streptococcus*, and unclassified *Gemellaceae* but higher abundance of *Janthinobacterium, Pseudomonas, Stenotrophomonas,* and unclassified *Enterobacteriaceae* than non-users. Calcium users still had higher abundance of unclassified *Comamonadaceae* and lower abundance of *Enhydrobacter* than non-users. Vitamin C supplement users had higher abundance of *Actinomyces* than non-users.

4.4.5 Sensitivity analysis among the 721 prenatal multivitamin supplement users throughout all trimesters of pregnancy

We conducted a sensitivity analysis among the 721 multivitamin supplement users throughout all trimesters of pregnancy (88.5% of 815) because the consistent intake of multivitamins creates a relatively steady state in the body for each of the micronutrients found in the multivitamin and provides a more homogenous background to assess the role of additional individual micronutrient on the microbiota (162).

Among the 721 multivitamin users throughout pregnancy, use of the following individual supplements was reported: 22.3% vitamin D, 16.4% folate, 18.7% calcium, 19.6% fish oil, 12.8% iron and 4.7% vitamin C.

Fish oil supplement users had lower alpha diversity (mean ±SD: 1.62 ±0.65 Shannon index) than non-users (1.79 ±0.67, *P*=0.007), while vitamin C supplement users had higher alpha diversity (2.02 ±0.58) than non-users (1.71 ±0.65, *P*=0.005) (Figure 4.8).

Vitamin D and vitamin C supplement use was associated with abundance of genera in milk (Figure 4.9). Compared to non-users, vitamin C supplement users had higher relative abundance of *Veillonella* (mean ±SD: 3.76% ±4.69 vs. 2.52% ±4.34, *P=0.04*), but lower abundances of *Stenotrophomonas (0.53*% ±2.29 vs. 1.41% ±5.63, *P=0.04*), *Finegoldia (0.03*% ±0.10 vs. 0.13% ±0.92, *P=0.04*), and *Janthinobacterium (0.003*% ±0.02 vs. 0.18% ±3.86, *P=0.04*). Vitamin D supplement users had lower abundance of unclassified *Comamonadaceae* (0.09% ±0.21 vs. 0.16% ±0.89, *P=0.03*) than non-users.

Adjusting for batch of analysis, maternal parity and HEI-2010 scores, and child age, breastfeeding exclusivity and mode of breast milk feeding at the time of sample collection, use of the supplements was not associated with the 35 genera in milk (data not shown).

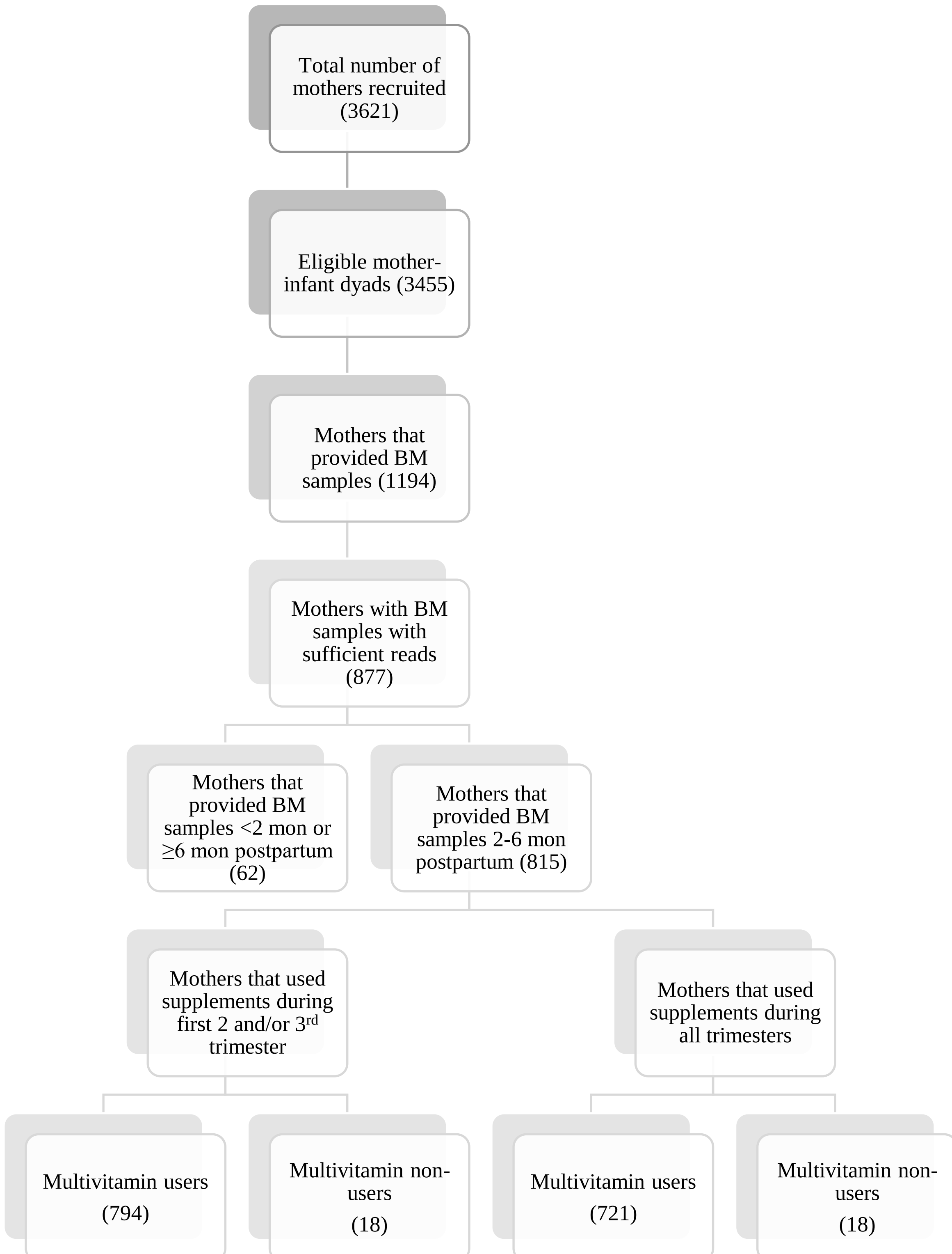
Table 4.1 Characteristics of mother-infant dyads among multivitamin users during the first two and/or the third trimester of pregnancy (n=794)

Study sample characteristics		**n (%) or mean ±SD**
Maternal characteristics		
Age at delivery (years)		32.87 ±4.32
Ethnicity	Caucasian	632 (79.6)
	Asian	110 (13.85)
	Other	51 (6.42)
Parity	Nulliparous	453 (57.05)
	Multiparous	338 (42.57)
Pre-pregnancy BMI		24.31 ±5.25
	Underweight	21 (2.64)
	Normal	356 (44.84)
	Overweight	117 (14.74)
	Obese	67 (8.44)

Table 4.1 continued

Study sample characteristics (cont.)		n (%) or mean ±SD
Child characteristics		
Sex	Boy	441 (55.54)
	Girl	353 (44.46)
Mode of delivery	Vaginal	578 (72.80)
	C-section	206 (25.94)
Gestation age	Preterm	88 (11.08)
	Term	695 (87.53)
Age (months)*		3.65 ±0.83
	2-3.99	570 (71.79)
	4-5.99	224 (28.21)
Breastfeeding exclusivity*	Exclusive	406 (51.13)
	Partial	368 (46.35)
Breastfeeding mode*	Fed directly from the breast	297 (37.41)
	Some expressed breast milk in the bottle	471 (59.32)
Prenatal diet scores		
Diet quality (HEI-2010)		74.0 ±7.92
	Low	377 (47.48)
	High	382 (48.11)
Plant-based pattern		0.04 ±1.02
	Avoiders	446 (56.17)
	Adherers	309 (38.92)
Western pattern		-0.03 ±1.00
	Avoiders	443 (55.79)
	Adherers	312 (39.29)
Balanced pattern		0.03 ±1.01
	Avoiders	416 (52.39)
	Adherers	339 (42.70)
Prenatal supplement use		
Multivitamin supplement users		794 (100)
Vitamin D supplement users		295 (37.15)
Folate supplement users		279 (35.14)
Calcium supplement users		270 (34.01)
Fish oil supplement users		231 (29.09)
Iron supplement users		217 (27.33)
Vitamin C supplement users		133 (16.75)
Vitamin B-complex supplement users		44 (5.54)
Vitamin B-6 supplement users		35 (4.41)
Vitamin E supplement users		18 (2.27)
Zinc supplement users		18 (2.27)
Vitamin A supplement users		6 (0.76)
Beta-carotene supplement users		4 (0.50)
Selenium supplement users		3 (0.38)
Niacin supplement users		2 (0.25)

*At time of breast milk sample collection



BM: Breast milk

Figure 4.1 Flowchart of study sample size

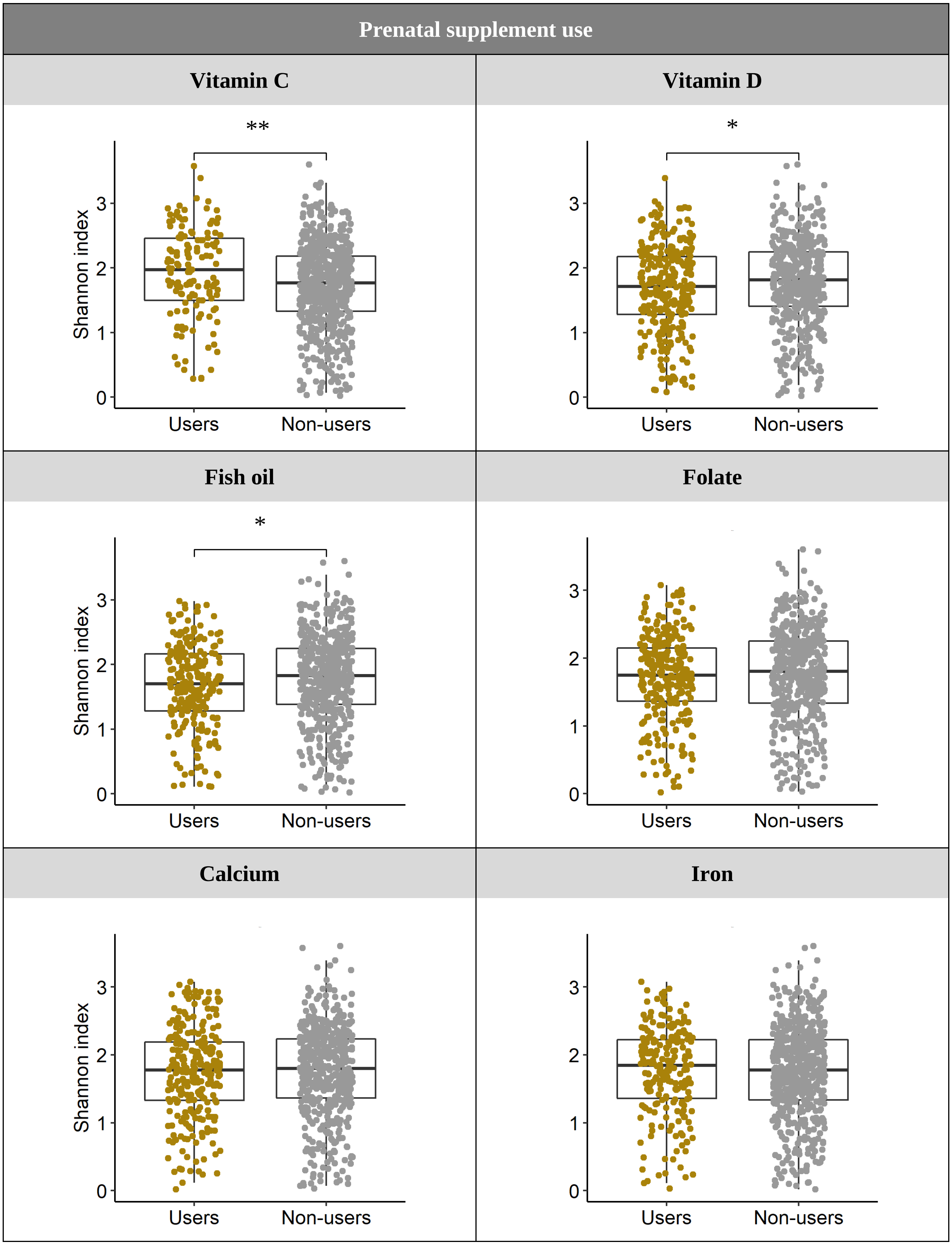


Figure 4.2 Microbial alpha diversity by prenatal supplement use among multivitamin users during the first two and/or the third trimester of pregnancy (n=794)

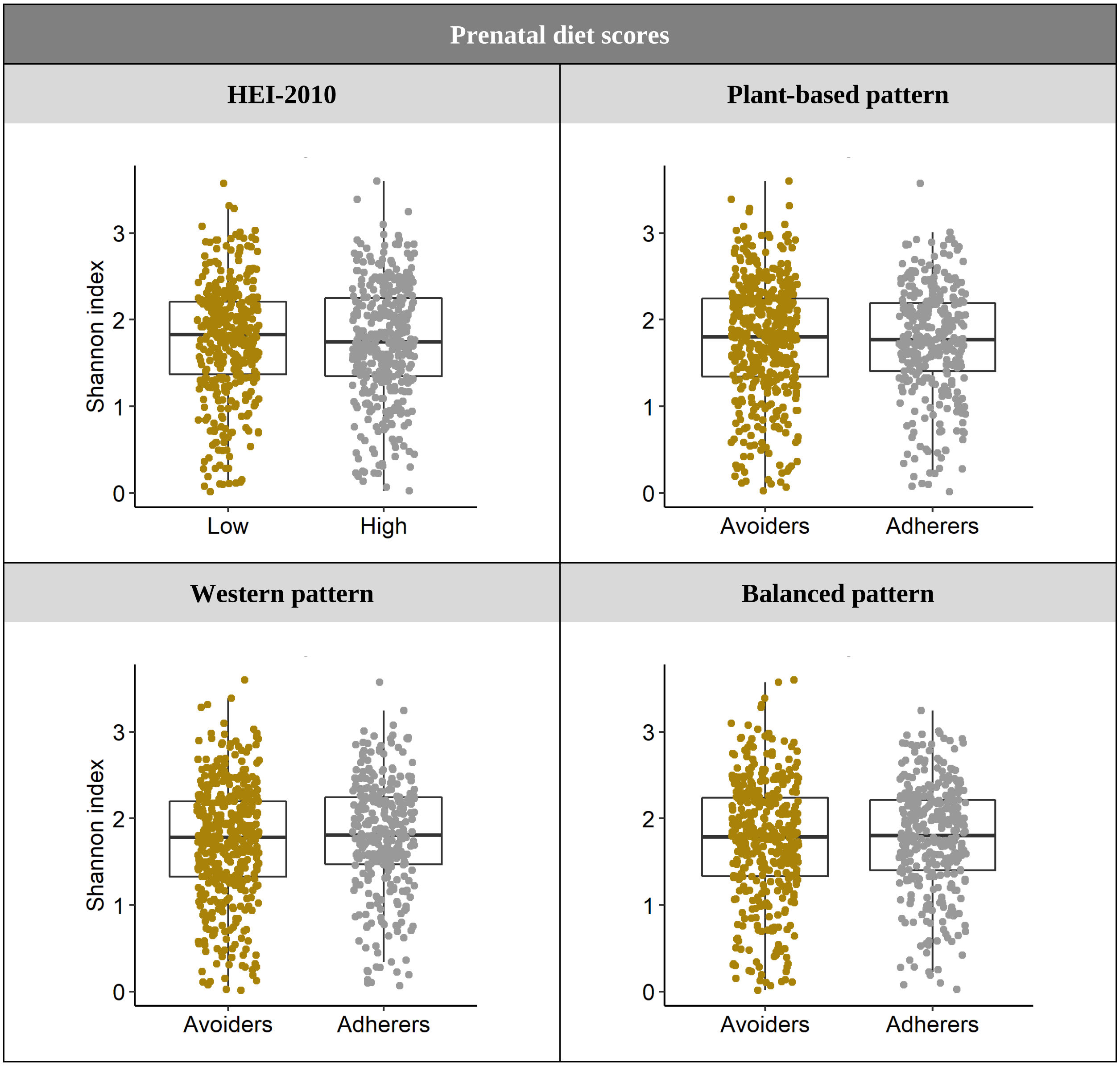


Figure 4.3 Microbial alpha diversity by prenatal diet scores among multivitamin users during the first two and/or the third trimester of pregnancy (n=794)

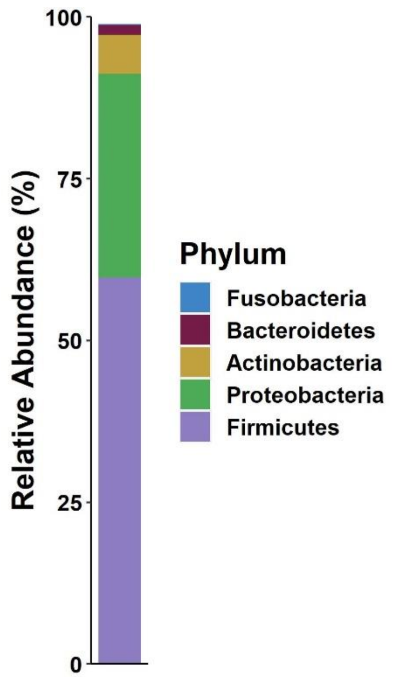
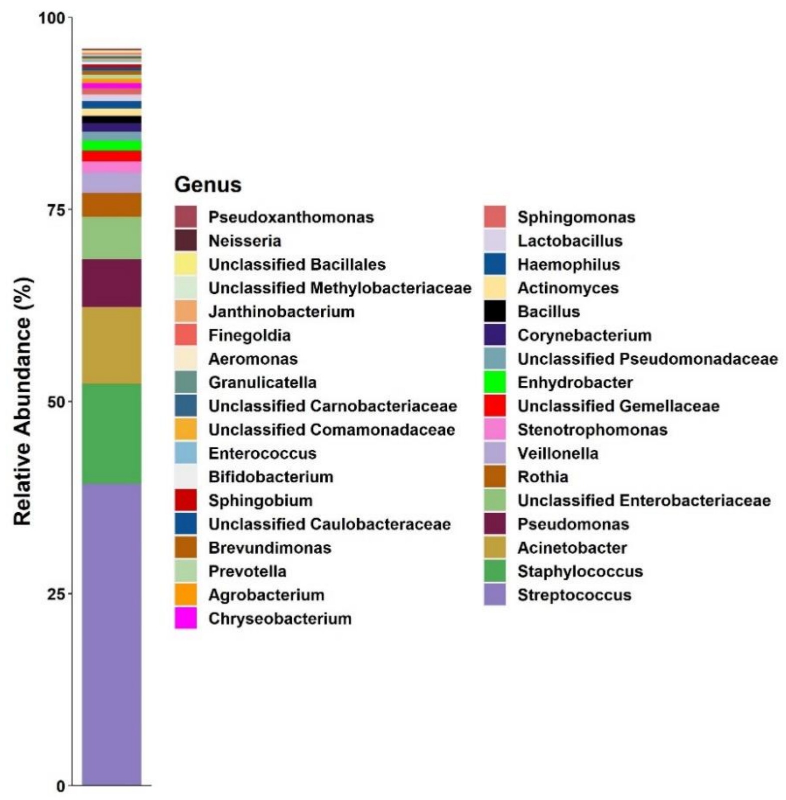
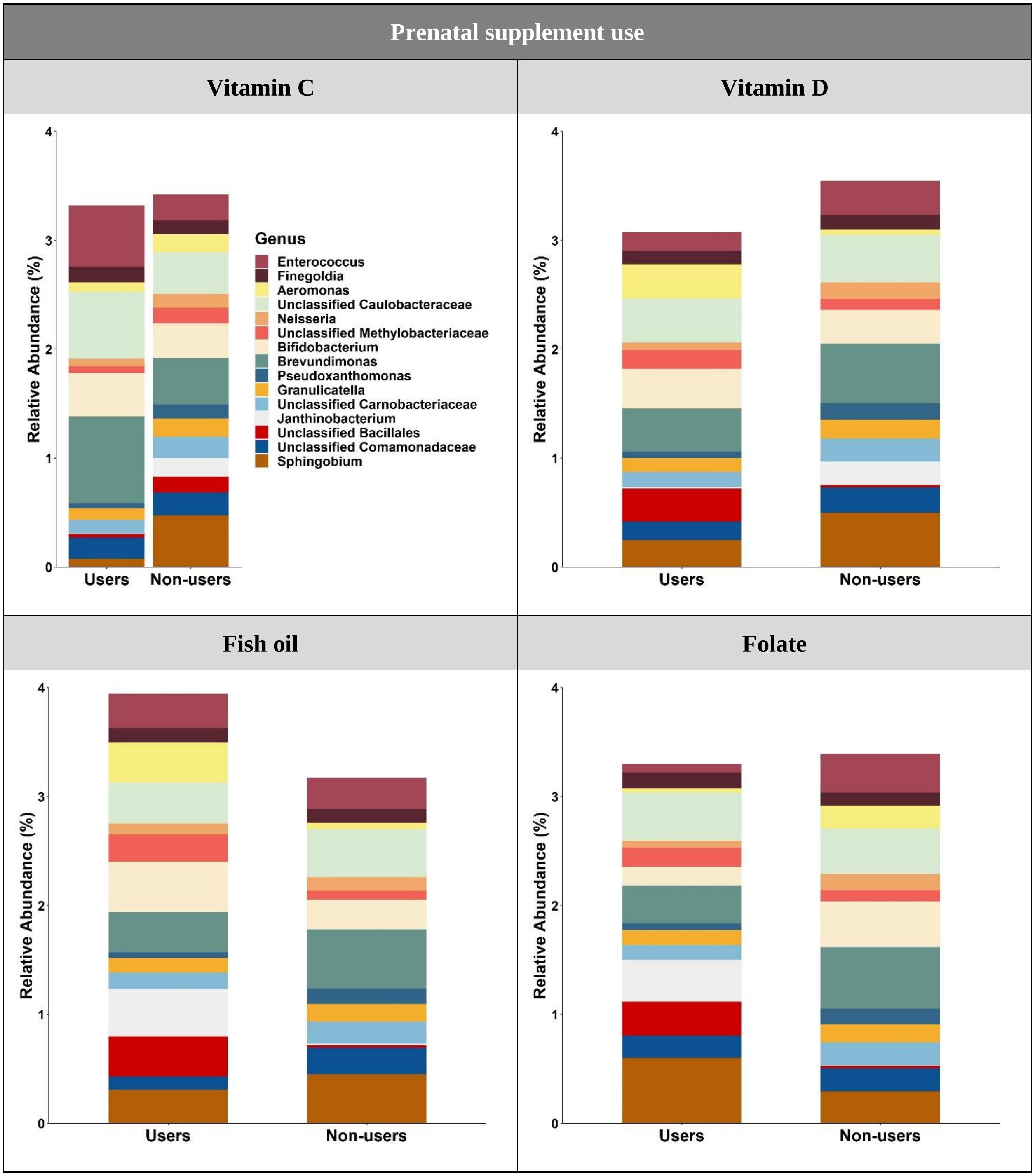
Phylum relative abundance	Genus relative abundance
	

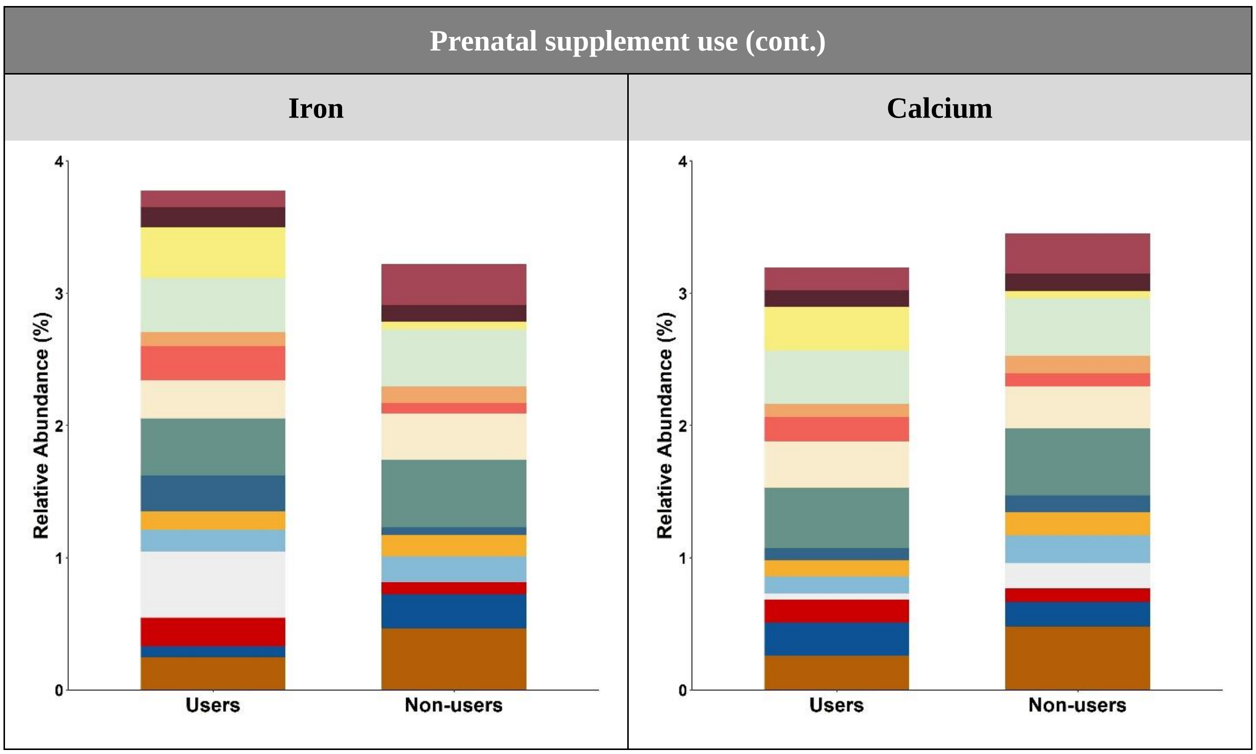
Figure 4.4 Relative abundance of the most abundant phyla and genera in the breast milk of multivitamin users during the first two and/or the third trimester of pregnancy (n=794)



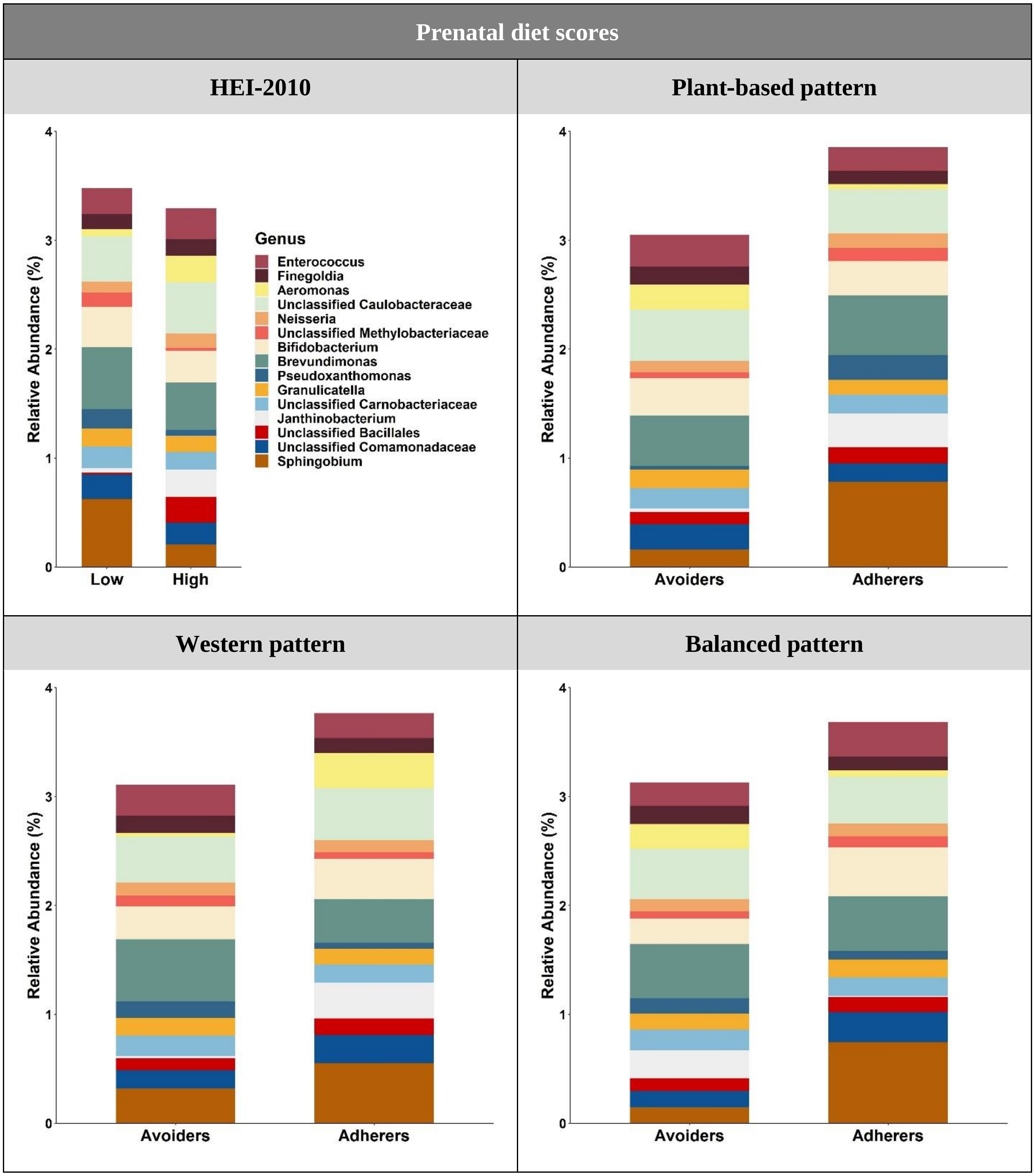
*Visualizing the genera with mean relative abundance between 0.1-0.5%

Figure 4.5 Relative abundance of the most abundant genera* by prenatal supplement use in the breast milk of multivitamin users during the first two and/or the third trimester of pregnancy (n=794)

Figure 4.5 continued

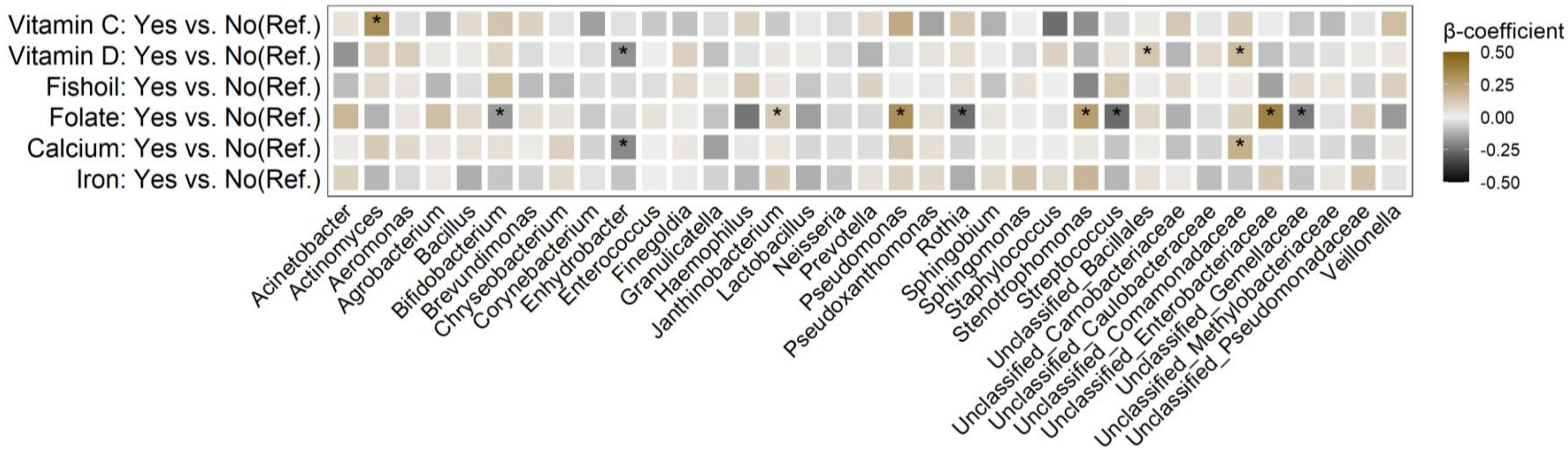


*Visualizing the genera with mean relative abundance between 0.1-0.5%



*Visualizing the genera with mean relative abundance between 0.1-0.5%.

Figure 4.6 Relative abundance of the most abundant genera* by prenatal diet scores in the breast milk of multivitamin users during the first two and/or the third trimester of pregnancy (n=794)



Each of the supplements were tested separately in a model that adjusted for batch of analysis, maternal parity and HEI-2010 scores, as well as child age, breastfeeding exclusivity and mode of breast milk feeding at the time of sample collection; *: $P_{FDR}<0.05$

Figure 4.7 Linear permutation regression models of prenatal supplement use with most abundant genera in milk of multivitamin users during the first two and/or the third trimester of pregnancy (n=794)

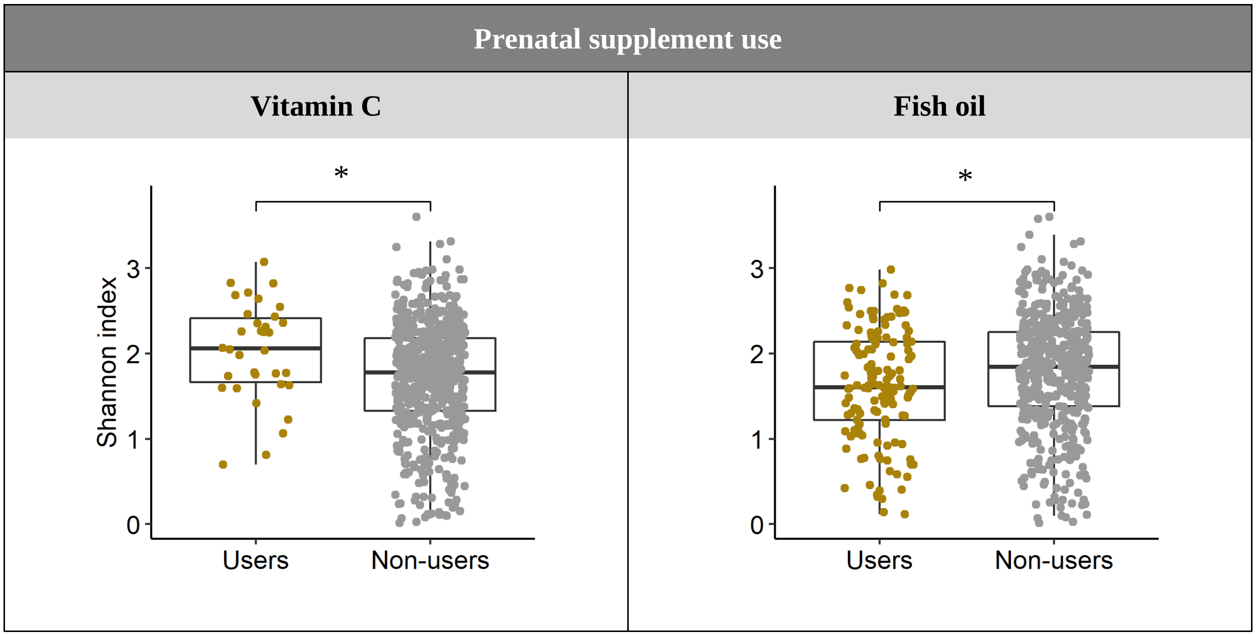
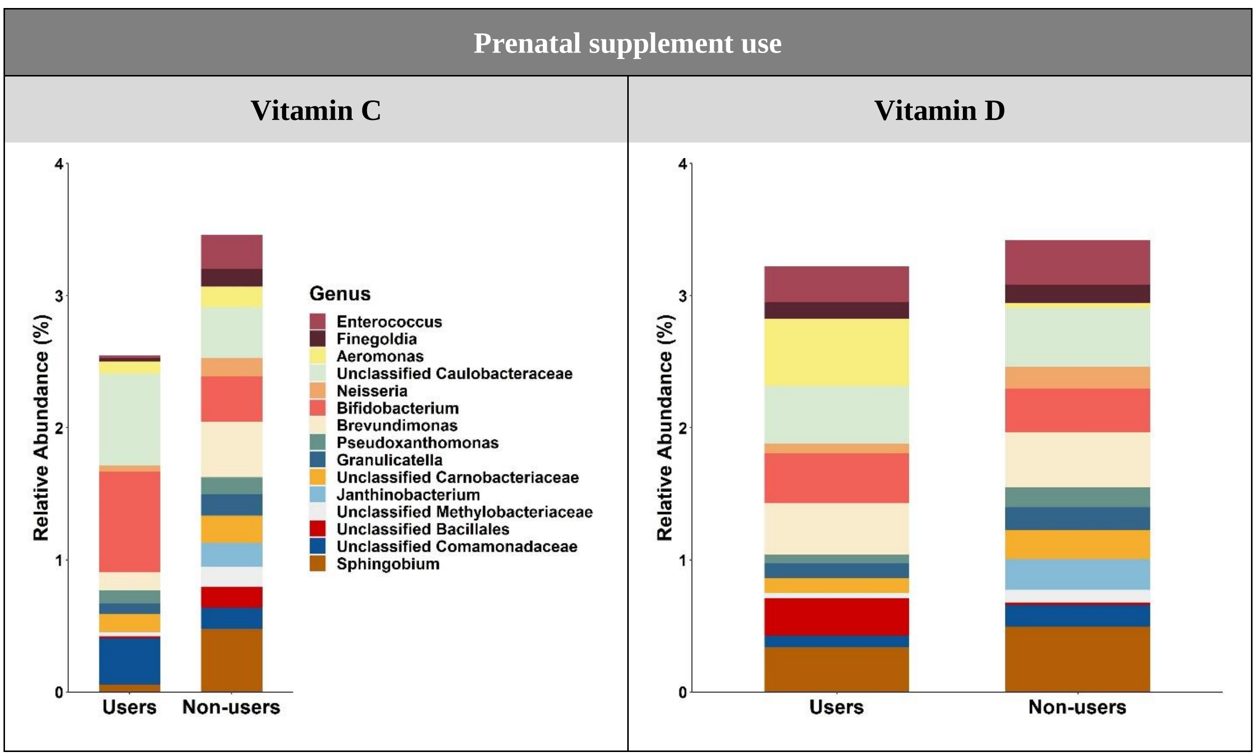


Figure 4.8 Sensitivity analysis of microbial alpha diversity among multivitamin users throughout all trimesters of pregnancy (n=721)



*Visualizing the genera with mean relative abundance between 0.1-0.5%.

Figure 4.9 Sensitivity analysis of relative abundance of the most abundant genera among MV users throughout all trimesters of pregnancy (n=721)

4.5 Discussion

In one of the largest samples of women from the CHILD cohort study in Canada, we report for the first time that individual maternal prenatal supplement use over and above use of multivitamin mineral supplementation is associated with the diversity and composition of the breast milk microbiota, even after adjustment for covariates. Specifically, vitamin D, fish oil, and vitamin C supplement use during the first two and/or the third trimester of pregnancy among multivitamin supplement users was associated with differential microbial diversity. Further, vitamin D, folate, calcium and vitamin C supplement users had differential genus abundance, compared to their respective non-users, which persisted after adjustment for covariates. In a sensitivity analysis of daily multivitamin supplement users throughout pregnancy, fish oil and vitamin C supplement use throughout all trimesters of pregnancy was associated with breast milk microbiota diversity, while vitamin D and C supplement use was associated with genera abundance only in the unadjusted analysis. Our study did not detect associations between maternal diet quality or dietary patterns and the breast milk microbiota diversity or composition. To our knowledge, this is the first study that examines supplement use and the breast milk microbiota.

No associations between diet and the breast milk microbiota were observed, potentially because the focus was on diet quality from HEI-2010 scores and dietary patterns. Future analysis should examine the association between diet nutrient composition and the breast milk microbiota, given findings from a few studies of the associations with dietary fatty acids, fibers and some micronutrients including vitamin C (129, 142-144). Findings by Padilha et al. (142) among 94 healthy women in São Paulo city revealed differences in the breast milk microbiota diversity and composition at one month postpartum by dietary intake of vitamin C during pregnancy but not during lactation. Particularly, the intake of vitamin C during pregnancy was positively correlated with the presence of *Staphylococcus* genus. Most of the correlations during the lactation period were found for genera present at low relative abundance in the samples with nutrients including polyunsaturated fatty acids (PUFA), linoleic acid and B vitamins. Further associations were noted between prenatal dietary intake and the breast milk microbiota composition at 7-15 days postpartum among 120 healthy mothers from the MAMI cohort study in the Spanish Mediterranean area (144). Intake levels of dietary carbohydrates, total protein, lipids, PUFA, monounsaturated fatty acids (MUFA) as well as polyphenols, selenium and zinc were associated with specific breast milk microbial genera. These findings reveal the effect of the prenatal diet on the breast milk microbiota composition.

The consistent association between vitamin D and C supplement use among multivitamin users and the breast milk microbiota is intriguing, and requires further investigation to decipher the mechanism as well as the effect on child health. One of the proposed mechanisms is through the supplements' effect on the milk's nutrient composition. Several studies have indicated that the intake of vitamin D and C from diet or supplements influences the breast milk concentrations of these two vitamins (136, 138, 140). Although prenatal fish oil supplement use was associated with breast milk's fatty acid composition (137), which was in turn associated with milk microbiota composition in the CHILD cohort study (139), we did not detect associations between fish oil supplement use and milk taxa abundances. This requires further investigation along with accounting for sources of fatty acids from the diet. As for the proposed implications on child health, increasing intake of vitamins D and C during pregnancy has been associated with lower risk of allergy and wheezing (163-165). Both vitamins seem to be present in the breast milk at levels dependent on prenatal maternal intake and are protective against similar respiratory conditions in children. The CHILD Cohort study offers the opportunity to further investigate these associations and the role of the breast milk microbiota as a mediator given the primary aim of this study is to examine the developmental origins of pediatric asthma and allergy (146).

Our study has a number of strengths and limitations. The CHILD Cohort Study is conducted among a diverse, representative sample of mother-infant dyads with a large sample size and is among the largest studies with available breast milk microbiota data to date. The longitudinal prospective cohort study design provided the opportunity to examine prenatal diet with breast milk microbiota before and after adjustment for covariates known to influence the breast milk microbiota. However, this is an observational study with no repeated measures as maternal intake was assessed once during pregnancy and only one breast milk sample was collected from each mother. Further, our data is of limited generalizability because it was conducted among a Canadian cohort of women whose ethnicity and place of residence might have influenced both maternal intake and the breast milk microbiota. Despite the current limitations, our exploratory study begins to fill a gap in the understanding of the associations between maternal prenatal supplement use and the breast milk microbiota.

4.6 Conclusions

In this exploratory analysis with a large sample from the CHILD cohort study, use of specific prenatal individual supplements, namely vitamins D and C, but not diet quality and patterns, modulated the breast milk microbiota composition. These associations were observed among multivitamin supplement users in addition to the indicated individual supplements used during pregnancy. Further research is warranted to replicate these findings in other populations and to examine the effects of frequency and dose of supplement use on the associations with the breast milk microbiota. Future research is also needed to examine the associations with other breast milk components and the infant gut microbiota and overall health. Such findings can inform future hypotheses and intervention trials to elucidate mechanisms linking maternal supplement use to the breast milk microbiota. A better understanding of the mechanisms and effects of the associations between prenatal supplementation and the breast milk microbiota will help inform recommendations on maternal supplementation.

Acknowledgments

Special thanks to the CHILD Cohort Study participants for their dedication and commitment to advancing health research: childcohort.ca

Author's contributions

The authors' responsibilities were as follows: RFC conceptualized the research aims, performed the statistical analysis and wrote the manuscript. MRF provided guidance with the statistical analysis and write-up. MBA, SM and KF provided guidance with the statistical analysis. KF conducted the microbiome sequencing and data pre-processing. MBA, TH, PM, RJS, ST, PS and EK designed research (project conception, development of overall research plan, and study oversight). All authors read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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CHAPTER 5. THE GUT MICROBIOTA: A PROMISING TARGET IN THE RELATION BETWEEN COMPLEMENTARY FEEDING AND CHILD UNDERNUTRITION

Chehab RF, Cross TL, Forman MR. The Gut Microbiota: A Promising Target in the Relation between Complementary Feeding and Child Undernutrition. Adv Nutr. 2020 Nov 19:nmaa146. doi: 10.1093/advances/nmaa146. Epub ahead of print. PMID: 33216115.

5.1 Abstract

Child undernutrition is a major public health challenge that is persistent and disproportionately prevalent in low- and middle-income countries. Undernourished children face adverse health, economic, and social consequences that can be intergenerational. The first 1000 days of life, from conception until the child's second birthday, constitute the period of greatest vulnerability to undernutrition. The transition process from milk-based diets to solid, semi-solid, and soft food and liquids other than milk, referred to as complementary feeding (CF), occurs between the age of 6 months and 2 years. CF practices that do not meet the World Health Organization's guiding principles and are lacking in both quality and quantity increase susceptibility to undernutrition, restrict growth, and jeopardize child development and survival. The gut microbiota develops toward an adult-like configuration within the first 2-3 years of life. Recent studies suggest that significant changes in the gut microbial composition and functional capacity occur during the CF period, but these studies were conducted in high-income countries. Research in low- and middle-income countries, on the other hand, has implicated a disrupted gut microbiota in child undernutrition, and animal experiments reveal the potential for a causal relationship. Given the growing body of evidence for a plausible role of the gut microbiota in the link between CF and undernutrition, microbiota-targeted complementary food may be a promising treatment modality for undernutrition management. The aims of this paper are to review the evidence for the relation between CF and undernutrition and to highlight the potential of the gut microbiota to be a promising target in this relation. Our summary of the current state of the knowledge in this area provides a foundation for future research and helps inform the design of interventions targeting the gut microbiota to combat child undernutrition and promote healthy growth.

Keywords: malnutrition; child growth; first 1000 days; infant and young child feeding; solid food; gut microbiome; low- and middle-income countries

Summary to be used in the table of contents: Complementary feeding interventions targeting the gut microbiota are promising for undernutrition management among children in low- and middle-income countries.

5.2 Introduction

Undernutrition refers to insufficient or imbalanced intake of energy and nutrients necessary to maintain growth and good health (10). Child undernutrition manifests in three forms: stunting, wasting, and underweight (10), which are defined using weight and height (length) measurements in the index child compared to age- and sex- specific child growth standards by the World Health Organization (WHO) (166). Despite efforts to eradicate undernutrition, it remains the most common "disease" among children under the age of five (167). According to recent 2020 estimates, 144 million (21.3%) children younger than five years are stunted and 47 million (6.9%) are wasted (168). Child undernutrition is disproportionately prevalent in low- and middle-income countries (LMIC) with South Asia and Sub-Saharan Africa bearing the greatest disease burden (168).

Nearly half of all deaths in children under the age of five are attributable to undernutrition (169). Undernourished children are at a higher risk of death from common child illnesses such as diarrhea, pneumonia, and malaria (170), which together topped the list of the leading causes of death among children under five in 2015 (171). In the long-term, undernutrition is linked to poorer overall health, impairments in intellectual performance, and reduced economic productivity (172). Further, a woman who was undernourished in childhood has higher odds of delivering a malnourished low birthweight newborn, rendering undernutrition an intergenerational problem (167). Interventions to combat undernutrition and promote healthy growth and development among children in LMIC are essential to meet the sustainable development goals, namely those related to hunger eradication and reduction of inequalities (173).

Undernutrition is associated with a web of risk factors, with inadequate dietary intake and infectious diseases being proximal determinants in LMIC (174). About 100,000 deaths due to undernutrition in children under five could be prevented each year if infant and young child feeding (IYCF) was adequate (175). Environmental enteric dysfunction (EED) due to exposure to enteropathogens is another common risk factor in LMIC with poor sanitation and unhygienic conditions (176). Diarrhea, one particularly visible clinical manifestation of EED, is the second leading cause of death among children under five contributing to nearly 9% of deaths in 2015 (171). The interaction between inadequate dietary intake and enteric infections may impair gut and immune functions and can lead to and/or exacerbate pre-existing undernutrition (176).

The first 1000 days of life, from conception until the child's second birthday, constitute a critical window for child growth and development when rapid maturation of metabolic, endocrine, neural, and immune systems occurs, according to the developmental origins of health and disease (DOHaD) (177). Birth cohort studies have consistently demonstrated that the first 1000 days are the period of greatest vulnerability to undernutrition (178), and that undernutrition during this period has profound irreversible effects with limited capacity for catch-up growth (179). The high prevalence and burden of child undernutrition confirm the need to scale up interventions during this vulnerable window, including promotion of optimal age-specific IYCF practices (175, 180).

The human gut microbiota, which refers to the ecological community of commensal, symbiotic, and pathogenic microorganisms in the gastrointestinal tract (181), is established during the first 2-3 years of life (48). A growing body of evidence has linked disruptions in the establishment and maturation of the gut microbiota to child undernutrition (182-185). Gut microbiota disruptions in early life might result from adverse environmental insults such as suboptimal IYCF and contribute to lifelong and intergenerational deficits in growth and development (51). Although several studies have examined the effects of breastfeeding on the gut microbiota (186-190) and risk of undernutrition (191, 192), few have focused on the effects of the following stage of IYCF, notably complementary feeding (CF). The aims of this paper are to review the evidence for the relation between CF and undernutrition and to highlight the potential of the gut microbiota to be a promising target in this relation.

5.3 Methods

PubMed was searched using combinations of the following keywords: complementary feeding or complementary food or solid feeding or solid food and gut microbiome or microbiota for section B that addresses the changes in the gut microbiota during the CF period. The search yielded research in the HIC not LMIC. We chose to highlight the studies that examined the effects of CF and BF on the gut microbiota. For section C on the gut microbiota and undernutrition, we searched for the papers that describe a novel approach to describe the disrupted gut microbiota as immature. We also reference a number of papers that describe other definitions of a disrupted microbiota. In section D, based on the definition of the immature microbiota above, we searched for articles on complementary feeding interventions that specifically target the microbiota for undernutrition management. As for our selection criteria, primary research studies were included if they (1) were conducted in humans (rather than a focus on all animal research, but we describe an illustrative animal study that enhanced our understanding of the human research); (2) collected (the majority of) fecal samples from infants/ children during or around the complementary feeding period; (3) examined the effects of complementary feeding on the gut microbiota rather than the inherent variation in the process of microbial succession associated with age; (4) examined undernutrition in any of its three anthropometric forms: stunting, wasting, underweight; and (5) used next generation sequencing techniques for microbiome analysis.

5.4 Current Status of Knowledge

5.4.1 Inadequate CF practices increase susceptibility to undernutrition

The WHO defines CF as “the process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed along with breast milk” (193). The transition process from milk-based diets to solid, semi-solid, and soft food and liquids other than milk typically covers the period between 6 months and 2 years of age (193). Infants have high nutritional needs for their rapid growth, so optimal CF practices are essential to provide key nutrients to maintain healthy growth and development (3). However, unlike the WHO’s recommendation for exclusive breastfeeding for the first 6 months (94), there is no single recommendation for CF. CF encompasses several dimensions including the age of introduction, amount, energy density, nutrient content, frequency of consumption, food consistency, safe preparation and storage of complementary food, and responsive feeding (4).

In 2003 and 2005, the WHO published detailed guiding principles covering the CF dimensions among breastfed (4) and non-breastfed infants (194), respectively. The guiding principles have had an important impact on policies especially in LMIC and have increased attention to nutrition and growth during the CF period (34). Afterwards in 2008, the WHO used 10 existing datasets from Africa, Asia, and Latin America to define and validate a set of eight core and seven optional indicators to assess IYCF based on the guiding principles (195). Five of the eight core indicators focus on specific dimensions of CF including age of introduction, energy and nutrient content, and frequency of consumption of the complementary food (Table 5.1), while the remaining 10 indicators relate to breastfeeding. Other dimensions of CF such as adequate consistency and texture of food and responsive feeding were deemed too complex to assess and require more work to develop valid and reliable indicators (195).

The indicators have been used to assess CF practices worldwide, especially in LMIC. The first published application of the indicators appeared in the country profiles by the WHO using 2002-2008 Demographic and Health Survey (DHS) data from 46 countries (196). Most countries had grossly inadequate CF practices, especially those in South Asia and sub-Saharan Africa with the highest prevalence and burden of child undernutrition (168). In a more recent analysis of the UNICEF global database of national-level household surveys of 85 countries conducted between 2010-2016, CF practices were still poor worldwide and were the poorest in the aforementioned two regions (197). Specifically, less than 50% of children in South Asia and sub-Saharan Africa achieved minimum meal frequency, less than 25% achieved minimum diet diversity, and less than 15% achieved minimum acceptable diet.

Other than assessing CF practices, the WHO indicators have been used to examine the associations between CF and undernutrition susceptibility in different populations in LMIC (198-204), demonstrating the applicability of the indicators for their intended purposes and in populations for which they were originally developed (195). In Malawi for example, a sub-Saharan African country severely burdened by child undernutrition (205), 13-23-month-old children who did not meet the minimum meal frequency or the minimum acceptable diet indicators were more likely to be underweight (203). In Bangladesh, which has one of the highest burdens of child undernutrition worldwide (206) and where CF practices remain grossly inadequate (207), untimely introduction of complementary food and failure to meet the minimum diet diversity indicator were associated with stunting among 6-23-month-old children (204). Association between the consumption of a diverse diet and lower likelihood of stunting was also noted in a pooled analysis of 74,548 children aged 6-23 months using the 2010-2014 DHS from 39 LMIC (208). In addition, consumption of animal source food, which is not currently a WHO indicator but has emerged as a critical dimension related to protein quality (34), was inversely associated with stunting after adjustment for socioeconomic and other covariates (208).

CF interventions that are effective at reducing undernutrition in LMIC are a high priority (33). Systematic reviews and meta-analyses assessing the effectiveness of CF interventions are valuable, yet variations in study populations, type of interventions, reported outcomes, and grade quality of the reviewed studies render calculation of pooled estimates challenging. Heidkamp (209) synthesized the evidence in 2017 from recent systematic reviews and meta-analyses of the impact of CF interventions on linear and ponderal growth of children aged 6-23 months in LMIC (210-212). The systematic reviews of interventions with CF education alone (210, 212) and those of interventions with provision of food and other supplements with or without education (211, 212) revealed modest effects on height and weight gain. In another review, CF education and supplementary feeding interventions did not have significant effects on stunting and wasting prevalence, whereas CF provision had significant positive effects on the prevalence of stunting (213). Other systematic reviews have noted similar small and mixed effects of CF interventions on child growth in LMIC (32, 214).

The association between the multiple dimensions of CF and undernutrition coupled with the limited catch-up growth following CF interventions in undernourished children highlight the complexity of this relation. Further, adequate CF to maintain child growth and development depends not only on what, when, and how children are fed but also on the extent to which their body is capable of efficiently digesting, absorbing, and utilizing these food (51), three functions in which the gut microbiota plays a critical role.

5.4.2 Significant changes in the gut microbiota of children occur during the CF period

The gut microbiota develops and matures toward an adult-like configuration during the first 2-3 years of life (48). Gut microbiota development has prominent effects on long-term health (51) and is affected by several factors including mode of delivery, antibiotic exposure, and IYCF (186, 187, 189). While the differential effects of milk-based diets (breast- versus formula- versus mixed-feeding) on the infant microbiota have been examined (186-190), less research has been conducted on the effects of CF (215). Further, some studies have suggested that breastfeeding in terms of exclusivity, amount, duration, and cessation is the main driver of the changes in the gut microbiota during the CF period rather than CF itself (186, 187). The following section reviews the studies (Table 5.2) examining the association of CF with the changes in the gut microbiota and addresses whether the aforementioned breastfeeding-related variables influence these changes.

Prospective cohort studies with multiple fecal sample collections during the CF period help inform how the introduction to new food components influences the changes in the gut microbial structure and function in early life. Introduction of solid food (SF) among infants in the United States (US) was associated with increased alpha (within sample) diversity as well as changes in the composition of bacterial taxa and predicted function, with observed differences by exclusive breastfeeding status prior to SF introduction (216). Specifically, alpha diversity was higher following SF introduction in infants who were not previously exclusively breastfed compared to those who were. SF introduction was associated with overrepresentation of 24 functional genes in exclusively breastfed infants compared to 230 functional genes in non-exclusively breastfed infants. The changes in predicted function following SF introduction were corroborated by a metagenomic analysis of the gut microbiome of a full-term, vaginally delivered, healthy, and exclusively-breastfed boy (217). The boy’s microbiome was enriched with functional genes characteristic of the adult gut microbiome including genes for vitamin biosynthesis, plant polysaccharide utilization, and breakdown of xenobiotic compounds (foreign substances to the human body, e.g. antibiotics, pharmaceuticals). Further, fecal concentrations of individual short chain fatty acids (acetate, propionate, and butyrate) were higher following introduction of table food. However, since this is a case-study of one child, it is important to replicate the results among a larger number of infants.

The effect of the age of introduction and the composition of the complementary food on the gut microbiota of infants and children have been also studied. Pannaraj et al. (218) examined the trajectory of microbiota maturity among US infants using a random forest (RF) model, which is a machine-learning algorithm suggested by Subramanian et al. (219) to determine a ranked list of all bacterial taxa in the order of age-discriminatory importance. Early CF (<4 months) compared to later CF (≥4 months of age) was associated with a rapid maturation of the gut microbiota and increased predicted function related to xenobiotics biodegradation and metabolism. In the Nurture cohort study, early (≤3 months) versus late CF (>3 months) was associated with higher alpha diversity and differential abundance of 29 bacterial taxa at 3 and 12 months of age after adjusting for potential confounders including breastfeeding (220). Early CF was also associated with greater concentrations of fecal butyrate and total short chain fatty acid levels at 12 months.

Unlike the studies conducted among infants in the US, a study by Laursen et al. (221) among Danish infants suggested that the age of SF introduction (3-6 months) was not associated with alpha diversity or microbiota composition at 9 months. Nevertheless, the duration of exclusive breastfeeding (0-6 months) was associated with these microbiota indicators. The authors concluded that exclusive breastfeeding duration, rather than CF timing, was a stronger determinant of the gut microbiota in early life. Studies by Backhed et al. (186) among Swedish infants and Stewart et al. (187) among infants and children in three European countries (Germany, Sweden and Finland) and US states (Colorado, Georgia and Washington) had a similar conclusion. They argued that breastfeeding cessation rather than SF introduction is the major driver in the development of an adult microbiota. It is worth noting however that these studies (186, 187) base their conclusion on the effects of breastfeeding status and did not examine the effect of the CF dimensions, including age of introduction and composition of SF, on the infants' microbiota.

To decipher the effect of breastfeeding versus that of CF, Laursen et al. (222) stratified the Danish infants by breastfeeding status at 9 months. They found that protein and fiber content of SF and progression toward family food were associated with higher alpha diversity in both groups of infants, suggesting that these changes were independent of breastfeeding status. Additionally, whereas meat, cheese and rye bread represented the specific food groups most strongly associated with alpha diversity among all infants as well as those no longer breastfed at 9 months, porridge (primarily oatmeal) was most strongly associated with alpha diversity among infants still breastfed. Progression to family food was associated with a decrease in the abundance of several bacterial genera including Bifidobacterium and an increase in the abundance of others including Faecalibacterium and Ruminococcus among all infants (221). Of note, Bifidobacterium is known for its capacity to utilize lactose and human milk oligosaccharides in breast milk (223) and has been referred to as 'milk-oriented' taxon in the study by Gehrig et al. (224). Faecalibacterium and Ruminococcus on the other hand increase with the addition of dietary fiber or protein to the diet (225, 226) and have been thus referred to as 'weaning-phase' taxa. Hence, the changes in the gut microbiota during the CF period are likely to be driven by a combination of breastfeeding- and CF-related variables.

Randomized controlled trails examined the effects of CF on the gut microbiota of infants. Eighty-seven exclusively breastfed infants were randomly assigned to receive either iron-fortified cereal, iron-fortified cereal with fruit, or meat as their first complementary food (227). The age of SF introduction varied by the parents' preference and ranged between 4-6 months, but all infants consumed their assigned diets for 2-4 weeks. In a subset of 56 infants, microbiota analysis revealed increased alpha diversity following introduction of meat or cereal with fruit, but not cereal alone. No significant changes in microbiota composition were noted after introduction of any of the SF. In another trial, 45 exclusively breastfed 5-month-old infants were randomized to receive commercially available pureed meats, iron- and zinc-fortified infant cereals, or iron-only fortified infant cereals as the first and primary complementary food through 9-10 months of age (228). In a subsample of 14 infants, no significant change in alpha diversity was observed following introduction of any SF. Changes in microbiota composition were apparent among infants introduced to the iron-fortified cereal compared to those introduced to the meat or iron- and zinc-fortified cereal.

The studies in this section suggest that significant changes in the gut microbiota occur during the CF period and are potentially driven by breastfeeding as well as CF. All the studies were conducted in HIC. No similar studies were conducted among LMIC populations, which are the focus of this review, or in the context of undernutrition in which other factors including deficient diets, EED, and poor sanitation might also drive the changes during the CF period. Moreover, none have examined the effect of CF adequacy as assessed by the WHO indicators on the gut microbiota. More research is needed to (1) understand the nature and magnitude of the link between CF and the gut microbiota; (2) the extent to which such a link can be beneficial to improve children's growth and nutrition; and (3) the means in which the knowledge gained from items (1) and (2) can translate to the context of undernutrition and LMIC.

5.4.3 Gut microbiota disruptions are associated with, and can even cause, child undernutrition

Disruptions in the gut microbiota in early life are likely to have detrimental effects on children's nutritional status and health later in life. Studies linking the gut microbiota to undernutrition have different definitions for gut microbiota disruptions. One novel approach, described in the section below, was proposed by Jeffrey Gordon's team who suggested that undernourished children have immature gut microbiotas (49, 219, 229, 230). Such an approach provides potential insight and testable hypotheses on the role of the gut microbiota in child undernutrition and inform the development of promising interventions.

Smith et al. (49) studied 317 Malawian twin pairs during the first 3 years of life, some of whom were discordant for kwashiorkor, a form of severe acute malnutrition (SAM) characterized by peripheral edema, abdominal distension, and hepatic metabolic disruptions (231). Twin studies are especially valuable in gut microbiota research as they help distinguish the effects of genetic from those of environmental factors. Compared to the gut microbiota of the healthy co-twin, the microbiota of the twin with kwashiorkor was immature and resembled that of younger children (49). Even when the twin with kwashiorkor was treated with ready-to-use therapeutic food (RUTF), an energy-dense and micronutrient-enriched paste used in the treatment of SAM, the divergent trajectory of gut microbiota maturation was not corrected.

To further investigate the role of the gut microbiota in undernutrition, Smith et al. (49) transplanted fecal communities from several discordant twin pairs to C57BL/6J germ-free mice, which lack microorganisms living in and on them. The recipient mice were fed a typical Malawian diet that is nutrient-deficient and low in calorie density for 3 weeks. Mice that received transplants from the kwashiorkor-diagnosed twin lost more weight, had overrepresentation of pro-inflammatory taxa, and exhibited perturbed metabolism of amino acids and carbohydrates compared to mice with fecal transplants from the healthy co-twin. One proposed mechanism resulting from these observations in which the gut microbiota might be implicated in kwashiorkor is through the generation of products such as inhibitors of enzymes in the tricarboxylic acid cycle, which can compromise effective energy metabolism and eventually nutritional status. This proof-of-concept study suggests that an immature gut microbiota coupled with a nutrient-deficient diet may be included among the causal risk factors of undernutrition.

Gut microbiota immaturity was further examined in a study by Subramanian et al. (219) among Bangladeshi children younger than 2 years using an RF machine-learning algorithm. The relative abundance of the operational taxonomic units (sequences that are clustered together based on similarity to inform taxonomy) was regressed against the chronological age of each child at the time of fecal sample collection to identify age-discriminatory taxa. Among the 24 most age-discriminatory taxa were the weaning-phase taxa including Faecalibacterium prausnitzii and Ruminococcus sp. and the milk-oriented taxa including Bifidobacterium longum. Two metrics to assess microbiota maturity of children were subsequently defined using the sparse 24-taxon model. The first metric, relative microbiota maturity, compared the microbiota age of the child to that of healthy children of similar chronologic age. The second, microbiota-for-age z-scores (MAZ), is similar to the anthropometric z-scores used to assess nutritional status such as height-for-age, weight-for-age, and weight-for-height z-scores.

Applying the 24-taxon model to the fecal samples of a group of 64, 6-20-month-old Bangladeshi children with SAM revealed that undernourished children had gut microbiota immaturities and lower MAZ compared to age-matched healthy children (219). The microbiota immaturities were only partially repaired when the children with SAM were treated with RUTF or a locally-produced alternative, supporting previous findings that existing nutrition therapies fail to completely repair immature gut microbiotas (49). The RF model among Bangladeshi children was applicable to the population of Malawian twin pairs (229) assessed in the study by Smith et al. (49). F. prausnitzii, R. sp., and B. longum were also age-discriminatory in the Malawian sparse 25-taxon model (229). In addition, MAZ among Malawian twins at 12 months was significantly positively correlated with weight-for-height z-scores and weight-for-age z-scores at 18 months, suggesting that MAZ may be useful for predicting future ponderal growth.

Although the use of RF models to identify age-discriminatory taxa is helpful, it typically describes a ‘parts list’ as it focuses on the abundance of community members without accounting for the interactions between them (230). Analysis of conserved bacterial taxon-taxon covariance in the gut microbiota of healthy Bangladeshi children younger than five years revealed a network of 15 covarying bacteria termed an “ecogroup” (230). F. prausnitzii and B. longum were among the 15 covarying taxa, along with others that were identified as age-discriminatory in the RF model among Bangladeshi children. The ecogroup confirmed the existence of a program of gut microbial community maturation that was completed by the second year of life in healthy Bangladeshi children. The ecogroup further supported findings that undernourished Bangladeshi children had impaired gut community development characterized by perturbed interactions between the 15 covarying taxa.

Other definitions have been proposed for microbiota disruption including lower alpha diversity and/or higher abundance of pathogenic taxa (232, 233). Different forms of undernutrition have been also studied, ranging from chronic undernutrition (234) to moderate (224) and severe acute malnutrition (49, 219, 229). Further, associations between gut microbiota disruptions and undernutrition have been examined using different microbiota analytic methods (e.g. 16S rRNA 454 pyrosequencing versus 16S rRNA Illumina sequencing versus shotgun metagenomic sequencing) in various populations residing in different geographic locations with distinct cultural practices and traditional diets. Such variations in definition, analysis, and study population characteristics possibly explain the contrasting results related to the nature and strength of the association between gut microbiota disruption and undernutrition. All the studies, however, hint at the potential of the disrupted gut microbiota to be a key player in the web of risk factors of undernutrition. Interestingly, the opposite direction of the relation where child undernutrition may cause gut microbiota disruption has been suggested in a few review papers (183, 184), but has not been not fully studied in children or animal models.

5.4.4 Complementary food targeting the gut microbiota are promising interventions for undernutrition

With the advancement in our knowledge of the paramount role of the gut microbiota in child undernutrition, a number of interventions targeting the microbiota have emerged as promising treatment modalities. Prebiotics, probiotics, and synbiotics have gained significant attention following findings of their potential ability to modulate the gut microbiota of undernourished children (235, 236). A systematic review of the effects of probiotics on child growth suggests that probiotics might have more significant effects improving child nutritional status and growth among undernourished children and those living in LMIC (237). There are a number of challenges, however, to implementing probiotic-based approaches including the need to closely monitor safety, particularly among undernourished children with impaired immune function, and to gain culture acceptability to supplement children with live microorganisms (238).

Locally produced complementary food that target the gut microbiota immaturities among undernourished children can help overcome such challenges. Gehrig et al. (224) conducted a pilot of a randomized controlled trial to compare the efficacy of microbiota-directed complementary food (MDCF) to the traditional rice- and lentil-based ready-to-use supplementary food (RUSF) used in the treatment of moderate acute malnutrition (MAM). The 1-month pilot trail tested three MDCF formulations with different proportions of four ingredients: peanut flour, chickpea flour, soy flour, and banana among 12-18-month-old Bangladeshi children with MAM. The ingredients were selected based on their growth-promoting effects in gnotobiotic mice and piglets (animals with defined microbial communities) colonized with members of the gut microbiota from undernourished Bangladeshi children. MDCF-1 had all four ingredients but at lower concentrations than MDCF-2, and MDCF-3 had chickpea flour and soy flour only. MDCF-1 and RUSF contained milk powder. All MDCF formulations were supplemented with a micronutrient mixture designed to provide 70% of the recommended daily allowances for 12-18-month-old children. The MDCF formulations and RUSF had similar protein energy ratio, fat energy ratio and macro- and micronutrient content and provided 250 kcal/day.

At the end of the trial, all children had improved weight-for-height z-scores regardless of the treatment group (n= 14-17 children per group) (219). Children randomized to MDCF-2, compared to MDCF-3, had a significantly greater increase in mid-upper arm circumference, an independent diagnostic criterion of MAM (239). Further, MDCF-2 induced an increase in the relative abundance of several weaning-phase taxa including F. prausnitzii and a decrease in that of milk-oriented taxa such as B. longum compared to the three other treatments. However, there was no improvement in MAZ with any of the treatments, which the authors attributed to the possible confounding by unexpectedly high baseline microbiota maturity scores in the children with MAM in this study (219). MDCF-2 was uniquely associated with covariation of the ecogroup taxa towards a more complete community repair (230). MDCF-2 also increased the abundance of proteins that are higher in plasma from healthy children and that are positively correlated with height-for-age z-scores, and it reduced the levels of proteins elevated in plasma of undernourished children and those that are negatively correlated with height-for-age z-scores (219). Therefore, despite the small group size and the short length of the study, MDCF-2 elicited a marked shift in the microbiota profiles and plasma proteome toward those of healthy children.

Findings from this pilot trial informed the design and implementation of a proof-of-concept efficacy trial, whose protocol has been recently published (240), with similar aims but among a larger group of 248 Bangladeshi children with MAM for a longer 3-month period of time. Another study protocol has been published for the MALINEA project that aims to compare the effects of three strategies of renutrition for MAM on the gut microbiota of 840, 6-24-month-old children in four African countries (Madagascar, Niger, Central African Republic, and Senegal) (241). The children are randomized to receive enriched flour alone, enriched flour with prebiotics (combination of inulin and fructo-oligosaccharides), or enriched flour coupled with a 4-day antibiotic treatment (azithromycin) for 12 weeks. It is hoped that results from these studies will serve as a stepping-stone for the development of complementary food targeting the microbiota of undernourished children for sustainable benefits on child growth.

Table 5.1 Indicators to assess complementary feeding adequacy

Indicators*	Definition	Notes
1. Timely introduction to solid, semi-solid, or soft foods	Proportion of infants 6–8 months of age who receive solid, semi-solid or soft foods	
2. Minimum diet diversity	Proportion of children 6–23 months of age who receive foods from 4 or more of the 7 food groups	The 7 food groups are: grains, roots and tubers; legumes and nuts; dairy products; flesh foods; eggs; vitamin-A rich fruits and vegetables; other fruits and vegetables.
3. Minimum meal frequency	Proportion of children 6–23 months of age who receive solid, semi-solid, or soft foods (but also including milk feeds for non-breastfed children) the minimum number of times or more according to the infant's age	The minimum number of times is defined as: 2 times/day for 6–8 month old breastfed infants; 3 times/day for 9–23 month old breastfed children; 4 times/day for 6–23 month old non-breastfed children.
4. Minimum acceptable diet	Proportion of children 6–23 months of age who had at least the minimum dietary diversity and the minimum meal frequency	This is a summary or composite indicator of the previous two indicators.
5. Consumption of iron-rich or iron-fortified foods	Proportion of children 6–23 months of age who receive an iron-rich food or iron-fortified food that is specially designed for infants and young children, or that is fortified in the home.	Suitable iron-rich or iron-fortified foods include flesh foods, commercially fortified foods specially designed for infants and young children that contain iron, or foods fortified in the home with a micronutrient powder containing iron or a lipid-based nutrient supplement containing iron.

*All indicators are assessed based on the child's dietary intake in the day preceding the survey
Source: World Health Organization (195)

Table 5.2 Summary of the studies examining the changes in the gut microbiota during the CF period

Reference	Study design	Study location	Sample size	Age at fecal sample collection	Examined variable related to CF	Examined effect of BF in relation to CF	Examined variable related to the gut microbiota				
							Alpha diversity	Microbial composition	Fecal SCFAs	Functional genes	Relative microbiota maturity[1]
Thompson et al., 2015 (216)	prospective cohort	Atlanta, US	9	periodically between 13 days-14 mo.	introduction (4-6 mo.)	✓	✓	✓	×	✓[2]	×
Koenig et al., 2011 (217)	case study	US	1	periodically between birth-2.5 years	introduction (~5.5 mo.)	✓	✓	✓	✓	✓	×
Pannaraj et al., 2017 (218)	prospective cohort	California & Florida, US	119	periodically between 1-331 days	introduction (4-6 mo.) & age of introduction (<4 vs. ≥4 mo.)	✓	✓	✓	×	✓[2]	✓
Differding et al., 2020 (220)	prospective "Nurture" cohort	North Carolina, US	67	at 3 & 12 mo.	age of introduction (≤3 vs. >3 mo.)	✓	✓	✓	✓	×	×
Laursen et al., 2016 & 2017 (221, 222)	prospective cohort	Denmark	227	at 9 & 18 mo.	age of introduction (3-6 mo.) & composition	✓	✓	✓	×	×	×
Qasem et al., 2017 (227)	randomized controlled trial	Manitoba, Canada	87	before and after introduction between 4-6 mo. in 56 infants	introduction (4-6 mo.) & composition	✓	✓	✓	×	×	×
Krebs et al., 2013 (228)	randomized controlled trial	Colorado, US	45	monthly between 5-9 mo. in 14 infants	introduction (5 mo.) & composition	✓	✓	✓	×	×	×

BF: breastfeeding; CF: complementary feeding; mo.: month; SCFA: short chain fatty acids; SF: Solid food; US: United States

[1]Calculated using a random-forest model which is a machine-learning algorithm to determine a ranked list of all bacterial taxa in the order of age-discriminatory importance

[2]Predicted functional genes

5.5 Conclusions and Future Research

The persistent high prevalence of child undernutrition in LMIC and its long-term health and societal costs are a call for action. This review paper provides evidence supporting a plausible relation between CF, the gut microbiota, and child undernutrition (Figure 5.1). An association between inadequate CF and child undernutrition was suggested in numerous studies in different LMIC. In contrast, studies examining the changes in the gut microbial composition and function during the CF period were largely conducted in HIC. Further, a disrupted gut microbiota has been associated with, and causally linked to, child undernutrition. Indeed, the direction of the causality is not fully examined and potentially exists in both directions depending on genetic and environmental conditions. The current state of knowledge supporting the potential of the gut microbiota to be a key player in the relation between CF and undernutrition has ignited interest in developing microbiota-directed interventions during the CF period for undernutrition management.

More research is required to further our knowledge of the interplay between the different dimensions of CF and the gut microbiota especially amongst populations in LMIC, and to accordingly inform an update of the WHO guiding principles and indicators of IYCF that were published over a decade ago (185). Research is also necessary to decipher the mechanisms involved in the association between inadequate CF and undernutrition, including those related to the microbial communities residing in the child's gut. Studies using animal models and those conducted among children in LMIC can address how CF and the gut microbiota influence growth and will contribute to the development of an updated algorithm of the risk factors of undernutrition. The potential of the disrupted gut microbiota as a key player in this algorithm is evident. Such a holistic view of the risk factors of undernutrition can help better inform the design of interventions to create persistent beneficial effects on the maturation process of the gut microbiota and child growth. This in turn can help decrease the prevalence and burden of undernutrition and promote health and well-being.

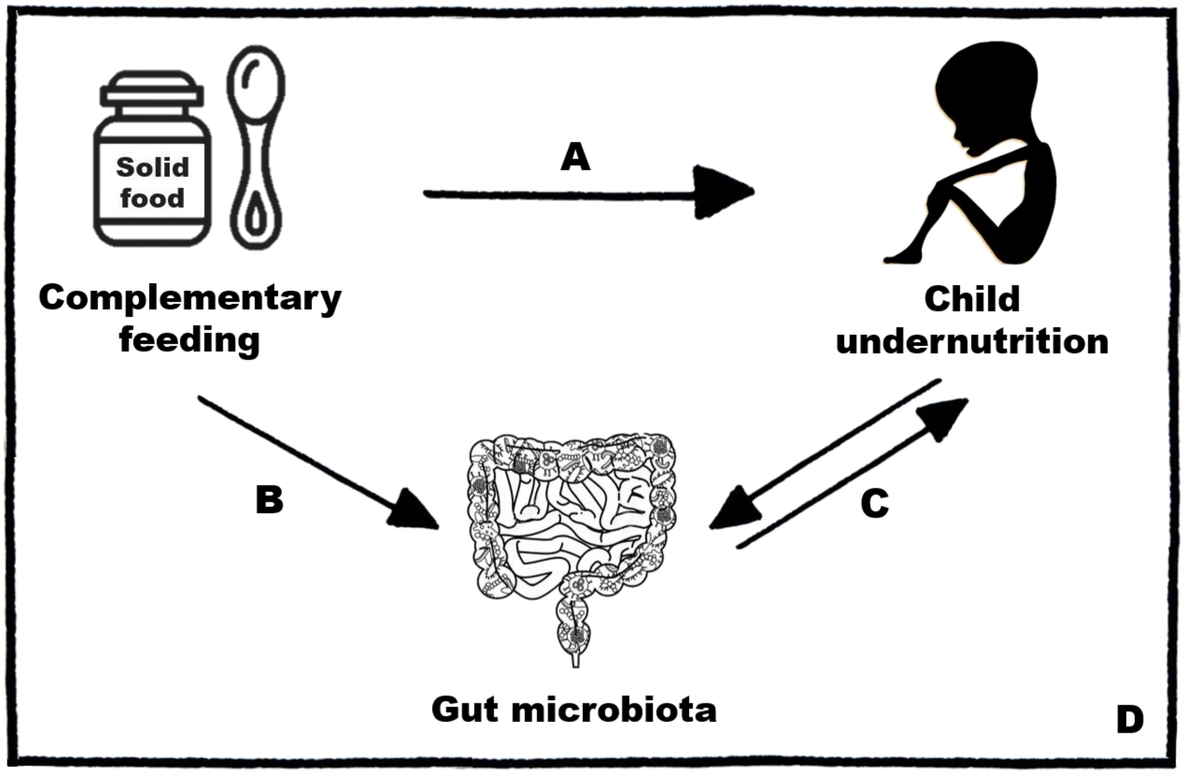

Figure 5.1 Summary of the plausible links between CF, the gut microbiota, and child undernutrition

A: Inadequate CF is associated with child undernutrition. These associations were mostly examined in LMIC. CF interventions often have limited effects on child growth, hinting at the complexity of the relation between CF and undernutrition. B: Significant changes in the gut microbiota occur during the CF period. The introduction of new food components and the age of introduction and composition of the complementary food influence the gut microbiota. However, evidence in this area originates solely from HIC. C: Gut microbiota disruptions are associated with, and causally linked to, child undernutrition. A bidirectional relation is plausible where a disrupted gut microbiota can cause undernutrition and vice versa. D: Interventions for undernutrition targeting the gut microbiota during the complementary feeding period are promising. More research is needed to further our knowledge of the interplay between complementary feeding, the gut microbiota, and undernutrition as this can help better inform the design of interventions with long-lasting benefits to combat child undernutrition and promote health and well-being. CF: Complementary feeding; HIC: high-income countries; LMIC: low- and middle-income countries.

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None declared

List of acronyms

CF: Complementary feeding

DOHaD: Developmental origins of health and disease

DHS: Demographic and health survey

EED: Environmental enteric dysfunction

HIC: High-income countries

IYCF: Infant and young child feeding

LMIC: Low- and middle-income countries

MAM: Moderate acute malnutrition

MDCF: Microbiota-directed complementary food

RF: Random forest

RUSF: Ready-to-use supplementary food

RUTF: Ready-to-use therapeutic food

SAM: Severe acute malnutrition

SF: Solid food

US: United States

WHO: World Health Organization

CHAPTER 6. CONCLUSIONS

6.1 Summary

The studies in this dissertation provide evidence informing the paradigm linking socio-demographic, maternal and child determinants including prenatal intake to infant feeding, the breast milk and infant gut microbiome, and child growth, all within the first 1000 days of life (Figure 1.1).

I first examined the socio-demographic, maternal and child determinants of child growth and breastfeeding in Lebanon, a middle-income country in the Middle East undergoing nutrition transition. In chapter 2 of the dissertation, I report that the determinants of child growth classified using a hierarchical conceptual framework differed by child sex. Among the distal sociodemographic determinants, higher maternal education among boys and lower crowding index among girls were associated with higher length-for-age z-scores. On the other hand, higher paternal education among boys was associated with lower weight-for-length z-scores. At the intermediate level, maternal obesity among girls was associated with lower length-for-age z-scores. Among the proximal child determinants, birth length among girls was positively associated with length-for-age z-scores and birthweight among boys and child's age among boys and girls were positively associated with weight-for-length z-scores. On the contrary, being a third or later child among girls was associated with lower weight-for-length z-scores. Longer breastfeeding duration was among the proximal determinants associated with lower length-for-age z-scores among girls, a common finding in middle- and high- income countries compared to low-income countries.

In chapter 3, I report on suboptimal breastfeeding practices in Lebanon. Less than half (41.5%) of the infants were exclusively breastfed during the 40-day rest period, a common cultural and religious practice in countries of the Middle East characterized by a period of rest, seclusion, and ritual that helps establish and maintain breastfeeding. An even lower proportion of infants (12.3%) were exclusively breastfed during the WHO recommended duration of 6 months. Higher socioeconomic status, as reflected by a larger number of cars owned, and C-section delivery were persistently inversely associated with lower odds of exclusive breastfeeding for 40 days and 6 months. Belonging to a family with more children was associated with higher odds of exclusive breastfeeding for 40 days. In contrast, maternal overweight and obesity were associated with lower odds of exclusive breastfeeding for 6 months.

I then examined the role of the microbiome as a potential player in the relation between prenatal intake and infant feeding practices and child growth. In chapter 4, I report for the first time that prenatal supplement use, but not prenatal dietary quality and patterns, modulate the breast milk microbiota composition in the CHILD cohort study in Canada. Specifically, use of vitamin C and D supplements in addition to multivitamins during any trimester in pregnancy was consistently associated with milk microbial diversity and genus composition before and after adjustment for socio-demographic, maternal and child covariates. Use of other supplements such as fish oil, folate and calcium was less consistently associated with the breast milk microbiome. Prenatal diet was not associated with breast milk microbiota composition, potentially because the analysis focused on diet quality and patterns rather than nutrient composition due to differences between the American and Canadian nutrient composition databases.

In chapter 5, I reviewed the evidence on the potential of the infant gut microbiome to mediate the association between complementary feeding and child undernutrition in LMIC. The effects of complementary feeding on the infant gut microbiome are less commonly studied than those of breastfeeding, with most research conducted in HIC but not LMIC. In contrast, associations between inadequate complementary feeding and undernutrition have been examined in LMIC where undernutrition is most prevalent. Further, a disrupted gut microbiota has been associated with, and causally linked to, child undernutrition. Indeed, the direction of the causality is not fully examined and potentially exists in both directions depending on genetic and environmental conditions. In light of the current state of knowledge described in our review supporting the potential of the gut microbiota as a key player in the relation between complementary feeding and undernutrition, the development of microbiota-directed interventions during the complementary period offers a promising route for undernutrition management.

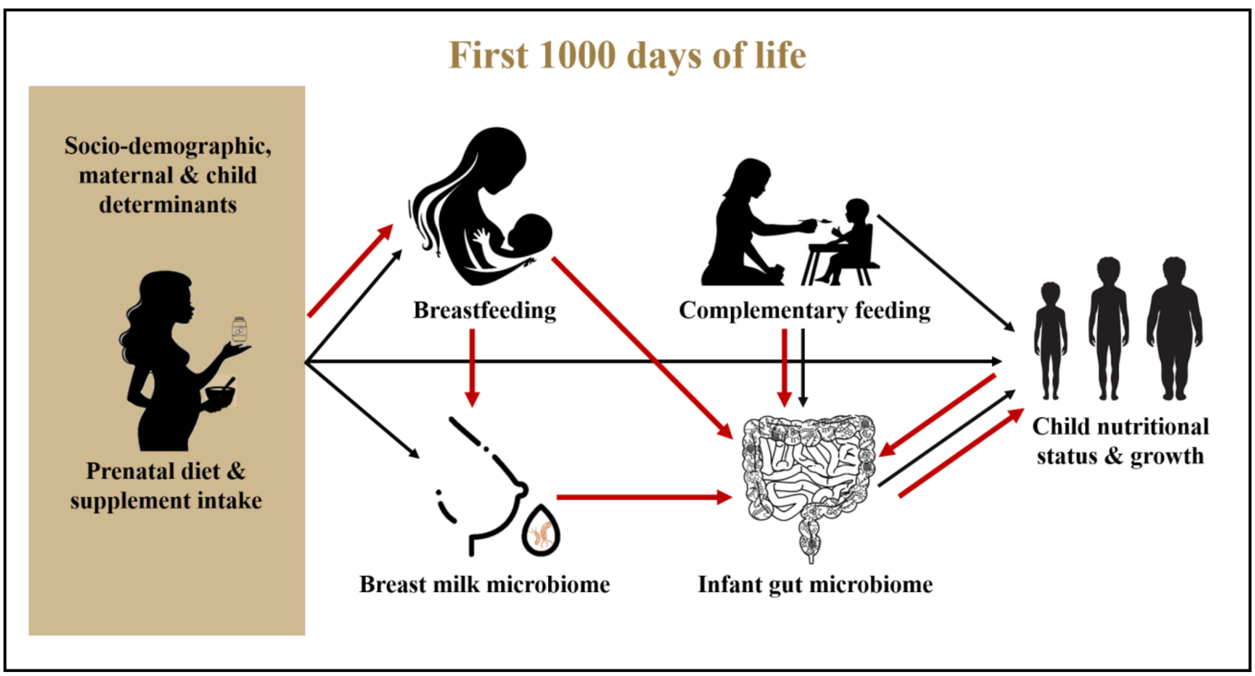
6.2 Future directions

Findings from the studies presented in this dissertation highlight several culture-specific determinants of breast feeding and child growth in Lebanon. Such determinants can be the target of future interventions and can help inform policies aiming to scale up breastfeeding and improve child growth and health later in life. 'Supportive measures are needed at many levels to improve breastfeeding practices, from legal and policy directives to social attitudes and values, women's work and employment conditions, and health-care services to enable women to breastfeed. The best outcomes are achieved when interventions are implemented concurrently through several channels' (30). In Lebanon where C-section rates far exceed the recommended rate suggested by the WHO, policies as well as awareness raising among health care professionals are needed to limit elective and unnecessary C-sections. Further, interventions targeting overweight and obese women of child bearing age are critical to improve not only breastfeeding rates, but also child growth parameters.

Future studies using longitudinal prospective cohorts, intervention trails and animal models are needed to provide evidence for the proposed links to complement our paradigm (Figure 6.1). The determinants of child growth and breastfeeding should be further investigated in longitudinal prospective cohort studies with repeated measures and attention to covariates like the country-specific cultural practices. Technological advancements in the field of the microbiome have made it easier and cheaper to examine the multiple dimensions of the microbiota composition and function. Therefore, the future cohort studies should include metagenomic analysis of the microbiome of mothers and infants to allow for the examination of the microbiome as a mediator in the association between infant feeding practices and child growth. Specifically, future studies should concurrently examine the effect of prenatal intake on the maternal gut and breast milk microbiome. In addition, future integrated analysis should investigate associations between prenatal intake and the breast milk composition in terms of nutrients, bioactive components and microbiota, given that the milk microenvironment has been proposed as a significant modulator of its microbiome. Furthermore, analysis of the infant gut microbiome can further contribute to this proposed pathway as it links breastfeeding (in terms of the various milk constituents including the microbiome) and complementary feeding with the child nutritional status.

Intervention trials and animal studies are needed to clarify the directions of the associations between the microbiome and nutritional status and to help establish causality. Intervention trials in pregnant women might randomly allocate individual prenatal supplements of differing doses of vitamins C and D in addition to prenatal multivitamins as that is a universal recommendation for pregnant women, collect breast milk samples, and determine whether and how individual supplementation altered the breast milk microbiome. Such trials can inform recommendations on maternal intake during pregnancy, which is an understudied area. With the current evidence in the area of the gut microbiome and undernutrition, it is unclear whether a disrupted gut microbiome causes child undernutrition or vice versa. Studies of animal models can help clarify the direction of this association as well as provide evidence in an experimentally controlled setting that a specific pathway links inadequate complementary feeding, gut microbiome disruptions and child undernutrition.

Such a holistic view of the determinants of and pathways between infant feeding and child growth are of great potential public health significance to improve the health of children throughout their lives.



Black arrows represent links in the paradigm that were explored in the current dissertation. Red arrows represent links to be examined in future research.

Figure 6.1 Future areas of research within the first 1000 days of life

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VITA

RANA CHEHAB

PhD candidate, Master of Public Health, Registered Dietitian

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EDUCATION

Purdue University, West Lafayette, IN, USA **Aug. 2017 - Jul. 2021**
PhD in Nutrition Science, Population Nutrition and Health Promotion

- Research focus: Infant and young child nutrition, microbiome, and health across the life course
- Advisor: Michele R. Forman, PhD, FACE, FASN, Former Chair and Distinguished Professor
- Relevant coursework: Nutrition Epidemiology, Microbiome Analysis, Statistics, Data Science

American University of Beirut, Beirut, Lebanon **Sep. 2015 - May 2017**
Master of Public Health, Health Promotion and Community Health
Research projects:

- Accuracy, Credibility, and Readability of Webpages Describing Pregnancy-related Nutrition Information: A Content Analysis; Mentor: Marco Bardus, PhD, MA, Assistant Professor
- A Pilot Intervention to Promote Breastfeeding among Grade 10 Students in Public Schools in Akkar, Lebanon; Mentor: Sawsan Abdulrahim, PhD, Associate Professor

American University of Beirut, Beirut, Lebanon **Sep. 2010 - Jun. 2014**
BS and US Accredited Coordinated Program in Nutrition and Dietetics

CERTIFICATIONS

- **Registered Dietitian** (Registration # 86082783), Commission on Dietetic Registration **2015 - present**
- **Licensed Dietitian,** Ministry of Public Health, Lebanon **2014 - present**
- **Certificate of Foundations in College Teaching,** Purdue University, IN **Mar. 2020**
- **Certificate of Practice in College Teaching,** Purdue University, IN **Dec. 2020**

RESEARCH EXPERIENCE

Purdue University, West Lafayette, IN, USA **Aug. 2017 - present**
Graduate Research Assistant

- Develop a package for anthropometric training for basic, intermediate, and advanced levels for the LANI study as part of the USAID-funded project in Laos co-led by Dr. Gerald Shively, PhD, Associate Dean and Director of the International Programs in Agriculture and Dr. Michele Forman, PhD, FACE, FASN, Formerly Chair and Distinguished Professor
- Examine associations between maternal diet quality and patterns and breast milk microbiota in the Canadian CHILD cohort study[1]
- Examined infant feeding practices and growth patterns among a nationally representative sample of children in Lebanon[2,3]
- Developed structured questionnaire to assess food insecurity among Purdue students

Beirut Arab University, Beirut, Lebanon **Feb. 2017 - Feb. 2019**
Part-time Research Assistant

- Analyzed data from a national cross-sectional survey and prepared a UNFPA report on "Knowledge, Attitude and Practices of Syrian Refugees on Family Planning"; PI: Issam Shaarani, MD, MPH, Assistant Professor at the Faculty of Medicine, Medical Director of the Healthcare Center
- Mentored and trained medical students on research skills and statistical analysis

American University of Beirut, Beirut, Lebanon **May - Aug. 2017**
Project Coordinator

- Planned and launched a project on the components and benefits of the Lebanese Mediterranean diet in coordination with the Food and Agriculture Organization; PI: Nahla Hwalla, PhD, RD, Professor and Former Dean of the Faculty of Agriculture and Food Science
- Authored a report on prevalence, characteristics and disease burden of nutrition transition in the Middle East used in the ongoing project "Towards the enhancement of the Mediterranean diet in the Mediterranean region"

American University of Beirut, Beirut, Lebanon **Sep. 2015 - Dec. 2016**
Graduate and Research Assistant

- Authored a scoping review of medical databases on gender equity in planning, development and management of human resources for health[4]
- Conducted interviews with physicians and explored their perceptions of female surgeons in Lebanon
- Coordinated with "Kafa" NGO to develop a policy brief on domestic violence in Lebanon

American University of Beirut, Beirut, Lebanon **Dec. 2014 - Sep. 2015**
Research Consultant

- Interviewed caregivers to collect data on food security, dietary intake and physical activity as part of a national study on childhood obesity in Lebanon
- Examined dietary data from food frequency questionnaires and 24-hour recalls
- Trained 5+ consultants on field work and data entry on NutriPro, SPSS, and Microsoft Access

PUBLICATIONS, PRESENTATIONS, & POSTERS

Peer-reviewed publications

1. Forman MR, **Chehab RF.** A Glimmer of Hope for Medically Indicated Preterm Delivery. J Womens Health (Larchmt). 2021 Feb 11. doi: 10.1089/jwh.2021.0004. Epub ahead of print. PMID: 33577393.

2. **Chehab RF,** Nasreddine L, Forman MR. Determinants of nutritional status during the first 1000 days of life in Lebanon: Sex of the child matters. Paediatr Perinat Epidemiol. 2021 Jan 11. doi: 10.1111/ppe.12747. Epub ahead of print. PMID: 33428236. [2]

3. **Chehab RF,** Cross TL, Forman MR. The Gut Microbiota: A Promising Target in the Relation between Complementary Feeding and Child Undernutrition. Adv Nutr. 2020 Nov 19:nmaa146. doi: 10.1093/advances/nmaa146. Epub ahead of print. PMID: 33216115.[1]

4. El Sayed Ahmad R, Baroudi M, Shatila H, Nasreddine L, Chokor FAZ, **Chehab RF**, Forman MR, Naja F. Validity and Reproducibility of a Culture-Specific Food Frequency Questionnaire in Lebanon. Nutrients. 2020 Oct 29;12(11):E3316. doi: 10.3390/nu12113316. PMID: 33137973.

5. **Chehab RF**, Nasreddine L, Zgheib R, Forman MR. Exclusive breastfeeding during the 40-day rest period and at six months in Lebanon: a cross-sectional study. Int Breastfeed J. 2020 May 19;15(1):45. doi: 10.1186/s13006-020-00289-6. PMID: 32430076.[3]

6. El Arnaout N, **Chehab RF**, Rafii B, Alameddine M. Gender equity in planning, development and management of human resources for health: a scoping review. Hum Resour Health. 2019 Jul 11;17(1):52. doi: 10.1186/s12960-019-0391-3. PMID: 31296235.[4]

7. Kharroubi S, **Chehab RF**, El-Baba C, Alameddine M, Naja F. Understanding CAM Use in Lebanon: Findings from a National Survey. Evid Based Complement Alternat Med. 2018 Jul 25;2018:4169159. doi: 10.1155/2018/4169159. PMID: 30147730.

8. Makhoul J, **Chehab RF**, Shaito Z, Sibai AM. A scoping review of reporting 'Ethical Research Practices' in research conducted among refugees and war-affected populations in the Arab world. BMC Med Ethics. 2018 May 15;19(1):36. doi: 10.1186/s12910-018-0277-2. PMID: 29764456.

Publications in preparation

1. **Chehab RF**, Moossavi S, Fehr K, Azad M, Subbari PJ, Moraes T, Mandhane P, de Souza RF, Turvey S, Khafipour E, Forman MR. Maternal prenatal supplementation, but not dietary patterns, is associated with the breast milk microbiota composition in the CHILD cohort study.[1]
2. Shatila H*, **Chehab RF***, Baroudi M, Ahmad R, Forman MR, Abbas N, MoezAlIslam F, Naja F. Impact of Ramadan on dietary intakes among fasting healthy adults in Lebanon: A year-round comparative study.
3. **Chehab RF***, Shatila H*, Forman MR, Naja F. Development of a socioeconomic status score in Lebanon.

*Authors with equal contribution to the manuscript.

Oral presentations

1. **NUTRITION LIVE ONLINE,** American Nutrition Society **Jun. 2021**
   Maternal Prenatal Supplement Intake, but Not Dietary Patterns, Is Associated with Human Milk Microbiota Composition in the CHILD Cohort Study[1]
2. **Office of Interdisciplinary Graduate Programs, Purdue University,** Online **May 2021**
   Prenatal Supplement Intake is Associated with the Breast Milk Microbiome[1]
3. **American College of Epidemiology,** Online **Sept. 2020**
   Nutritional Status Determinants during the First 1000 Days of Life in Lebanon[2]

Posters

1. **American Society of Nutrition,** LIVE Online **Jun. 2020**
   Child Growth during the First 1000 Days of Life in Lebanon: Sex of the Child Matters[2]
2. **Health and Disease Poster Session,** Purdue University **Mar. 2020**
   Nutritional status determinants during the first 1000 days in Lebanon: A hierarchical regression analysis[2]
3. **Women's Health Symposium,** Purdue University **Nov. 2019**
   Exclusive breastfeeding during the 40-day rest period and 6 months in Lebanon[3]
4. **American Society of Nutrition,** Baltimore, Maryland **Jun. 2019**
   Exclusive breastfeeding during the 40-day rest period and 6 months in Lebanon[3]
5. **Office of Interdisciplinary Graduate Programs 2019 Spring Reception** **May 2019**
   C-section delivery is a barrier to and demographic-maternal-child factors have mixed effects on the length of exclusive breastfeeding in Lebanon[3]

TEACHING EXPERIENCE

Purdue University, West Lafayette, IN, USA
Teaching Assistant

1. NUTR 424: Communication Techniques in Food and Nutrition **Aug. - Dec. 2020**
   Instructor of record: Marie Allsopp, DrPH, RD, LD, CHES, Clinical Assistant Professor
   - Organized weekly modules of course lectures, assignments, and announcements on Brightspace
   - Prepared grading sheets and graded assignments and video presentations

2. NUTR 107: Introduction to Nutrition Science **Aug. - Dec. 2018 & 2019**
Instructors of record: Michele Forman, PhD, FACE, FASN, Formerly Chair and Distinguished Professor; Laura Bowers, PhD, RD, Assistant Professor
    - Graded and provided feedback on assignments

International College, Beirut, Lebanon **Oct. 2011 - Jun. 2017**
Private Tutor
- Tutored 2000+ hours of Science, Math and Literature subjects to high school students

CLINICAL EXPERIENCE

Private Clinic, Beirut, Lebanon **Jul. 2014 - Aug. 2017**
Licensed Dietitian
- Provided comprehensive nutrition counseling tailored to the patients' health conditions

American University of Beirut Medical Center **Jul. - Oct. 2014**
Clinical Dietitian
- Assessed patients' nutrition status, devised meal plans, and provided nutritional care and education
- Executed a "Nutrition Screening and Collaborative Care Audit" in different hospital units

INTERNSHIPS

Practicum Intern, Save the Children, Beirut, Lebanon **Jan. - May 2017**
- Advisor: Jihad Makhoul, Dr.PH, MPH, Professor, Chairperson of Department of Health Promotion & Community Health, American University of Beirut
- Project: Hygiene Promotion Interventions: A Community Participatory Approach to Evaluate their Relevance and Impact

Dietetic Intern, American University of Beirut Medical Center, Beirut, Lebanon **Aug. 2013 - Jun. 2014**
- Assessed nutrition status of patients and tailored medical nutrition therapy accordingly
- Developed patient educational materials on pregnancy and kidney dialysis
- Trained 25+ food service employees on food safety and diet restrictions

Project Assistant and Dietetic Intern, Diet Center, Beirut, Lebanon **Jun. - Aug. 2013**
- Prepared a report on components and benefits of the Lebanese Mediterranean Diet as part of a project funded by the European Union
- Developed interactive activities for school children on the Mediterranean Diet
- Assessed patients' body composition and planned weight loss diets accordingly

ONLINE TRAINING

- **Life Course Nutrition: Maternal and Child Health Strategies in Public Health** **May 2020**
  by University of Washington, Seattle, WA
- **Breast Milk Scientific,** by Microbirth School **Feb. 2020**
  featuring 7 professors including Dr. Meghan Azad, Dr. Lars Bode, and Dr. Gregor Reid
- **Maternal and Infant Nutrition in the First 1000 Days** **Aug. 2019**
  by Kathleen Rasmussen, ScD, RD, The Nancy Schlegel Meinig Professor of Maternal and Child Nutrition, Cornell University

SCHOLARSHIPS

- **Evelyn Enrione Graduate Scholarship,** Dept. of Nutrition Science, Purdue University **2020**
  $1000 awarded to registered dietitians pursuing a doctoral degree in nutrition science
- **Mary E Fuqua Graduate Scholarship,** Dept. of Nutrition Science, Purdue University **2019**
  $300 awarded to graduate students conducting research in nutrition science
- **Rickard Family Graduate Scholarship,** Dept. of Nutrition Science, Purdue University **2018**
  $4000 awarded to graduate students conducting research related to infant and childhood nutrition
- **Lynn Fellowship,** Graduate School, Purdue University **2017 - 2018**
  $17,645 administered to PhD students admitted to an interdisciplinary program
- **Mary E Fuqua Graduate Scholarship,** Dept. of Nutrition Science, Purdue University **2017 - 2018**
  $2655 awarded to graduate students conducting research in nutrition science
- **Graduate Assistantship,** American University of Beirut, Lebanon **2015 - 2016**
  100% tuition fee remission for students with excellent academic achievement
- **Merit Scholarship,** Hariri Foundation for Sustainable Human Development, Beirut **2010 - 2012**
  25% tuition fee remission
- **Merit Scholarship,** Hariri High School II, Beirut, Lebanon **2007 - 2010**
  25% tuition fee remission

AWARDS

- **Winner of Three-Minute Thesis Competition[1],** American Society for Nutrition **Jun. 2021**
- **Robert Suskind and Leslie Lewinter-Suskind Pediatric Nutrition Student Award[1],** American Society for Nutrition **May 2021**
- **Emerging Leaders in Nutrition Science Abstract Recognition Award[1],** American Society for Nutrition **Apr. 2021**
- **People's Choice Award[1],** Three-Minute Thesis Competition, Purdue University **Apr. 2021**
- **Travel Award, Purdue Graduate Student Government** **Mar. 2021**
- **Trainee Award for highest ranking abstract[2],** American College of Epidemiology **Sept. 2020**
- **Academic Achievement Award,** Faculty of Health Sciences, AUB **May 2017**
- **Dean's Honor List,** Faculty of Agriculture & Food Science, AUB **2010 - 2014**
- **Excellent Academic Achievement in Lebanese Official Exams,** Hariri Foundation for Sustainable Human Development, Lebanon **2007 & 2010**

PROFESSIONAL SERVICE

- Journal of Nutrition, Scientific Peer Reviewer **Mar. 2021 - present**
- Advances in Nutrition, Scientific Peer Reviewer **Jan. 2021 - present**

PROFESSIONAL ASSOCIATIONS

American Society of Nutrition

- **Community and Public Health Research Interest Section** **Feb. 2021 - present**
  Graduate Student Representative

- **Nutrition Education Behavioral Science Research Interest Section** **Oct. 2020 - present**
  Graduate Student Representative

- **Student Interest Group** **July 2020 - present**
  At-Large Delegate

Purdue University

- **Purdue University Nutrition Science Alumni Network (PUNSAN)** **May 2020 - present**
  Graduate Student Representative

- **Purdue Graduate Student Government (PGSG)**
  Symposium and Graduate Student Organization Grant Allocation Vice Chair **May 2020 - present**
  Student Senator representing the Department of Nutrition Science **Aug. 2019 - present**

- **Nutrition Science Graduate Student Organization (NSGSO)**
  Career Advocate **Apr. 2020 - present**
  First Year Advocate **Mar. 2018 - Mar. 2019**

- **Lebanese International Organization (LIO)** **Mar. 2019 - present**
  President

Other associations

- **Western Indiana Academy of Nutrition and Dietetics (WIAND)** **May 2019 - present**
  Treasurer

- **INJAZ El-Arab,** Beirut, Lebanon **2014 - 2015**
  Mentor

Membership in Societies

- **The International Society for Research in Human Milk and Lactation** **Jun. 2020 - present**
- **Society for Nutrition Education and Behavior (SNEB)** **May 2020 - present**
- **American Society for Nutrition (ASN)** **Mar. 2018 - present**
- **Health Science Student Society,** American University of Beirut, Lebanon **2015 - 2017**
- **Nutrition Society,** American University of Beirut, Lebanon **2011 – 2012**

REFERENCES

Available upon request