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The Effect of a Maternal Dairy-Free Lebanese Mediterranean Diet on Colic and Allergy Symptoms in Exclusively Breastfed Infants

By

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Dedication Page

This thesis is dedicated to my parents and brother whose love and support knows no bounds.

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ABSTRACT

The origins of infantile colic are yet to be confirmed, however, multiple mechanisms have been proposed including cow's milk protein allergy and presence of gut inflammation. The Mediterranean diet has numerous protective roles in multiple diseases, including its protective role observed in inflammatory and allergic diseases. In this study, we compared healthy exclusively breastfed infants whose mothers were either on their own typical diet (n=10) or on a dairy free Lebanese Mediterranean diet (LMD) (n=7). Mothers were recruited during pregnancy and assessed at weeks 2, 4 and 7 postpartum. Infant allergy and colic symptoms and behavior, including crying duration, were assessed using a questionnaire. Infant fecal sample were collected, and concentrations of eosinophil cationic protein (ECP) and pH were determined. Differences between the two groups were analyzed using the two sample independent t test and Mann-Whitney test depending on distribution of the variable while fisher's exact test was used to evaluate the difference in categorical variables. Mothers in the intervention group scored higher on the dairy free LMD index (p=0.05). Daily consumption of dairy was lower for mothers on a dairy free LMD (p=0.01) while that of the remaining food groups was not statistically different. Infant fecal profile (ECP, p=0.971; pH, p=0.665), allergy and colic symptoms (p=0.447) were not statistically different between the two groups, but a trend towards lower crying duration in the second week was observed (p= 0.174). Although adherence to a dairy free LMD in exclusively breastfeeding mothers did not show substantial effects on infant allergy or colic symptoms, it is difficult to draw conclusions due to our small sample size. Further investigating the effects of maternal adherence to a LMD in exclusively breastfed infants is needed.

Keywords: Lebanese Mediterranean Diet, Cow's Milk, Allergen, Infantile Colic, Allergy, Breastfeeding, Maternal Diet, Inflammation, Crying, Eosinophil Cationic Protein

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LIST OF ABBREVIATIONS

DHA: docosahexaenoic acid

FODMAP: fermentable oligo-, di-, mono-saccharides and polyols

IgE: immunoglobulin E

IgA: immunoglobulin A

FPIAP: food protein-induced allergic proctocolitis

LMD: Lebanese Mediterranean Diet

ECP: Eosinophil Cationic Protein

ELISA: enzyme-linked immunosorbent assay (ELISA)

BMI: body mass index

HPR: horseradish peroxidase

Chapter One

Introduction

Infantile colic is a common problem that impacts early infancy with symptoms including excessive crying, fussiness, and irritability, as defined by the "Wessel Criteria", and more recently by Rome IV criteria for infantile colic. "Wessel Criteria" defines infantile colic simply as crying episodes that last for >3 hours per day, for ≥3 days per week and for ≥3 weeks, while the Rome IV criteria defines infantile colic as infant fussing or crying ≥ 3 hours per day during ≥ 3 days in 7 days as well as several other factors (Hyman et al., 2006, Okan, Gunduz, Okur, Akgun, & Esin, 2015, Benninga et al., 2016 & Iacovou et al., 2017). Multiple mechanisms have been proposed in the etiology and pathophysiology of infantile colic, including intestinal inflammation, increased intestinal permeability, excessive intestinal gas, and food protein allergy, including cow's milk protein allergy and many others (Camilleri et al., 2017; Gordon et al., 2018). In some infants where excessive crying, fussiness and irritability are encountered, food allergens may play a role. Cow's milk and soy protein are found to be the most common food allergens, in specific bovine immunoglobulin G and β-Lactoglobulin, which are found in breastmilk after maternal consumption of cow's milk. These allergens may result in an immunological response and disturbances in gastrointestinal tract leading to symptoms of infantile colic (Hiscock & Jordan 2004, Ghosh & Barr, 2007 Camilleri et al., 2017 & Gordon et al., 2018). Multiple studies have assessed different maternal diets during lactation in order to reduce symptoms of colic in infants. Although more research is needed to draw a conclusion, some maternal diets such as a diet low in multiple allergens, a diet free of cow's milk, and a low FODMAP diet have shown to improve symptoms in colicky infants (Gordon et al., 2018). The Mediterranean diet is a well-known traditional and healthy diet derived from Mediterranean countries. It includes a high consumption of vegetables, fruits and plant-based foods such as legumes, seeds, nuts, and whole grains. This diet has numerous health benefits including improving mental health, decreasing cancer risk, as well as protecting against cardiovascular diseases, hypertension, diabetes and the metabolic syndrome (Gotsis et al., 2015).

Through numerous observational studies, the Mediterranean diet has also been found to have a protective role in allergic diseases, mediated by its antioxidant and anti-inflammatory properties (De Batlle et al., 2008 & Sewell, et al., 2017). Studies have assessed adherence to the Mediterranean diet prenatally and during lactation in mothers and the subsequent infant outcomes during childhood, as well as adherence during childhood and adulthood. Such studies have shown that a higher adherence to the traditional Mediterranean diet may play a role in chronic inflammatory processes and allergies by decreasing the risk or symptoms, particularly in the case of asthma (Nurmatov, Devereux, & Sheikh, 2011 & Sewell, et al., 2017). However, the evidence is still inconclusive, and more research is needed (Chatzi et al., 2008, De Batlle et al., 2008, Barros et al., 2008 & Sewell, et al., 2017). The Lebanese Mediterranean diet is a diet native to the country of Lebanon and has specific cultural aspects. When compared to other Mediterranean diets from European countries, the Lebanese Mediterranean diet included more burghul, dried fruits and eggs but did not include fish as an essential component. Benefits of the Lebanese Mediterranean diet have been documented. Higher adherence to the Lebanese dietary pattern decreased risk of type 2 diabetes, while overweight and obese individuals who followed this pattern were more likely to be metabolically healthy (Naja et al., 2012, Naja, et al., 2014 & Matta et al., 2016). Despite its anti-inflammatory properties, no study, to our knowledge, have examined the relationship between maternal adherence to a Lebanese Mediterranean diet and colic and allergy symptoms in exclusively breastfed infants. Additionally, and as stated above, maternal diet, specifically in regard to the intake of cow's milk during breastfeeding, may contribute to allergy and colic symptoms in exclusively breastfed infants via an immune and inflammatory response. Thus, this study aims to test the effect of maternal adherence to dairy-free Lebanese Mediterranean diet on colic and allergy symptoms in exclusively breastfed infants.

Chapter Two

Literature review

2.1- Importance and Benefits of Breastfeeding

According to the World Health Organization, it is recommended to exclusively breastfeed infants for up to 6 months and to continue breastfeeding thereafter alongside appropriate complementary feeding until the infant is 2 years of age or more (WHO, 2003). The importance of breastfeeding is well recognized due to its association with many short- and long-term health benefits for the infant, as well as the mother. Breastfeeding decreases the risk of infant morbidity and mortality as reported in a systemic review conducted by Horta & Victora (2013) which found that breastfeeding reduces the risk of hospital admission for respiratory infections and diarrhea in children by 57% and 72%, respectively. In children under the age of 2 years, breastfeeding also reduces the risk of developing otitis media (Victora et al., 2016). Moreover, breastfeeding is strongly associated with sudden infant syndrome, reducing its occurrence by 36% (Chung et al., 2007). In addition, studies have shown that breastfeeding provides health benefits in the long term as well, including decreasing the odds of type 2 diabetes and overweight/obesity, a positive effect on cognition and an enhanced performance on intelligence tests in childhood and adolescence (Horta, Loret de Mola, & Victora, 2015a, 2015b). A meta-analysis that included 113 studies found that longer durations of breastfeeding were negatively associated with overweight or obesity, reducing the risk by 26% (Victora et al., 2016). Breastfeeding was also found to protect against childhood cancer, whereby breastfeeding for 6 months or longer was found to reduce the risk for childhood leukemia by 20% (Amitay, & Keinan-Boker, 2015). These benefits stem from bioactive compounds found in human milk, as well as a human milk composition that evolves with the age of the infant in order to meet the needs of his/her development and growth. Human milk begins as colostrum, and changes to transitional and then mature milk. Colostrum, the first milk produced after birth, contains a higher concentration of protein, immunoglobulins and a lower concentration of lactose and fat when compared to mature milk. In addition, colostrum is found to have a high concentration of antioxidants, oligosaccharides and bioactive compounds such as lactoferrin, secretory immunoglobulin (IgA) and leukocytes, indicating colostrum's essential role in immunity. Starting day 6 postpartum colostrum is progressively replaced by transitional milk, which is similar to colostrum in some features. By the second week postpartum, human milk is mostly mature and becomes fully mature as of the 4^{th} to the 6^{th} week postpartum (Ballard, & Morrow, 2013; Mosca & Giannì, 2017).

2.2- Factors Affecting Breastmilk Composition

Multiple factors may affect human milk composition for each woman, including genetics, geography, ethnicity, lifestyle, body composition and diet (Geddes & Perrella, 2019). When comparing African and Italian women's milk composition, African women had higher concentrations of immune active molecules suggesting that the environment plays a role in human milk composition to further protect an infant from infection (Munblit et al., 2018). In a systemic review conducted by Amaral et al. (2019), human milk of mothers who were overweight pre-pregnancy had a higher omega 6 to omega 3 ratio and increased concentration of omega 6 compared to eutrophic women of normal weight range. Other factors also play a role in human milk polyunsaturated fatty acid composition such as smoking, gestational weight gain, maternal age, supplementation of omega 3 and food consumption (Jørgensen et al., 2001, Mäkelä et al., 2013, Keikha et al., 2017 & Moro et al., 2019). Maternal body composition also affects the amount of saturated fatty acids in human milk. The quantity of saturated fatty acids in human milk is found to be higher in overweight women compared to normal weight women even after adjusting for maternal diet (Mäkelä et al., 2013). Studies examining the effect of maternal diet on human milk composition and infant development are scarce. It has been found that maternal diet mainly affects fat composition, while protein and carbohydrates may not be affected. Furthermore, long chain polyunsaturated fatty acids such as linoleic acid and docosahexaenoic acid (DHA) in human milk are largely derived from maternal dietary intake. Therefore, it is recommended that the mother consumes a minimum of 200 mg/day of DHA to supply 100 mg of DHA to an exclusively breastfed infant, for his/her metabolic requirements (Mosca & Giannì, 2017; Keikha et al., 2017; Jørgensen et al., 2001). Additionally, many studies have assessed the maternal dietary contribution to certain micronutrients' concentration in human milk (Moro et al., 2019). In particular, the content of vitamin B1, vitamin C, fat soluble vitamins, and other micronutrients in human milk composition was related to maternal dietary intake (Keikha et al., 2017). In another study, human milk retinol was significantly correlated with the frequency of vitamin A rich food consumption, in particular sweet potatoes (Ettyang et al., 2004). Maternal dietary intake of copper and calcium is also associated with human milk content (Keikha et al., 2017). Furthermore, maternal consumption of eggs affects human milk composition as it increases ovalbumin levels (Keikha et al., 2017).

2.3- Infantile Colic

Infantile colic is a problem that may appear in early infancy, characterized by excessive unexplained crying, fussiness and irritability. As defined by the "Wessel Criteria", infantile colic is an excess of crying episodes that last for >3 hours per day, for ≥3 days per week and for ≥3 weeks (Iacovou et al., 2012). In 2006 the Rome III Criteria for infantile colic was published and was defined as crying for >3 hours per day, for >3 days per week, for a minimum of 1 week, with fits of crying, irritability, or fussing that begin and end without an apparent reason and without failure to thrive (Hyman et al., 2006). However, in 2016, the Rome III Criteria was revised and updated to the Rome IV consensus, which differentiates the diagnostic criteria of infantile colic for research and clinical purposes. In regards to clinical purposes, the infant must have all of the following features: symptoms must begin and end in the time when the infant is less than 5 months of age, caregivers reporting recurring and persistent episodes of infant crying, irritability, or fussing that take place without an apparent reason and cannot be resolved or prevented and no failure to thrive, illness or fever are present in the infant (Zeevenhooven et al., 2017). For research purposes, a diagnosing infantile colic must include all of the previous criteria mentioned as well as caregiver reporting through a face-to-face screening interview or telephone with a clinician or researcher. The caregiver should indicate that the infant has fussed or cried ≥ 3 hours per day during ≥ 3 days in 7 days and a minimum of one, prospectively kept, 24-hour behavior diary measuring and confirming crying and fussing to have occurred for 3 hours or more (Zeevenhooven et al., 2017). Excessive crying reportedly ranges from around 14% to 30% in infants up to 3 months old, and colic symptoms typically arise at 6 to 8 weeks postpartum and naturally resolve by the 3rd to 4th month postpartum (Iacovou et al., 2012, Okan, Gunduz, Okur, Akgun, & Esin, 2015, Benninga et al., 2016 & Iacovou et al., 2017). The exact cause of infantile colic is still poorly understood. Multiple causes, risk factors and pathophysiological mechanisms have been proposed. These include intestinal cramping, gut inflammation, increased intestinal permeability, excessive intestinal gas, milk protein allergy, changes in gastrointestinal microbiota, and an increase in gut hormones such as motilin and ghrelin leading to colonic hyperperistalsis. Also being a first born child, maternal smoking and increasing age, as well as maternal fatigue and a stressful pregnancy increase the risk of infantile colic (Savino et al., 2006; Savino, 2007; Kurth et al., 2011; Camilleri et al., 2017 & Daelemans et al., 2018). Furthermore, an infant swallowing air has been proposed as another contributing factor. Neurodevelopmental factors have also been proposed in the pathophysiological mechanisms underlying infantile colic, specifically that the endogenous opioid system is altered in infants with colic, as they have an increased release of excitatory neurotransmitters (Zeevenhooven et al., 2018). Intestinal microbial patterns and inflammation have also been associated with infantile colic. Studies have shown that the gut microbial composition in infants with colic are different from those without colic in regard to microbial stability, diversity and patterns in colonization. Infants with colic were found to have decreased levels of lactobacilli, bifidobacteria and butyrate producing species, as well as species with anti-inflammatory properties, and increased levels of Escherichia Coli and Klebsiella species, in addition to proteobacteria species that produce inflammation and gas (Daelemans et al., 2018). This intestinal dysbiosis present in infants with colic has been hypothesized to play a role in the manifestation of symptoms in infantile colic. The microbiota–gut–brain axis connects the brain with peripheral intestinal functionalities and this connection is involved in multiple pathways such as immune, neural and endocrine signaling pathways, affecting detection of pain and leading to excessive crying in such infants (Zeevenhooven et al., 2018). Furthermore, intestinal dysbiosis may cause an increase in gut extension and gas production, as it causes an increase in fermentation of proteins, carbohydrates, and lactose (Zeevenhooven et al., 2018). Another factor that was proposed to play a role in infantile colic is gut permeability. For all infants, the start of life begins with an inadequate gut integrity and immature intestinal mucosa for a few months after birth, which allows large molecules to pass into the blood. However, for infants with colic, human milk α-lactalbumin transfer is increased across the gut in comparison to healthy infants (Daelemans et al., 2018). This elevated gut permeability may increase gut, systemic and mucosal low-grade inflammation (Zeevenhooven et al., 2018). A study conducted by Pärtty et al., in 2017 found that infants with colic have high levels of Monocyte Chemoattractant Protein-1, macrophage inflammatory protein 1b and Interleukin 8, indicating that there is an association between infantile colic and low-grade systemic inflammation. In addition, major constituents found in the healthy gut microbiota, C leptum and C coccoides, were found to be negatively associated with proinflammatory biomarkers, which supports a proposition that colic in infants is an inflammatory gastrointestinal condition associated with intestinal dysbiosis (Pärtty et al., in 2017). Still, it has not yet been confirmed whether the change seen in the intestinal microbiota composition of an infant with colic is the consequence or the cause of an inflammatory response.

Certain dietary factors have been implicated in the pathophysiology of infantile colic. Cow's milk protein intolerance is a proposed cause of colic in infants as an allergic reaction may occur following the introduction of these proteins (Savino, 2007; Zeevenhooven et al., 2018). Carbohydrates that are not absorbed completely in the small intestine lead to bacterial fermentation resulting in excessive intestinal gas and subsequent potential colic, given the intestinal immaturity in infants (Zeevenhooven et al., 2018). Moreover, lactose intolerance has been hypothesized to play a role in infantile colic as enzyme insufficiency leads to malabsorption and subsequent fermentation, and by 3 months of age there is an increase in the expression of lactase enzyme in the gut, which is around the same time infantile colic usually resolves (Savino, 2007; Zeevenhooven et al., 2018).

2.4- Maternal Diet and Infantile Colic

For some colicky, fussy or irritated infants, food allergens may be the culprit. In exclusively breastfed infants, allergens may be transmitted from the mother via the breast milk. The food allergens that are more commonly reported in the literature include cow's milk and soy protein (Hiscock & Jordan 2004). In specific, the most potentially antigenic proteins, casein, bovine immunoglobulin G and β-Lactoglobulin, pass on to the breastmilk when the mother has consumed cow's milk (Ghosh & Barr, 2007). Such antigenic proteins may cause the infant's intestinal mucosa to produce immunological responses and disturbances in gastrointestinal motility leading to colic (Gupta, 2002, Heine, 2006 & Gordon et al., 2018). Various maternal diets have been of interest, in order to decrease these symptoms. In one study (Oggero, Garbo, Savino, & Mostert, 1994), researchers compared dietary intervention to pharmacological intervention in infants with colic. The dietary intervention consisted of soymilk or hydrolyzed milk formula for bottle-fed infants and a maternal diet free of cow's milk and its products, fish and eggs for breastfed infants. Results showed that dietary modifications provided more reduction in symptoms than pharmacological treatment (Oggero, et al., 1994). Another study found a high correlation between infantile colic in infants who are breastfed and their mothers' intake of cow's milk protein and that the elimination of cow's milk from the diet of breastfeeding mothers reduced colic symptoms. (Jakobsson & Lindberg, 1983). In 2005, a randomized control trial conducted by Hill et al. reported that a low allergen diet for breastfeeding mothers for 1 week resulted in a significant decrease in the cry and fuss duration in their colicky infants. The low allergen maternal diet eliminated the following foods: soy, wheat, eggs, peanuts, fish, tree nut and dairy products. Another study by Iacovou et al. (2018) compared the effect of a low FODMAP diet to that of a typical Australian diet in breastfeeding mothers on cry-fuss durations in infants with colic. The crying-fussing time's median change was found to be 32% with the low FODMAP diet, in comparison to 20% for the typical Australian diet. In addition, an observational study indicated a possible association between colic symptoms in infants and cow's milk, cruciferous vegetables, chocolate, and onion in the maternal diet (Lust et al., 1996).

2.5- Allergies

There have been many attempts at preventing food allergies throughout the years, and despite this, food allergy in children continues to rise, specifically immunoglobulin E mediated food allergies in westernized countries (Lack, 2012). This has become a global public health concern as sensitization rates to allergens amongst school children are approaching up to 50% globally (Fujimura et al., 2019). The National Institute of Allergy and Infectious Diseases, along with multiple professional organizations, patient advocacy groups, and federal agencies published a report titled “Guidelines for the Diagnosis and Management of Food Allergy in the United States”. According to the report, food allergens are defined as “specific components of food or ingredients within food that are recognized by allergen-specific immune cells and elicit specific immunologic reactions, resulting in characteristic symptoms”. On the other hand, a food allergy is defined as “an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food”. Such a reaction to food from a food allergen derives from an immunological response and can include non-immunoglobulin E (IgE), IgE and combined IgE mediated allergy pathways. In IgE-mediated allergies, symptoms typically arise shortly after exposure, within 1 to 2 hours. For non–IgE-mediated food allergies, symptoms usually develop at a later stage, within multiple hours or even after several days of consuming the allergen (Meyer et al., 2020). Adverse reaction to food may also occur as non-immune mediated reactions or food intolerances through metabolic (such as lactose intolerance), toxic, pharmacologic and idiopathic mechanisms, which may elicit similar reactions as an immunologic response (Brill, 2008). The commonly seen food allergens in a diet are milk, soy, eggs, wheat, tree nuts, fish and crustacean shellfish, with cow’s milk being the most common allergen (Meyer et al., 2020). The food proteins in wheat, soya, eggs and cow’s milk consumed by the mother are found in breastmilk and remain there for hours to days after maternal consumption and for some infants this may elicit an allergic response (Meyer et al., 2020). As symptoms of non-IgE-mediated food allergies may take days to develop, the diagnosis should be based on these symptoms and signs as they occur with the consumption of the food allergen, the disappearance of the symptoms with food allergen avoidance and very often, the presence of an immunologically mediated process, such as eosinophilic inflammation in the GI tract (Panel, 2010). Symptoms of a food allergy may range from mild to severe, some of which are reflux, vomiting, blood and mucus in stool, abdominal pain, diarrhea, failure to thrive, itching or tingling of the tongue, roof of the mouth, lips and throat, acute urticarial, itching and eczematous flares. Infants with continuous irritability and reflux, especially if accompanied by atopic symptoms should consider a non-IgE mediated food allergy as the cause (Meyer et al., 2020). In regards to a milk protein allergy, it can occur as an IgE-mediated (milk antigens bind to IgE antibodies releasing the inflammatory mediator histamine from the mast cell) and a non–IgE-mediated allergy response (IgG or IgA antibodies immune complexes bound to milk antigens that directly stimulate T cells resulting in cytokine release and elevated antibody production) (Brill, 2008). Symptoms of an IgE mediated reaction to milk protein include rhinoconjunctivitis, asthma, laryngeal edema, otitis media, atopic dermatitis, urticaria, angioedema, nausea, vomiting, colic, and diarrhea (Brill, 2008). As for the symptoms of a non–IgE-mediated reaction, they include pulmonary hemosiderosis, atopic dermatitis, contact rash, failure to thrive, gastroesophageal reflux, protein-losing and transient enteropathy, colitis, constipation, and enterocolitis syndrome. Other reactions, which are not specific to either IgE-mediated or a non–IgE-mediated reactions to milk protein include anemia, arthritis, Henoch-Schönlein purpura and migraine (Brill, 2008). It is essential to distinguish between IgE-mediated and a non–IgE-mediated allergies as infants with IgE-mediated milk protein allergy may have an increased risk of developing other food allergies and atopic disorders as they grow older (Brill, 2008).

2.6- Maternal Diet and Allergies in Infants

In children, eczema prevalence varies from 10% to 30% in developed countries and approximately 16% in children in the first year of their life. Around one-third of infants with moderate to severe eczema may be diagnosed with IgE mediated food allergy. For breastfed infants, allergens in the maternal diet that are transferred to human milk, including cow's milk protein, aggravate and lead to atopic dermatitis flares (Rajani et al., 2020). Various researchers have described that the maternal dietary avoidance of common food allergens led to the reduction of atopic dermatitis development in breastfed infants (Cant et al., 1986; Chandra et al., 1986; Uenishi et al., 2011; Rajani et al., 2020). In a study comparing a food allergen avoidance diet to an unrestricted diet in a mother with a previous child with atopic illness, it was found that maternal dietary avoidance of food allergens was associated with lesser symptoms and lower incidence and of atopic eczema. This effect was particularly seen within the group of breastfed infants. The food allergen avoidance diet included an almost complete avoidance of milk and dairy products, fish, eggs, peanut and beef during both pregnancy and lactation (Chandra et al., 1986). A study conducted on 37 breastfed infants with eczema aged between 6 weeks and up to 6 months, examined how alterations in their mothers' diets impacted the infants skin condition. Eggs and cow milk were restricted from the diet for 2 weeks and then re-introduced. Following 2 trials, 46 % of infants displayed improvements in their eczema when their mothers where on the egg and milk restricted diet, and only six infants displayed improvements in symptoms of eczema during the restriction and after the foods were reintroduced. These results indicate that for a certain percentage of infants, food allergens in the maternal diet may lead to an allergic reaction in the infant (Cant et al., 1986). Another study conducted in 2011 by Uenishi et al. investigated the role of maternal dietary consumption of fermented foods such as yogurt, cheese, soy sauce, bread, fermented soy beans and miso soup, as well as tree nut related foods such as chocolate and coffee in 92 exclusively breastfed infants with atopic dermatitis. Around 73% of infants were found to show improvements in eczema symptoms when these foods were avoided, particularly yogurt, soy sauce, chocolate, and miso soup. In addition, it was noted that symptoms of eczema were further aggravated once these foods were reintroduced in the maternal diet, while long term maternal avoidance of foods that trigger an allergy led to continuous improvement of eczema symptoms in most of the infants (Uenishi et al., 2011). Although the number of studies are limited and are not based on general population, the research indicates that there is a role of food allergens, especially cow's milk protein, in inducing skin disorders in certain breast-fed infants through the maternal diet (Rajani et al., 2020). In addition, a common illness that occurs in breast-fed infants is food protein-induced allergic proctocolitis (FPIAP) and is characterized by inflammation of the distal colon as a response to certain food proteins. Symptoms include persistent vomiting, diarrhea, blood in stool and infantile colic. Up to 60% of FPIAP cases arise in exclusively breastfed infants and in many cases maternal dietary avoidance of soy and cow's milk protein resolves the issue (Nowak-Węgrzyn, 2015). Other foods such as egg, corn, and wheat have also been reported to trigger FPIAP in exclusively breastfed infants. It is hypothesized that the consumption of food allergen proteins through human milk leads to an inflammatory response in the colon. While food is the main trigger, FPIAP may also be caused by concomitant viral infections (Nowak-Węgrzyn, 2015; Erdem et al., 2017 & Rajani et al., 2020). In a retrospective study conducted by Erdem et al. (2017), on 77 infants with FPIAP, 53% of infants were being breastfed when symptoms of FPIAP appeared and it was found that 78% of these symptoms were due to a reaction to milk, 13% due to egg and cow's milk maternal consumption, and 5% due to egg consumption alone. A cohort study conducted by Kaya et al. (2015) assessed 60 infants with confirmed allergic proctocolitis. A total of 50 infants were exclusively breastfed, while the other 10 were fed both human milk and cow's milk formula. It was shown that 83.3% of the participants reacted to maternal intake of cow's milk, 6.6% to milk and egg, 3.3% to chicken and milk, and 1.7% to wheat and milk, while 3.3% has several food allergies. For breastfed infants with delayed symptoms (E.g. gastrointestinal symptoms and eczema) of food allergy it is particularly difficult to diagnose a food allergy and identify the allergen responsible. In contrast to the previously stated, there are studies that have also observed the opposite effect, where exposure to allergens has been seen to provide a protective effect against food allergies (Fujimura et al., 2019). For instance, in a nested cohort study conducted by Pitt et al., 2018, it was observed that children whose mothers had consumed peanuts during lactation and then introduced peanuts to the child before 12 months of age had the lowest incidence of peanut sensitization (1.7%). When comparing with this group, children whose mothers did not consume peanuts during lactation but who introduced peanuts to their infants before 12 months, had a significantly higher incidence of peanut sensitization (17.6%, OR: 12.45, 95% CI: 1.18-131.30). Furthermore, children also had significantly higher peanut sensitization when their mothers consumed peanuts during lactation but introduced peanuts to their infants after 12 months (15.6%, aOR: 8.30, 95% CI: 1.05-65.80). It has been proposed that this exposure to allergens reduces the risk of sensitization by development of tolerance through the priming of an infant's immune system (Pitt et al., 2018). Nevertheless, conclusions cannot be drawn regarding the role of breastfeeding in delaying or preventing the onset of food allergies in infants as further randomized controlled trials investigating maternal antigen avoidance are needed, in specific with longer follow up period and large sample sizes (Greer, Sicherer & Burks, 2019). Furthermore, at this time, there are no guidelines present in regard to the approach to be used for breastfed infants with symptoms related to a possible food allergy from human milk allergens derived from maternal diet (Rajani et al., 2020).

2.7- The Mediterranean Diet

The Mediterranean diet is a traditional, cultural and healthy diet based in Mediterranean countries. It consists of a high consumption of vegetables, fruits and plant-based foods such as legumes, seeds, nuts, and whole grains. It emphasizes the use of olive oil, the consumption of fish and a low consumption of processed and red meats (Sewell, et al., 2017). The benefits of the Mediterranean diet are numerous and have been well documented. These benefits include an increased life expectancy and lower risk of developing disorders including obesity, metabolic syndrome, diabetes, cancer, pulmonary diseases, chronic inflammation, atherosclerosis and cognitive disorders due to its antioxidant and anti-inflammatory properties (Gotsis et al., 2015 & Serra-Majem et al., 2019). The positive health benefits of the Mediterranean diet are not only restricted to adults, but have been observed in children, whereby its consumption has been associated with lower risks of childhood obesity, fatty liver, diabetes, allergies, asthma and attention deficit hyperactivity disorder, and with better academic performance and cardiorespiratory fitness (Mascarenhas, 2019). Mediterranean diet health benefits are due to some of the following mechanisms: 1)having a lipid lowering effect; 2)influencing metabolic health through intestinal microbiota mediated metabolite production; 3)modifying growth and hormonal factors related to the pathogenesis of cancer; 4) protecting against inflammation, oxidative stress and platelet aggregation; and 5) inhibiting nutrient sensing pathways such as mTOR and GCN2, given that mTOR inhibition may extend the life span (Tosti, Bertozzi, & Fontana, 2018). These benefits majorly derive from the combination of the diet's fatty acid composition (rich in monounsaturated and omega-3 fatty acids), low oxidative capacity and dietary antioxidant properties. This leads to enhanced molecular function and structure of plasma lipids and cellular membranes in a number of secretions and tissues (Moro et al., 2019). In line with this, a study which is yet to be completed by Moro et al. (2019) is assessing the role of a maternal Mediterranean diet on human milk properties. The study hypothesized that the breast milk of women adhering to the Mediterranean diet should contain increased anti-oxidative capacity, reduced levels of oxidative damage to human milk ingredients and a fatty acid profile which is rich in monounsaturated and omega-3 fatty acids. Studies, both observational and randomized controlled trials, have consistently shown that the Mediterranean diet and consumption of extra virgin olive oil have the potential to reduce low grade inflammation biomarker levels (Schwingshackl & Hoffmann, 2014; Schwingshackl, Christoph, & Hoffmann, 2015). In an observational study, the ATTICA study, the adherence to a traditional Mediterranean diet was found to be inversely associated with low grade inflammation biomarkers levels. In specific, those with the highest diet score had 17% lower interleukin-6 levels ($p=0.025$), 20% lower C reactive protein levels ($p=0.015$) and 14% lower white blood cell counts ($p=0.001$) compared with those with the lowest diet score (Chrysohoou et al., 2004). Similarly, in another study, an analysis nested within the Nurses' Health Study, observed reductions of 13% in e-selectin concentrations, 16% in interleukin-6 levels and 24% in C reactive protein levels when comparing the top to the bottom quintiles of the alternate Mediterranean Diet Index (Fung et al., 2005). Observational studies may be affected by residual confounding, however, similar results have been observed in clinical trials as well (Razquin & Martinez-Gonzalez, 2019). A multicenter randomized clinical trial assessing 772 adults with a high risk for cardiovascular disease compared a low-fat diet to two Mediterranean diet groups, one of which were given free nuts while the second group were given free virgin olive oil. Compared to the low-fat diet, interleukin-6 (OR: -1.6 ng/L; CI: -2.5 to -0.6 ng/L), adhesion molecules (OR: -178 ng/mL; CI: -277 to -79 ng/mL) and C reactive protein levels (OR: -0.54 mg/L; CI: -1.04 to -0.03 mg/L) were reduced in the Mediterranean diet. Nevertheless, C reactive protein levels decreased only in the Mediterranean diet group that was supplemented with olive oil, but not nuts

(Estruch et al., 2006). In a recent clinical trial, researchers sought to find out the effect of a Mediterranean diet with the addition of 40 g/day of extra virgin olive oil for a period of 3 months on overweight/obese participants and normal weight controls. Results indicated that markers of oxidative stress and inflammation significantly decreased in the case of both overweight/obese and normal weight subjects (Luisi et al., 2019).

2.8- The Mediterranean Diet and Allergic Diseases

Through observational studies, the Mediterranean diet has been shown to have a protective role in allergic diseases (Sewell, et al., 2017). This may be through fruits, vegetables and various antioxidants which are highly consumed in a Mediterranean diet. In regard to fruits and vegetables, some studies have found an inverse association between vegetable and fruit consumption and asthma symptoms (Kim et al., 2009). Antioxidants in the Mediterranean diet protect against oxidative damage in many areas, including the airways, and play a protective role in the immune system development throughout childhood (De Batlle et al., 2008). Prenatally, a mother's high adherence to a Mediterranean diet leads to high fetal exposure to antioxidant compounds which may decrease oxidative damage to lung tissue. Due to all these factors the Mediterranean diet may play a role in chronic inflammatory processes and allergies, including asthma (Chatzi et al., 2008 & De Batlle et al., 2008). Accordingly, the dietary inflammatory potential of a diet during pregnancy, be it a low dietary inflammation potential or a high dietary inflammation potential, may have an effect on offspring allergic diseases, particularly on asthma outcomes (Chen et al., 2020). This was furthermore elucidated in a recently published cohort study with a 10 year follow up of 862 mother-child pairs where a low-quality and pro-inflammatory maternal antenatal diet was found to be associated with a greater risk of offspring asthma (OR: 1.35; 95% CI: 1.10, 1.65) (Chen et al., 2020). The relationship between a Mediterranean diet supplemented with fatty fish and its resulting effect on inflammatory markers has been investigated in children with asthma (Papamichael et al., 2019). The 6-month randomized controlled trial resulted in a significant effect in the intervention group with a 14-unit decrease in bronchial inflammation. Authors concluded that a Mediterranean diet consisting of 2 fatty fish meals per week may be a possible approach in decreasing airway inflammation for children with asthma (Papamichael et al., 2019). Additionally, a cohort study conducted by Chatzi et al. in 2008 assessed 460 children after a 6.5 years follow up period and found that high level of maternal adherence to the Mediterranean diet during pregnancy is significantly related to a reduced risk of atopy and wheeze in childhood, even after adjusting for potential confounders. Consistently, a systematic review looking at nutrients and foods as primary prevention of allergy, found that the consumption of vitamins A, D, and E, zinc, fruits, vegetables and following the Mediterranean diet during pregnancy may be factors that reduce the risk of developing allergic diseases during childhood particularly in relation to asthma outcomes (Nurmatov, Devereux, & Sheikh, 2011). In line with this, the PANACEA study, a cross-sectional study was conducted on 700 children to assess association between childhood asthma and adherence to the Mediterranean diet. Results showed that a greater adherence to the Mediterranean diet was inversely associated with the prevalence of asthma, specifically with exercise wheeze ($p=0.004$), any wheeze ($p=0.001$), any asthma symptoms ($p<0.001$) and diagnosed asthma ($p=0.002$). In this cross- sectional study, the KIDMED score was used in order to assess adherence to the Mediterranean diet and one-unit increase in KIDMED score was associated with a reduced chance of having asthma symptoms by 14%, even after adjusting for potential cofounding factors (OR: 0.86, 95%, CI: 0.75–0.98) (Arvaniti al., 2011). Moreover, a cross-sectional study with 690 participants from ages 7 to 18 years examined the associations between rhinitis and wheeze and the adherence to the Mediterranean diet and consumption of vegetable and fruits. A questionnaire on allergic and respiratory symptoms, a food frequency questionnaire and a skin prick test were conducted. The results found that the intake of fresh tomatoes, oranges, grapes and apples were protective against rhinitis and wheezing, high intake of nuts was inversely associated with wheezing (OR: 0.46; 95% CI: 0.20 to 0.98) and a high level of adherence to a Mediterranean diet was significantly associated with decreased risk of allergic rhinitis during childhood (OR: 0.34; 95% CI: 0.18 to 0.64) (Chatzi et al., 2007). Benefits of the Mediterranean diet are seen in adults as well. A study assessing the association between the adherence to the Mediterranean diet and asthma control in 174 asthmatic adults with a mean age of 40 found that asthma is more likely to be under control in individuals who adhere to the traditional

Mediterranean diet. Participants with controlled asthma had a significantly higher Mediterranean diet scores and intake of fruits. Furthermore, even after adjusting for several cofounders a high adherence to the Mediterranean diet decreased the risk of uncontrolled asthma by 78% (Barros et al., 2008). To this date, no study, to our knowledge have examined the relationship between maternal adherence to a Lebanese Mediterranean diet during lactation and allergy symptoms in exclusively breastfed infants.

2.9- Lebanese Mediterranean Diet

As previously stated, definitions of Mediterranean diets mostly include vegetables, fruits and olive oil. However, there are substantial differences among various versions of the Mediterranean diet in relation to the consumption of fish, types of meats, the amount of oil or fats, the amount of alcohol, and the amount of sugar, as well as the types of desserts consumed. These differences mainly depend on the country of origin (Naja, et al., 2014). A study conducted by Naja et al., (2014) used factor analysis to identify a Lebanese traditional pattern. Foods specific to the Lebanese Mediterranean diet (LMD) in comparison to other Mediterranean diets from European countries included burghol, dried fruits and eggs. Also, fish was not found to be an essential component in the LMD as it is in all other Mediterranean diet, while alcohol was not used in the calculation. Results from this study found that the LMD index was most similar to the Italian Mediterranean index and least related to the French (Naja et al., 2014). A case control study conducted on dietary patterns and the odds of type 2 diabetes in Beirut, Lebanon found that the Traditional Lebanese Mediterranean dietary pattern was inversely associated with type 2 diabetes (OR: 0.46, CI: 0.22-0.97) (Naja et al., 2012). Furthermore, a cross-sectional study was conducted on the Lebanese adult population, which concluded that individuals who are overweight and obese and have a high adherence to the Traditional Lebanese dietary pattern are more likely to be metabolically healthy (Matta et al., 2016). Finally, a third study conducted on the rural Lebanese adult population assessed the diet pattern and its association with adiposity. Adherence to a Mediterranean diet was assessed via the Mediterranean diet score (MDS) and was found to be negatively associated with visceral adiposity and obesity, both of which are type 2 diabetes risk factors (Issa et al., 2010).

As previously stated, both the Mediterranean diet as well as the Lebanese Mediterranean diet have been found to contain anti-inflammatory properties, however, no study, to our knowledge, have examined the relationship between maternal adherence to a Lebanese Mediterranean diet and colic and allergy symptoms in exclusively breastfed infants. Furthermore, the maternal diet may affect allergy and colic symptoms in infants who are exclusively breastfed. This, in specific, has been observed in regard to maternal intake of cow's milk during breastfeeding which may contribute to allergy and colic symptoms in the infant via an immune and inflammatory response. Therefore, this study aimed to test the effect of maternal adherence to dairy-free Lebanese Mediterranean diet on colic, allergy symptoms and intestinal inflammation of exclusively breastfed infants. It was hypothesized that an exclusively breastfed infant whose mother is on a dairy free Lebanese Mediterranean diet would have a lower presence of colic and allergy symptoms, as well as better fecal profiles indicating lower gastrointestinal inflammation when compared to a mother on their typical diet.

Chapter Three

Methodology

3.1- Ethical Approval

This study was approved by the Lebanese American University Institutional Review Board (IRB number: LAU.SAS.MB2.11/Apr/2019; Appendix A). After recruitment, participants were provided with an informed consent (Appendix B), which included the purpose and benefits of the study, and ensured anonymity. Participants were asked to read the informed consent form thoroughly and any questions posed were answered verbally. All researchers involved in this study, including myself, have completed Collaborative Institutional Training Initiative (CITI) program training.

3.2- Study Design and Procedure

This study is a controlled pilot trial. Assessment of participant eligibility and recruitment began in May, 2019. Participants were provided with a nutrition education prenatally if assigned to the intervention group and all participants were provided with a lactation consultation postnatally and data was collected up until week 7 after the participant had given birth. Data collection is still ongoing. The primary endpoint is the presence of infantile colic symptoms, which were assessed using a Daily Crying Diary (Appendix C) that was translated to, and back-translated from Arabic by a certified translator (Hiscock & Jordan, 2004). The Daily Crying Diary indicates the total time an infant was awake, had cried, slept and been fed (Hiscock & Jordan, 2004). The secondary outcomes include Infant allergy symptoms which were assessed using a questionnaire (Appendix C) adapted from section "A-2 Health" of the CDC's Breastfeeding and Infant Feeding Practices postnatal questionnaire at 2 months (Centers for Disease Control and Prevention, 2017). This was also translated to and back-translated from Arabic by a certified translator. Additionally, Infant fecal stools were collected and tested for the presence of eosinophil cationic protein and stool pH (Saarinen, Sarnesto, & Savilahti, 2002 & Savino, et al., 2016) as detailed below.

3.2.1- Study Population

Recruited participants were first-time mothers who planned to exclusively breastfeed their infants. Our participants were not randomized into the groups, but rather conveniently assigned to a certain group as many participants were not willing to adhere to a dairy free diet and were therefore placed in a certain group based on willingness to adhere to a dairy-free Lebanese Mediterranean diet. Participants were recruited from a maternity center that provides childbirth and prenatal classes, as well as professional breastfeeding consultations. The center (Sophia Maternity) is located in Beirut, Lebanon. Participants were also recruited via advertising on social media platforms (Appendix D). Participants were screened between the first, second and beginning of the third trimester of pregnancy. Women included in the study were those who were pregnant for the first time (primigravida), had a healthy full-term pregnancy, and planned to exclusively breastfeed. Exclusion Criteria included multi-parity and any underlying medical conditions in the mother and/or infant. Participants were also excluded if the mother had stopped exclusively breastfeeding.

Following recruitment, mother-infant pairs were assigned into 2 groups, the intervention group, and the control group. The intervention group received an education session to follow a dairy free Lebanese Mediterranean diet in the 3rd trimester of pregnancy. After the education session, participants in the intervention group were asked to follow a dairy-free Lebanese Mediterranean diet immediately and for the remainder of the trial. The control group was instructed to continue their own personal lifestyle. The intervention groups' diet/guidelines were presented by a licensed dietician using a PowerPoint presentation and handouts. The PowerPoint and handouts for the intervention group contains the following points: early breastfeeding practices and risks of formula feeding, reasons to follow a dairy free diet and the benefits of a Mediterranean diet. In addition, the intervention group received an explanation of a dairy free diet including alternatives to dairy (calcium rich foods), and a description of the Lebanese Mediterranean diet and examples and tips for the Dairy Free Lebanese Mediterranean diet.

Based on data present in a study assessing the effect of a low FODMAP diet on infantile colic they concluded that in order to attain a medium effect size ($\eta p2 = 0.09$) with a power of 0.8 and α of 0.05, a sample size of 18 mother-infant pairs were required (Iacovou et al., 2018). Considering the previous information and that it is a pilot study, a sample size of 24 mother-infant pairs had initially been planned to be recruited with 12 mother-infant pairs assigned to arm of the study. However, we only ended up with 7 mother-infant pairs in the intervention group and 11 mother-infant pairs in the control group. This difficulty in recruitment and reaching the target sample size was partly due to the situation in Lebanon, namely the political unrest and the coronavirus pandemic. Approximately 95 individuals were assessed for eligibility. Of those, 65 individuals were excluded with 8 that did not meet the inclusion criteria, 54 that declined to participate and 3 due to travel purposes (Figure 1). Of the 30 participants recruited, 18 were assigned to the control group and 12 were assigned to the intervention group. In the control group, 8 mother-infant pairs either withdrew or were excluded from the study due to complications during birth (n=1), low birth weight (n=1), switching from exclusively breastfeeding to formula (n=1), participant feeling too overwhelmed to continue (n=4) and change in country of residence (n=1). We ended up with 10 participants that were included in the analysis for the control group (Figure 1). In the intervention group, 5 mother-infant pairs either withdrew or were excluded from the study due to the infant suffering from jaundice upon birth (n=1) and participants feeling too overwhelmed to continue (n=4). This has left us with 7 participants that were included in the analysis for the intervention group (Figure 1).

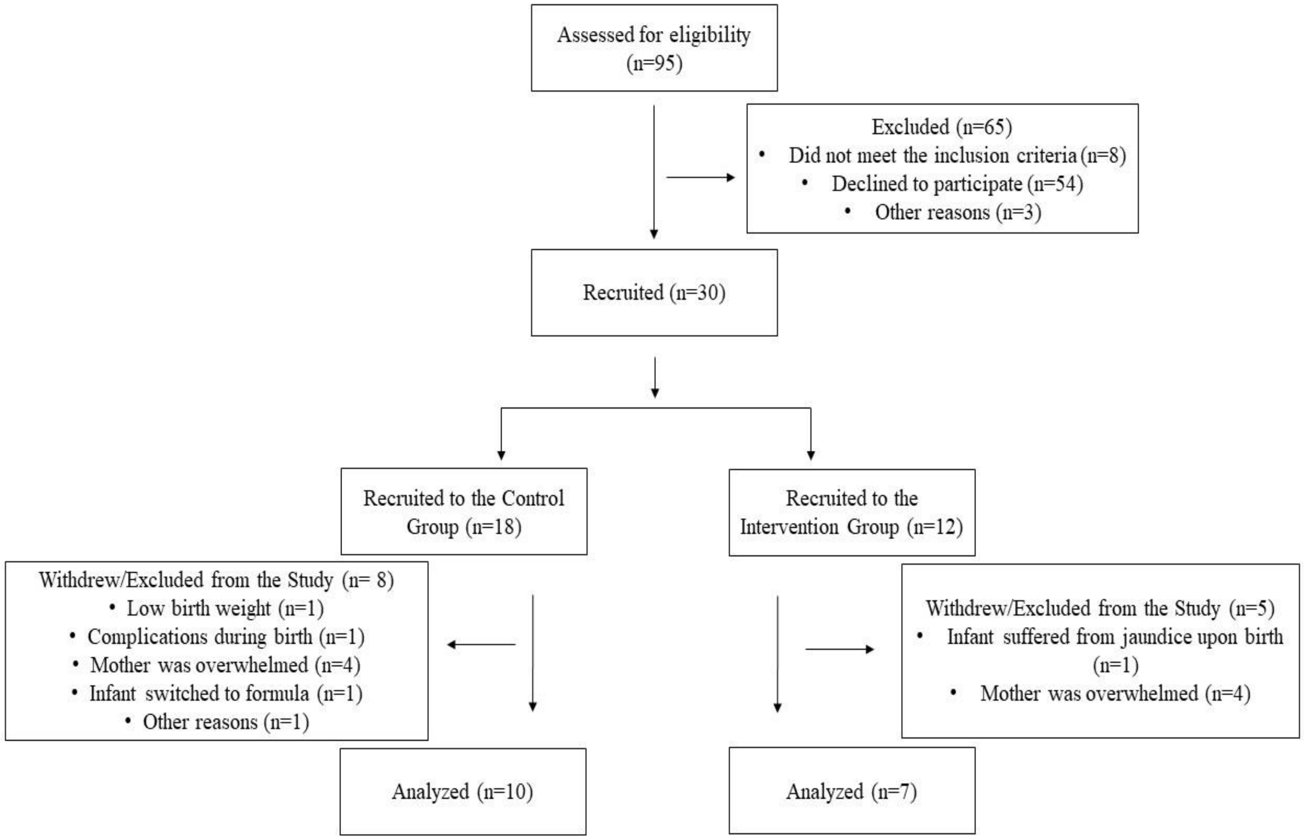

Figure 1. Participant flow diagram.

3.3- Data collection

After delivery, data was collected during the 2nd,4th and 7th week postnatal. On one day of every week, participants completed a questionnaire about infant colic and allergy symptoms (Appendix B) and provided stool samples of their infants. Participants also completed a 24-hour recall for 3 days each week, 2 week days and 1 day on the weekend, to ensure adherence to the dairy-free Lebanese Mediterranean diet by the intervention group. Infant stool samples were initially stored in the freezer at the participant's residence until pick up, which were then transported using a portable ice chest and stored at the Lebanese American University laboratory freezer at -1 degrees Celsius until analysis. Data from 24-hour recalls was entered into Nutritionist Pro software (Version 5.1.0, Axxya Systems, Texas, USA) to estimate food groups and nutrient analysis. All participants from both the control and intervention group received one educational lactation consultation with an experienced lactation consultant in order to avoid confounding factors related to breastfeeding positioning and techniques. This lactation consultation was provided for each participant postnatal at her own residence.

3.3.1- Adherence to the Lebanese Mediterranean Diet

Adherence to the Lebanese Mediterranean diet was assessed via an index derived in a study conducted by Naja et al., (2015), in which 9 food groups including fruits, vegetables, legumes, olive oil, burghol, dairy products, starchy vegetables, dried fruits and eggs, were assigned a score of 1, 2 or 3, based on their tertiles of consumption. As food groups were divided into tertiles, those in the first tertile received a score of 1, those in the second tertile received a score of 2 and those in the third tertiles received a score of 3 (Naja et al., 2015). However, in our case, as we are assessing the adherence to a dairy free diet, the scores of the "dairy products" food group were inverted and they were assigned as; a score of 3 for those in the first tertile, a score of 2 for those in the second tertile, and a score of one in the third tertile of consumption. The range of scores for this modified Lebanese Mediterranean diet index was between 9 and 27. A score of less than or equal to 18 was to be indicative of a low adherence while a score of greater than 18 to a high adherence (Jomaa et al., 2016).

3.3.2- Fecal Eosinophil Cationic Protein and pH

Eosinophil cationic protein in stool is used as an indicator of intestinal inflammation in different gastrointestinal and allergic diseases (Majamaa et al., 1999, Saarinen et al., 2002 & Hua et al., 2017). Furthermore, fecal eosinophil cationic protein was found to be helpful in diagnosing and monitoring food hypersensitivity reactions (Hua et al., 2017). A study conducted by Majamaa et al., (1999) aiming to evaluate the presence of allergic intestinal inflammation found that infants with atopic dermatitis, before an elimination diet, had higher fecal eosinophil cationic protein concentrations when compared to the healthy control group. For infants with a positive clinical response, an elimination diet leads to the decrease in eosinophil cationic protein fecal concentrations.

A human Eosinophil Cationic Protein (ECP) enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource, San Diego, USA) was used in order to determine the level on ECP in the infant's stool sample. The ELISA kit used the quantitative sandwich enzyme immunoassay technique. First, fecal samples were prepared by mixing them with a specified amount of phosphate-buffered saline, as per the kit manual. The ECP ELISA kit test began with a micro-elisa strip-plate, which was pre-coated with an ECP specific antibody. Then, standards and samples were added to the plate wells. In this step, ECP, when present, binds to the pre-coated wells. Most samples were run in duplicates, and results of the duplicated samples were similar. Horseradish peroxidase (HRP) reagent was then added to every well to “sandwich” the ECP. Next, the plate underwent incubation at 37 degrees Celsius for 60 minutes. Wells were washed 4 times to eliminate any free components. In the next step, two substrate solutions were added to each well, with the second solution having to be protected from light. The plate was then incubated for 15 minutes at 37 degrees Celsius. Wells that contained ECP changed in color as seen in Figure 2. In order to stop the enzyme substrate reaction, sulphuric acid solution was added to every well. Optical density was then measured spectrophotometrically at a wavelength of 450 nm with the ELISA reader instrument VarioskanFlash as seen in Figure 3. Averages of duplicated samples were determined and the concentration of ECP in each sample was calculated via a standard curve.

As for the level of pH in the stool samples, they were determined via direct insertion of an electrode pH probe into feces after homogenization. Infant fecal pH was measured as differences in pH and osmolarity may occur due to gut inflammation (Rhoads et al., 2009).

Figure 2. ECP ELISA kit plate wells that changed in color

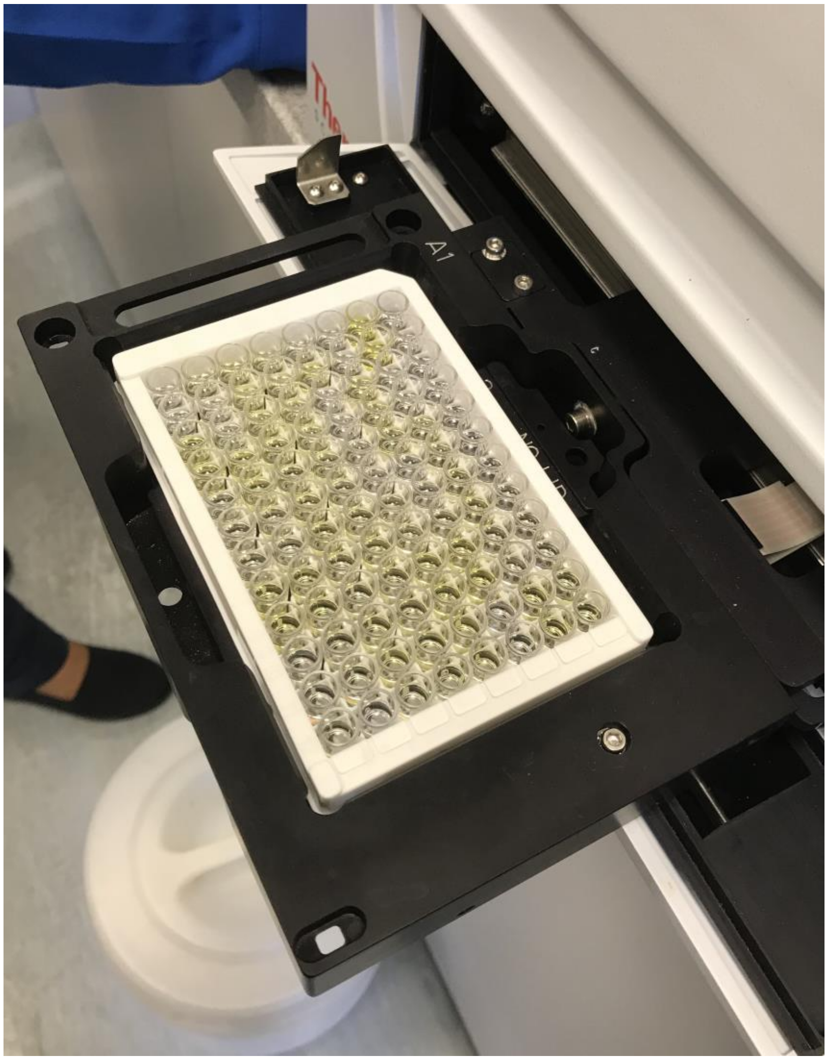

Figure 3. ECP ELISA kit plate before entering the ELISA reader instrument VarioskanFlash

3.6- Statistical Analysis

All statistical calculations were conducted via SPSS statistical software version 21. The independent variable in this study is adherence to the diet while the dependent variables are colic symptoms, infant allergy symptoms and the stool test results. Kolmogorov–Smirnov and Shapiro–Wilk tests were used to evaluate the assumption of normality. Continuous variables were described using means ± standard deviations (SD) for those with normal distribution and median (Interquartile) for those that with a skewed distribution. As for the categorical variables, they were described using proportions. A P-value of 0.05 or less was considered statistically significant. Two sample independent t tests and Mann-Whitney test were conducted to evaluate the difference in Eosinophil Cationic Protein and pH of the stool samples, infant behavior, allergy and/or colic symptoms and descriptive characteristics between the intervention and control group. Two sample independent t tests were conducted for continuous variables that follow a normal distribution and Mann-Whitney test for variables that follow a skewed distribution. Furthermore, Fisher's exact test was used to evaluate categorical variables. In regard to the Lebanese Mediterranean Diet Index, participants were grouped into tertiles based on the 9 food groups previously described, then ranked based on the tertile placed in. As for ECP concentrations, averages of duplicate samples were calculated and the concentration was calculated via a standard curve, which was plotted using the optical density and the standard concentrations (linear standard curve, r^2=0.95).

Chapter Four

Results

4.1- Participant Retention

Of the participants approached, 31.56% were included in the study. Overall, 10 (55.5%) of the 18 participants assigned to the control group and 7 (58.3%) out of the 12 participants assigned to the intervention group completed the study. Reasons for withdrawal or exclusion from the study have been previously stated.

4.2- Descriptive Characteristics

The study sample consisted of 17 mother- infant pairs. The mean age for mothers was 31.80 ± 3.85 and 29.50 ± 2.58 for the control and intervention group, respectively (p=0.22). In both groups, mothers either had a college education or a graduate school education with 20% and 33.3% having reached a college education and 80% and 66.7% having completed a graduate level education in the control and intervention group, respectively (p=0.49). Sixty percent of mother-infant pairs resided in Mount Lebanon and 40% resided in Beirut for the control group. For the intervention group, 50% resided in Beirut, 33.3% in Mount Lebanon and 16.7% in Beqaa (p=0.42). As for the mother's anthropometric measures, mean pre-pregnancy weight was 56.16 ± 5.12 kg for the control group and 60.63 ± 5.28 kg in the intervention group (p=0.12). Mean pre-pregnancy BMI was 21.80 ± 1.61 and 23.45 ± 2.86 in the control and intervention groups, respectively (p=0.16). At week 2, mother's weight was 63.65 ± 8.32 kg and 66.44 ± 7.09 kg (p=0.53) and had changed to 60.77 ± 7.92 kg and 64.46 ± 6.43 kg by week 7 (p=0.38) in the control and intervention group, respectively. As regards infant anthropometric measures, the infants mean birth weight was 3291.60 ± 404.19 g in the control group and 3100 ± 392.17 g in the intervention group (p=0.37). At week 2, infants weight was 3473.63 ± 357.14 g and 3085 ± 397.79 g (p= 0.12) and had increased to 4811 ± 641.26 g and 4277.50 ± 732.23 g by week 7 (p=0.20) in the control and intervention group, respectively. No difference was found between the control and intervention groups, in age, education, place of residence or any infant and mother anthropometric measures ($p>0.05$) (Table 1).

For the Lebanese Mediterranean diet score and as stated above, a score of ≤ 18 indicates a low adherence to the diet while a score of >18 indicated a high adherence. In the dairy-free Lebanese Mediterranean diet group (intervention group), 71.4% of participants scored higher than 18 while 28.6% scored ≤ 18, therefore 71.4% adhered to the diet given. In the control group 70% scored ≤ 18 and 30% score >18. The descriptive characteristics mentioned including demographic characteristics, anthropometric measurements and the Lebanese Mediterranean Diet Index score of mother-infant study participants are presented in Table 1.

When asked about infant medication use, no infant had taken antibiotics throughout all weeks in the control group. In the intervention group, one participant had given antibiotics at week 4 while in the other weeks no antibiotics were given to any infants. Medication other than antibiotics were more frequently taken with 20%, 50% and 30% taking some form of medication in weeks 2, 4 and 7, respectively, in the control group. A total of 33.3% and 16.1% were taking some form of medication in weeks 4 and 7, respectively, in the intervention group (Table 2). The mean number of infant stools in 24 hours were similar across all weeks and in both groups with no difference found between the control and intervention group ($p > 0.05$). Infant stool characteristics ranged from hard to watery. In the control group, stool form was most commonly noted down as semi-watery (60%) in week 2, soft (50%) and watery (50%) in week 4 and soft (50%) and semi-watery (60%). For the intervention group, stool form was most commonly noted down as soft (66.7%) and semi-watery (66.7%) in week 2, soft (66.7%) and semi-watery (50%) in week 4 as well, and soft (50%) in week 7. However, there was no statistical significance when comparing stool frequency and characteristics between the two groups ($p> 0.05$) (Table 2).

Table 1 Descriptive characteristics of study participants (n=16) [a]

Characteristics	**Control Group (n=10)**	**Intervention Group (n=6)**	**P-value**
Demographic Characteristics			
Mother's age (yrs)	31.80 ± 3.85	29.50 ± 2.58	0.22
Education			0.49
<7 years of school	0 (0)	0 (0)	
High school diploma	0 (0)	0 (0)	
Technical school	0 (0)	0 (0)	
College education	2 (20)	2 (33.3)	
Graduate school	8 (80)	4 (66.7)	
Place of residence			0.42
North of Lebanon	0 (0)	0 (0)	
Mount Lebanon	6 (60)	2 (33.3)	
Beirut	4 (40)	3 (50)	
South of Lebanon	0 (0)	0 (0)	
Beqaa	0 (0)	1 (16.7)	
Nabatieh	0 (0)	0 (0)	
Anthropometric measurements			
Mother's height (cm)	160.50 ± 6.13	161.17 ± 7.30	0.67
Mother's pre-pregnancy weight (kg)	56.16 ± 5.12	60.63 ± 5.28	0.12
Mother's pre-pregnancy BMI	21.80 ± 1.61	23.45 ± 2.86	0.16
Mother's weight at week 2 (kg)	63.65 ± 8.32	66.44 ± 7.09	0.53
Mother's BMI at week 2	24.73 ± 2.14	25.12 ± 3.36	0.80
Mother's weight at week 4 (kg)	61.46 ± 7.48	63.70 ± 6.21	0.55
Mother's BMI at week 4	23.79 ±1.82	24.61 ± 2.94	0.50
Mother's weight at week 7 (kg)	60.77 ± 7.92	64.46 ± 6.43	0.38
Mother's BMI at week 7	23.52 ± 1.94	24.36 ± 3.34	0.54
Infant birth weight (g)	3291.60 ± 404.19	3100 ± 392.17	0.37
Infant weight at week 2 (g)	3473.63 ± 357.14	3085 ± 397.79	0.12
Infant weight at week 4 (g)	4033.33 ± 447.21	4040 ± 705.41	0.98
Infant weight at week 7 (g)	4811 ± 641.26	4277.50 ± 732.23	0.20
Lebanese Mediterranean Diet Index (Score range 9-27)			
Low (≤ 18)	7 (70)	2 (28.6)	0.153
High (> 18)	3 (30)	5 (71.4)	

Continuous variables are presented as mean ± SD for those normally distributed and median (IQ) for those that are skewed and categorical variables as n (%)

[a] Significance is derived from independent t test for continuous variables that follow a normal distribution, Mann-Whitney test for variables that follow a skewed distribution and Fisher's exact test for categorical variables

Table 2 Description of infant medication use and stool characteristics (n=16)

	Control Group (n=10)			Intervention Group (n=6)		
	Week 2	Week 4	Week 7	Week 2	Week 4	Week 7
Infant medication use						
Antibiotics	0 (0)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)
Other medication	2 (20)	5 (50)	3 (30)	0 (0)	2 (33.3)	1 (16.7)
Stool frequency and characteristics						
Number of stool in 24 hours	4.55 ± 1.36	4.15 ± 1.45	4.25 ± 1.57	4.50 ± 1.05	4.42 ± 1.43	4 ± 1.41
Hard stool	1 (10)	1 (10)	0 (0)	0 (0)	0 (0)	0 (0)
Formed stool	2 (20)	1 (20)	2 (20)	0 (0)	0 (0)	0 (0)
Soft stool	3 (30)	5 (50)	5 (50)	4 (66.7)	4 (66.7)	3 (50)
Semi-watery stool	6 (60)	3 (30)	6 (60)	4 (66.7)	3 (50)	0 (0)
Watery stool	3 (30)	5 (50)	3 (30)	2 (33.3)	1 (16.7)	0 (0)

Continuous variables are presented as mean ± SD and categorical variables as n (%)

4.3- Dietary Intake

Percentage of carbohydrates, protein and fat intake, as well as fiber intake were found to be similar between both groups. However, energy intake was significantly higher in the control group when compared to the intervention group (p=0.044). In addition, mean calcium intake was found to be statistically different between the control and intervention group, as the control group's intake of calcium was higher (p=0.015) (Table 3).

Table 3 Energy and Nutrient Intake

	Control Group (n=10)	Intervention Group (n=6)	P-value
Energy (kcal)	2187.55 ± 272.35	1874.75 ± 312.88	0.044 [a]
Carbohydrates (%)	44.33 ± 4.33	41.91 ± 3.91	0.257
Protein (%)	18.47 ± 2.44	18.72 ± 4.19	0.875
Fat (%)	39.71 (6.60)	38.88 (6.46)	0.626
Fiber (g)	20.92 ± 5.77	20.53 ± 5.94	0.893
Calcium (mg)	980.62 ± 213.79	659.51 ± 269.47	0.015 [a]

Continuous variables are presented as mean ± SD for those normally distributed and median (IQ) for those that are skewed and categorical variables as n (%)
[a] Statistically significant

4.4- Lebanese Mediterranean Diet Index

The intervention group scored significantly higher in the Lebanese Mediterranean Diet Index (LMD) with a median score of 20 (4) in comparison to the control group that had a median score of 17 (3.75) (p=0.05). The difference in average daily grams of consumption of all food groups, except for dairy products, were not statistically significant between the two groups. Mean or median daily consumption of fruits, vegetables, legumes, olive oil, burghul, starchy vegetables, dried fruits and eggs was found to be 104.5 ± 35.41 vs. 84.68 ± 26.30, 73.56 (33.41) vs. 67.97 (19.08), 108.59 ± 47.81 vs. 83.87 ± 63.94, 12.52 (1.54) vs. 11.60 (5.44), 0 (22.38) vs. 1.70 (22.70), 85.92 ± 19.86 vs. 94.80 ± 27.82, 24.31 ± 29.52 vs. 27.58 ± 28.43 and 89.28 ± 27.58 vs. 67.08 ± 37.70, respectively, in the intervention group versus the control group. As predicted, median daily consumption of dairy products was found to be significantly higher in the control group when compared to the intervention group (p=0.01) (Table 4).

Table 4 Description of the Lebanese Mediterranean Diet Index score and average dietary intake of food groups in grams per day (g/d) (n=17) [a]

	Control Group (n=10)	**Intervention Group (n=7)**	**P-value**
Lebanese Mediterranean Diet Index – LMD (Score range 9-27)			
LMD score	17 (3.75)	20 (4)	0.05[b]
Lebanese Mediterranean Diet Index food groups			
Fruits (g/d)	104.5 ± 35.41	84.68 ± 26.30	0.23
Vegetables (g/d)	67.97 (19.08)	73.56 (33.41)	0.38
Legumes (g/d)	83.87 ± 63.94	108.59 ± 47.81	0.40
Olive Oil (g/d)	11.60 (5.44)	12.52 (1.54)	0.92
Burghol (g/d)	1.70 (22.70)	0 (22.38)	0.51
Dairy products (g/d)	72.85 (54.67)	5.88 (15)	0.01[b]
Starchy vegetables (g/d)	94.80 ± 27.82	85.92 ± 19.86	0.48
Dried fruits (g/d)	27.58 ± 28.43	24.31 ± 29.52	0.82
Eggs (g/d)	67.08 ± 37.70	89.28 ± 27.58	0.20

Continuous variables are presented as mean ± SD for those normally distributed and median (IQ) for those that are skewed

[a] Significance is derived from independent t test for continuous variables that follow a normal distribution and Mann-Whitney test for variables that follow a skewed distribution

[b] Statistically significant

4.5- Allergy and Colic Symptoms and The Infant Behavior Diary

One infant in the control group was diagnosed with infantile colic based on the Rome IV diagnostic criteria of infantile colic for research purposes (Zeevenhooven et al., 2017). The number of problems related to illness, allergy and colic symptoms were not statistically significant between the two groups ($p>0.05$). In regard to infant behavior, average hours of sleeping, feeding, and awake were not statistically significant between the two groups as well ($p>0.05$). When comparing crying duration between the two groups, those on the intervention group's dairy free Lebanese Mediterranean diet cried less with a borderline significance in the 2nd week ($p=0.1$). Median crying duration in the intervention group at week 2, 4 and 7 was 9 (17.50), 16.5 (26.25) and 8 (42.50) minutes, respectively. In comparison, crying duration in the control group at week 2, 4 and 7 was 33 (45.50), 22.5 (50.50) and 12.5 (46.25) minutes, respectively (Figure 4). The number of problems related to illness, allergy and colic symptoms and infant sleeping, feeding, awake and crying behaviors are presented in Table 5.

Table 5 Infant behaviors during week 2, 4 and 7 postnatal and cumulatively (n=16) [a]

	Control Group (n=10)	**Intervention Group (n=6)**	**P-value**
Infantile Colic			
Diagnosed Infantile Colic [b]	1 (10)	0 (0)	0.625
Illness, allergy and colic symptoms			
Number of problems			
Week 2	0 (1)	0.50 (2)	0.45
Week 4	0.90 ± 0.99	0.83 ± 0.75	0.89
Week 7	0.5 (1.25)	0.5 (1.25)	0.72
Infant Behavior Diary			
Sleep duration (minutes/day)			
Week 2	537.30 ± 161.40	599.50 ± 234.45	0.538
Week 4	549.50 ± 257.58	669.83 ± 186.01	0.337
Week 7	530 ± 260.43	668.50 ± 144.31	0.255
Total weeks	538.90 ± 197.34	646 ± 181.58	0.298
Feeding duration (minutes/day)			
Week 2	273 ± 125.38	296.83 ± 139.32	0.729
Week 4	274.10 ± 179.27	346.50 ± 133.04	0.408
Week 7	244.70 ± 145.40	286.83 ± 138.80	0.578
Total weeks	263.90 ± 141.34	310 ± 127.48	0.524
Awake duration (minutes/day)			
Week 2	127.5 (96.25)	107 (137.50)	0.329
Week 4	141.40 ± 80.44	160.33 ± 105.73	0.691
Week 7	158.60 ± 96.97	227 ± 128.21	0.245
Total weeks	152.60 ± 47.52	169.17 ± 88.64	0.631
Crying duration (minutes/day)			
Week 2	33 (45.50)	9 (17.50)	0.174
Week 4	22.5 (50.50)	16.5 (26.25)	0.586
Week 7	12.5 (46.25)	8 (42.50)	0.867
Total weeks	20 (69.75)	12 (23.5)	0.447

Continues variables are presented as mean ± SD for those normally distributed and median (IQ) for those that are skewed and categorical variables as n (%)

[a] Significance is derived from independent t test for continuous variables that follow a normal distribution and Mann-Whitney test for variables that follow a skewed distribution and Fisher's exact test for categorical variables

[b] Diagnosis based on the Rome IV diagnostic criteria of infantile colic for research purposes (Zeevenhooven et al., 2017).

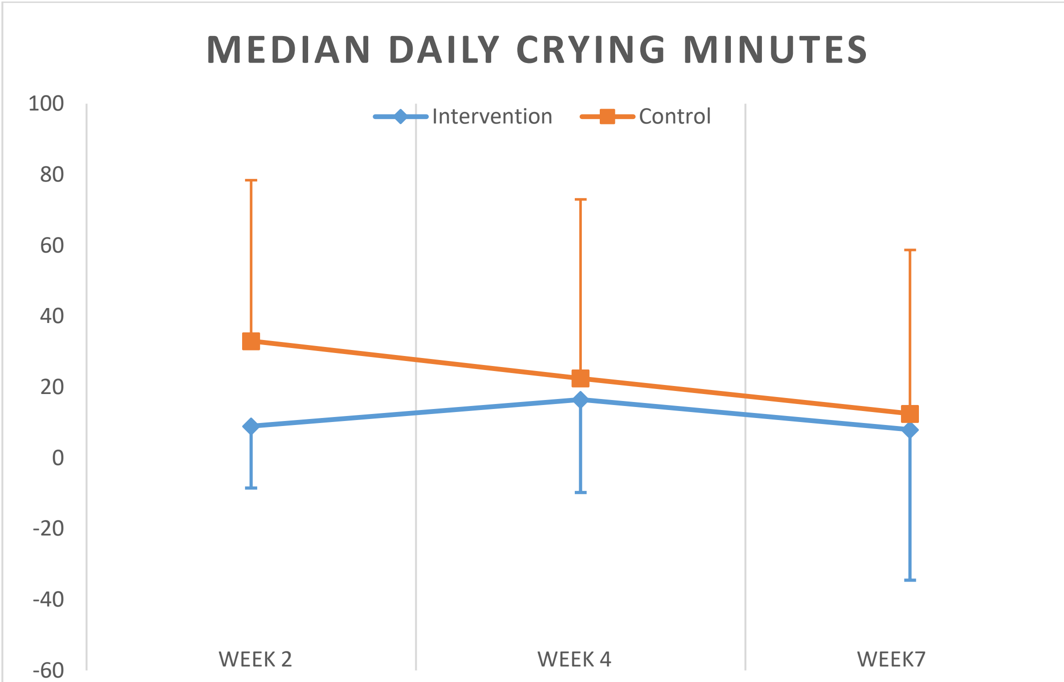

Figure 4. Median daily crying minutes at week 2, 4 and 7. The blue line represents the intervention group and the orange line represents the control group

4.6- Stool pH and Eosinophil Cationic Protein Presence

Eosinophil cationic protein concentrations were observed to increase from week 2 (59.74 ± 24.08 ng/ml and 53.85 ± 26.61 ng/ml in the control and intervention group, respectively) to week 7 (92.49 ± 28.47 and 90.80 ± 20.21 in the control and intervention group, respectively), however no significant differences were found when comparing the control and intervention groups. Similarly, fecal pH was not significantly different between the two groups ($p>0.05$) (Table 6).

Table 6 Stool Sample Test Results [a]

	Control Group (n=10)	**Intervention Group (n=6)**	**P-value**
Stool Sample Tests			
Eosinophil Cationic Protein (ng/ml) (n=17)			
Week 2	59.74 ± 24.08	53.85 ± 26.61	0.650
Week 4	82.03 ± 27.60	70.32 ± 35.65	0.505
Week 7	92.49 ± 28.47	90.80 ± 20.21	0.900
All weeks	71.96 ± 15.32	71.66 ± 18.90	0.971
pH (n=15)			
Week 2	6.84 ± 0.22	6.46 ± 0.44	0.098
Week 4	6.10 ± 0.34	6.70 ± 0.52	0.188
Week 7	6.31 ± 0.60	6.50 ± 0.74	0.737
All weeks	6.61 ± 0.46	6.49 ± 0.53	0.665

[a] Continuous variables are presented as mean ± SD

Chapter Five

Discussion

To our knowledge, the present study is the first that assessed the effect of a maternal adherence to a dairy-free Lebanese Mediterranean diet on colic and allergy symptoms in exclusively breastfed infants. Results showed no differences in colic and allergy symptoms or infant behavior, specifically in sleep, feed, awake or crying duration, between exclusively breastfeeding mothers who were on their own typical diet, versus mothers on a dairy-free Lebanese Mediterranean diet. Similarly, no differences were seen in infant fecal pH and presence of ECP between the two groups. This lack of significant difference may be attributed to a type 2 error given that our sample size was small, leading to an underpowered study. Furthermore, consumption of all food groups in the modified LMD, except for dairy products, were not different between the two groups indicating a fair adherence to the LMD in both groups which may contribute to the lack of significant difference. Our target sample size was not attained as difficulties in recruitment arose due to political unrest and the coronavirus pandemic that stuck Lebanon. In addition, challenges such as lack of interest and concerns for study duration and mother's own commitment to providing data due to lack of time were encountered. Similar to our study, the Mother and Infant Nutrition Assessment (MINA) birth cohort study conducted in Lebanon and Qatar only recruited 39.8% of pregnant women who were eligible and when compared to studies conducted in high income countries, retention rates were significantly lower (Maghera et al., 2014 & Ayoub et al., 2018). This indicates that there is a need for new approaches to improve recruitment of pregnant women, particularly in low to middle income countries such as Lebanon (Ayoub et al., 2018). Although limited, evidence has suggested that while pregnant women may possibly be interested in a research study, they are also careful about participating and may be overwhelmed and lack free time as they prepare for their newborn's arrival (Savitz et al., 1999). In a study conducted in Lebanon assessing the different factors that influence pregnant women participation in research, it was discovered that personal factors, contextual factors and study characteristics are the main influencers (Ayoub et al., 2018). Personal factors include altruism, level of self-confidence, interest in the topic, previous knowledge of research, education level and previous research experience. Contextual factors include societal factors such as exposure to research, where key informants included in the study stated that the Lebanese society is not as fully engaged in research like in western countries. Support of family and friends is also a contextual factor, especially the support of the partner or husband and this was also observed in our study, whereby more support was observed to incite more interest. In addition, study characteristics such as the burden of the study, ethical consideration, incentives, and the researcher's interpersonal skills and physician encouragement to participate were all factors that influence the decision to participate (Ayoub et al., 2018). In relation to the burden of the study, aspects including time commitment, type of data collected and logistical inconvenience were of concern. One of the main factors influencing decision to participate was observed to be time commitment and for some this was the only barrier (Ayoub et al., 2018). Similarly, this was of main concern in the pregnant women that we approached in the present study.

As for adherence to the dairy-free LMD, 71.4% of mothers recruited into the intervention group had a score greater than or equal to 18 indicating high adherence while 70% of mother's on their typical diet had a score less than 18 indicating a low adherence to a dairy-free Lebanese Mediterranean diet. Furthermore, mothers in the intervention group had statistically significant higher scores ($p=0.05$) than those in the control group. However, when assessing the dairy-free LMD food groups, only dairy was found to be significantly different between the two groups ($p=0.01$), with mothers in the intervention group consuming less dairy as predicted. This indicates that the difference in LMD scores was mainly driven by the difference in dairy intake. Indeed, mothers' consumptions of fruits, vegetables, legumes, olive oil, burghol, starchy vegetables, dried fruits and eggs were not different between groups. Adherence to a LMD before enrollment into our study was not assessed, however, since all food groups except dairy product consumption were not different between the two groups, it is possible that mothers in both groups may have been previously adherent to the protective LMD, contributing to the lack of significance observed.

Percentages of carbohydrates, protein and fat were found to be similar between both groups, whereas energy intake was significantly higher in the control group ($p=0.044$). This difference in energy intake between the two groups is mainly driven by one mother in the intervention group who had noticeably consumed lower kilocalories per day when compared to other mothers on a dairy free LMD as she stated that she would skip meals in order to sleep. When this participant was removed from the analysis, the difference in energy intake between the two groups was no longer significant ($p>0.05$). Nonetheless, this did not affect the mother or infant anthropometrics that were similar between groups throughout the study period. Moreover, maternal energy intake that is moderately restricted has not been found to affect a mother's milk production or human milk nutrient content (Mohammad et al., 2009 & Bravi et al., 2016; Boniglia et al., 2003 & Quinn et al., 2012). Moreover, previous studies have concluded that a maternal energy intake which is over 1,500 kilocalories is sufficient to maintain the volume of a mother's milk (Strode et al., 1986 & lovelady et al., 2000).

As for maternal calcium consumption, mothers on a dairy free LMD had consumed significantly less calcium. Although mothers were educated on dairy free calcium rich alternatives, future work should further stress and focus on the consumption of such alternatives.

When present, gut inflammation may alter fecal pH. For example, in cases of critically ill systemic Inflammatory Response Syndrome, and colorectal cancer patients, studies have observed higher pH levels when compared to healthy controls (Osuka et al., 2012, Ohigashi et al., 2013 & Yamada et al., 2015). In addition, it has been observed that an increased fecal pH may result in microbiome dysbiosis, as this increase has been associated with the abundance of Veillonellaceae Enterobacteriaceae, Peptostreptococcaceae, and Clostridiaceae species and a decrease in Bifidobacterium species in a breastfed infants gut (Henrick et al., 2018). Moreover, Infants who are formula fed have also been observed to have a higher fecal pH level when compared to breastfed infants (Holscher et al., 2012). In our study however, no differences were observed in fecal pH between a mother on her typical diet or on a dairy free LMD.

Similarly, in a study assessing probiotic supplementation and its effect on the microbiota and colic symptoms, no difference was observed in fecal pH values between colicky and non-colicky infants (Mentula et al., 2008).

As for fecal ECP, it is considered to be a good marker of eosinophilic inflammation and intestinal hypersensitivity in the case of eosinophil activation in gastrointestinal and allergic diseases (Hua et al., 2017). In infants, few studies have established fecal ECP levels for certain diseases such as atopic eczema and food allergy and bronchiolitis (Marjamaa & Isolauri, 1997, Majamaa et al., 1999, Kapel et al., 1999, Saarinen et al., 2002 & Silva et al., 2007). Kapel et al., (1999), compared 13 infants with confirmed or suspected cow's milk allergy to 10 healthy infants after conducting an oral cow's milk challenge and, similarly to our study, found no significant difference in baseline fecal ECP concentration between the groups, as well as no significant difference in fecal ECP post-challenge. In contrast, a study assessing 208 infants with symptoms that suggest the presence of cow's milk allergy, found that ECP levels were significantly higher for infants who were showing gastrointestinal symptoms and those who reacted to a cow's milk challenge after 24 hours rather than 1 hour. Furthermore, both serum and fecal levels of ECP have been observed to increase for infants and children with food allergies after conducting a cow's milk challenge. Also, a decrease in ECP level was observed when the culprit allergen was removed from their diet (Kosa et al., 1997). As for fecal ECP levels in healthy infants, data is scarce (Silva et al., 2007). Silva et al., (2007) assessed fecal ECP levels in 51 healthy children, ages ranging from 1 month to 12 years and a mean age of 2.9 ± 2.44. Mean fecal ECP concentration was found to be 1.93 µg/g and ranged from ranged 0.41 to 22.20 µg/g. Infant Fecal ECP levels of both groups in our study were found to be within the range of healthy infants assessed in Silva et al.'s (2007) study. Fecal ECP of infants in the control group ranged from 0.59 ± 0.24 µg/g to 0.92 ± 0.28 µg/g and from 0.54 ± 0.26 µg/g to 0.91 ± 0.20 µg/g in the intervention group. As for the lack of significance in our results, it may indicate that a dairy free LMD diet does not influence gastrointestinal inflammation. However, no conclusive finding may be drawn due to our small sample size.

Infant crying duration was observed to be higher for mothers on their typical diet when compared to a mother on a dairy free-LMD in all recorded weeks and reached borderline significance in week 2 (p=0.1). In other studies, assessing the removal of allergens in the maternal diet, reductions in colic symptoms such as crying durations were observed, however in these studies infants with diagnosed or suspected colic were recruited (Oggero et al., 1994 & Hill et al., 2005). Moreover, at 2.5 months postnatal, one infant in the control group was diagnosed with cow's milk protein allergy by a physician and another infant in the control group was diagnosed with infantile colic. Infantile colic was diagnosed based on the Rome IV diagnostic criteria of infantile colic for research purposes given that in a face-to-face interview, as well as a 24-hour infant behavior diary, the mother reported, recurring and persistent episodes of infant crying for ≥ 3 hours per day during ≥ 3 days in 7 days that occurred without an apparent reason and could not be resolved (Zeevenhooven et al., 2017). The infant had no failure to thrive, illness or fever. In summary, crying duration was observed to be lower in the dairy free LMD group and cases of diagnosed cow's milk allergy and infantile colic were seen in the group where mothers consumed a typical diet. Conclusions are yet to be draw as a study with a larger sample size is needed in order to confirm such results.

In regard to potential cofounding factors, all mother's in both groups were provided with a lactation consultation upon giving birth in order to avoid cofounding factors pertaining to breastfeeding techniques. Maternal stress and anxiety prenatally and postnatally were not assessed but may influence infantile colic symptoms such as crying (Søndergaard et al., 2003, Akman et al., 2006& Van der Wal et al., 2007). However, all participants were first time mothers and all participants who had completed the study were recruited from Sophia Maternity center, were they had attended a childbirth and prenatal preparation class which may lower anxiety levels. Maternal physical activity was not assessed neither in pregnancy or during lactation, however, physical activity is presumed to be similar postnatally due to the time constraints related to caring for a newborn.

Limitations of this study include its small sample size leading to limitations in the strength of the observations, as well as generalizability. Additionally, the questionnaires given are self-reported, therefore some response bias may have occurred. The 24-hour recalls were collected over the phone, therefore there may be the possibility of interviewer's bias. Furthermore, participants in the study were not be randomized into the groups but rather conveniently placed. In order to overcome limitations in future studies a larger sample size, assessment of maternal stress and prenatal assessment of adherence to a LMD should be acquired. Moreover, in order to attain more variability in participant dietary patters, an observational study with a larger sample size should be conducted. The strengths of this study include its design (controlled trial), as well as the objective measures used, as stool samples were tested for stool pH, and eosinophil cationic protein. In addition, all participants were first time mothers and were provided with a lactation consultation in order to avoid confounding factors related to breastfeeding techniques.

Ten to twenty percent of pediatrician visits in the first few months of life are related to infantile colic. Excessive crying is a symptom related to infantile colic and it has been found that it may lead to parental exhaustion, parents feeling incompetent, parents fear of harming the infant, and the mothers may reduce face to face interaction with her infant and discontinue breastfeeding early on (Iacovou et al., 2012). In addition, infant crying may pose negative effects on parental perception and bonding with the infant and parents may experience feelings of helplessness, frustration, anger, anxiety, and depression due to infant crying (Oldbury & Adams, 2015). Moreover, for numerous years the prevalence of allergic diseases has been on the rise and has developed into a public health concern worldwide (Fujimura et al., 2019). In addition, there are the limited studies conducted on the maternal diet in the management of infantile colic and allergy symptoms and lack of understanding in the role of breastfeeding in delaying or preventing the onset of food allergies. Taken together, it is important to further investigate, using randomized studies with large sample size, different maternal diets and dietary patterns and their effects on the infant who is exclusively breastfed.

Furthermore, evidence is lacking with regards to lactating mother's dietary patters and their effect on human milk composition (Moro et al., 2019.) A study yet to be published investigated the effect of maternal adherence to a Mediterranean diet on human milk composition. Similarly, future studies should investigate the effect of maternal adherence to a Lebanese Mediterranean diet on her milk composition (Moro et al., 2019 & Naja et al., 2015).

Another emerging contributing factor affecting human milk composition is its microbiota or soluble molecular content (Fernández et al. 2013). Research in this regard is still in its infancy; yet, it has been proposed that human milk microbiota composition is influenced by maternal factors and it may affect the infant's gastrointestinal microbiota. Animal studies have found that bacteria present in the maternal gut may migrate through dendritic cells to reach the mammary gland. This occurs during lactation and later stages of pregnancy (Fernández et al. 2013). In a cross-sectional study conducted in 2019, maternal intake of linoleic and polyunsaturated fatty acids during lactation were positively correlated with Bifidobacterium in the milk, indicating that maternal diet does affect human milk microbiota composition (Padilha et al., 2019). Further research is needed in order to assess the effect of different maternal diets and their influence on human milk microbiota composition and how this, in turn, may influence the breastfed infant's gastrointestinal microbiota. In particular, the effect of the Mediterranean diet needs to be explored, as studies have reported its beneficial effect on the gut microbiota and metabolome in adult populations (De Filippis et al., 2016 & Krznaric et al., 2019). Such work is of importance as an infant gastrointestinal microbiota has been proposed to play a role in infantile colic, as well as food allergies (Zeevenhooven et al., 2018 & Berni Canani et al., 2019).

Chapter Six

Conclusion

In conclusion, no differences were observed in allergy, colic symptoms, and fecal profile in exclusively breastfed infants whose mothers followed a dairy free LMD compared to controls. A trend indicating lower infant crying duration was observed among mothers consuming the intervention diet. Adherence to the dairy free LMD was high in the intervention group, however, only daily consumption of dairy was statistically different between the two groups while the remaining food groups were not. Difficulties in recruitment occurred as periods of political unrest and the coronavirus pandemic struck. In addition, factors such as the burden of the study, as well as women describing being overwhelmed and lacking free time were barriers to participating in the study. Subsequently, due to the small sample size, no definitive conclusion may be drawn. Future work must include a larger sample size and look into different maternal dietary patterns, including further investigation into maternal adherence to the LMD, and their effects on exclusively breastfed infants. Furthermore, mechanisms, through which maternal dietary patterns affect infant behavior, particularly crying duration, need to be further elucidated.

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Appendix A: Institutional Review Board Approval Letter

LAU
الجامعة اللبنانية الأميركية
Lebanese American University

Institutional Review Board (IRB) لجنة الأخلاقيات

NOTICE OF IRB APPROVAL

To: Dr. Maya Bassil
Associate Professor
School of Arts & Sciences

APPROVAL ISSUED: 11 April 2019
EXPIRATION DATE: 11 April 2020
REVIEW TYPE: EXPEDITED – Initial

Date: April 11, 2019
RE: ***IRB #:*** *LAU.SAS.MB2.11/Apr/2019*
Protocol Title: *The Effect of a Maternal Dairy-Free Lebanese Mediterranean Diet on Colic and Allergy Symptoms in Exclusively Breastfed Infants*

The above referenced research project has been approved by the Lebanese American University, Institutional Review Board (LAU IRB). This approval is limited to the activities described in the Approved Research Protocol and all submitted documents listed on page 2 of this letter. **Enclosed with this letter are the stamped approved documents that must be used.**

APPROVAL CONDITIONS FOR ALL LAU APPROVED HUMAN RESEARCH PROTOCOLS

LAU RESEARCH POLICIES: *All individuals engaged in the research project must adhere to the approved protocol and all applicable LAU IRB Research Policies. PARTICIPANTS must NOT be involved in any research related activity prior to IRB approval date or after the expiration date.*

PROTOCOL EXPIRATION: *The LAU IRB approval expiry date is listed above. The IRB Office will send an email at least 45 days prior to protocol approval expiry - Request for Continuing Review - in order to avoid any temporary hold on the initial protocol approval. It is your responsibility to apply for continuing review and receive continuing approval for the duration of the research project. Failure to send Request for Continuation before the expiry date will result in suspension of the approval of this research project on the expiration date.*

MODIFICATIONS AND AMENDMENTS: *All protocol modifications must be approved by the IRB prior to implementation.*

NOTIFICATION OF PROJECT COMPLETION: *A notification of research project closure and a summary of findings must be sent to the IRB office upon completion. Study files must be retained for a period of 3 years from the date of notification of project completion.*

IN THE EVENT OF NON-COMPLIANCE WITH ABOVE CONDITIONS, THE PRINCIPAL INVESTIGATOR SHOULD MEET WITH THE IRB ADMINISTRATORS IN ORDER TO RESOLVE SUCH CONDITIONS. IRB APPROVAL CANNOT BE GRANTED UNTIL NON-COMPLIANT ISSUES HAVE BEEN RESOLVED.

If you have any questions concerning this information, please contact the IRB office by email at irb@lau.edu.lb

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The IRB operates in compliance with the national regulations pertaining to research under the Lebanese Minister of Public Health's Decision No.141 dated 27/1/2016 under LAU IRB Authorization reference 2016/3708, the international guidelines for Good Clinical Practice, the US Office of Human Research Protection (45CFR46) and the Food and Drug Administration (21CFR56). LAU IRB U.S. Identifier as an international institution: FWA00014723 and IRB Registration # IRB00006954 LAUIRB#1

Institutional Review Board
Lebanese American University
11 APR 2019
APPROVED

Dr. Joseph Stephan
Chair, Institutional Review Board

DOCUMENTS SUBMITTED:

LAU IRB Exempt Application	Received 19 February 2019
Research Proposal Submission form	Received 26 February 2019
Approval Letter: Sophia Maternity center	Received 3 March 2019
Protocol	Received 19 February 2019, amended 18 March 2019
Consent Form For Intervention Group English	Received 19 February 2019, amended 18 March 2019
Consent Form For Intervention in Arabic	Received 19 February 2019, amended 18 March 2019
Consent Form for Control Group in English	Received 19 February 2019, amended 18 March 2019
Consent Form For Control in Arabic	Received 19 February 2019, amended 18 March 2019
Advertisement English	Received 19 February 2019
Advertisement Arabic	Received 19 February 2019
Allergy Survey English	Received 19 February 2019
Allergy Survey Arabic	Received 19 February 2019
IRB Comments sent: 25 February 2019 5 March 2019 21 March 2019	**PI response to IRB's comments dated:** 25 February 2019 18 March 2019 4 April 2019
NIH Training – Maya Bassil	Cert.# 2536094 Dated (16 January 2018)
NIH Training – Marya Hanna	Cert.# 2919781 Dated (11 September 2018)

Appendix B: Consent Form for the Intervention and Control Group, Respectively

LEBANESE AMERICAN UNIVERSITY

Informed Consent Form To Participate in a Research Study
Title of Study: The effect of maternal diet on colic and allergy symptoms in exclusively breastfed infants **Version date:19-12-2018**

Principal Investigator: Maya Bassil, Lebanese American University

Telephone and E-mail: 01786456 Ext. 2469 , mbassil@lau.edu.lb

Co-Investigator (s) or Advisor:

Telephone and E-mail:

Location where the study will be conducted: Lebanon

Why are you being asked to take part in this study?

You are being asked to take part in a research study to assess the effect of maternal diet on colic and allergy symptoms of exclusively breastfed infants

Why is this research study being done?

The purpose of this study is to investigate whether following a specific diet by the mother would reduce colic and allergy symptoms in exclusively breastfed infants

What is the duration of the research study?

The study will take 5 months to be completed. Your participation will require one visit from you to Sophia Maternity Center after delivery of your baby.

What will happen during the study?

If you take part in this study, you will be asked to attend during your second or third pregnancy trimester an education session about a healthy diet, which you will be asked to follow for the remainder of the trial. You will also receive one lactation consultation. You will also be required to fill up a questionnaire and provide a stool sample of your baby on one day of week 2, 4 and 7 after delivery of your baby. You will also be contacted 3 times per week during week 2, 4 and 7 postnatal to collect information about your daily food intake (24-hour recall). Once the stool sample tests are completed, you will have the option to receive the results. However, no counseling regarding the results of the test will be provided.

What are other alternatives?

You have the alternative to choose not to participate in this research study.

What are the potential benefits if you decide to take part in this study?

The potential benefits of participating in this study are that if your future infant has colic and/or allergy symptoms, they might decrease. However, in the case that you do not receive direct benefit from your participation, the results of this study may ultimately benefit future research aiming to mitigate infant colic and allergy through dietary measures, thus improving the overall health of the mother and child.

What are the potential risks and discomfort if you decide to take part in this study?

This research study is considered to be of minimal risk. That means that the risks associated with this study are the same as what you face every day. There are no additional risks to those who take part in the study.

What will it cost you if you take part in this study?

There will be no cost to you from taking part in this study, except for the time involved in participating in the study. The stool test to be conducted and lactation consultation to be provided will be free of charge.

Will you be paid for taking part in this study?

You will not be paid to participate in this study.

How will you keep my information confidential?

We will keep your study records as confidential as possible. We will carefully protect the information you provide about yourself and your child. What we learn from your surveys will be described only in a way that does not identify you. To protect your privacy, the surveys will be linked to a secret code. Your name will only be recorded on the informed consent form. We will keep the secret code carefully protected in a locked file. The surveys will be stored unless you ask to have them destroyed after the study is completed. Access is only by the principal investigator of the study and authorized personnel. In addition, study records may be reviewed by the Institutional Review Board at the Lebanese American University. Your records will be monitored and may be audited without violating confidentiality. We may publish what we learn from this study. If we do, any published information resulting from the study will not mention the names of the people who participated in this study.

Voluntary Participation or Withdrawal

You should only take part in this study if you want to volunteer. You are free to participate in this study or withdraw at any time. If you choose not to be in the study or to withdraw later on from the study, this will not affect you in any way. There will be no penalty or loss of benefits you are entitled to receive if you stop taking part in the study. If you wish to withdraw from the study, you can contact Dr. Maya Bassil by phone Tel: 01786456 Ext, 2469 or by email: mbassil@lau.edu.lb. The researcher may decide to discontinue your participation in this study without your permission if they feel it may be bad for you.

Whom to contact to get answers to your questions, concerns and complaints.

If you have any questions, concerns or complaints, please call the Principal Investigator / researcher of the study, listed on the first page of this informed consent document

If you have any questions about your rights or welfare as a participant in this study, or you want to talk to someone outside the research group, please contact the Institutional Review Board Office at the Lebanese American University at (01-786456 ext. 2546).

If you have any questions regarding the study, please contact any of the doctors listed on the first page of this informed consent document

CONSENT TO TAKE PART IN THE STUDY

I have carefully read the above information about this study. All of my questions have been answered to my satisfaction. I know that I may refuse to take part in or withdraw from the study at any time. **I freely give my consent to take part in this study.** I understand that by signing this form I am agreeing to take part in the study. I have received a copy of this form to take with me

____

Name of Subject

_________________________________ _____________

Signature of Subject Date

STATEMENT OF PERSON OBTAINING CONSENT

I certify that I have fully explained to the person taking part in the study the nature of the above research study, the potential risks and benefits and I have offered to answer any question that he/she may have.

_______________________________ _____________

Signature of Principal Investigator/Designate Date

____

Name of Principal Investigator/Designate

Informed Consent Form To Participate in a Research Study
Title of Study: The effect of maternal diet on colic and allergy symptoms in exclusively breastfed infants **Version date:19-12-2018**

Principal Investigator: Maya Bassil, Lebanese American University

Telephone and E-mail: 01786456 Ext. 2469 , mbassil@lau.edu.lb

Co-Investigator (s) or Advisor:

Telephone and E-mail:

Location where the study will be conducted: Lebanon

Why are you being asked to take part in this study?

You are being asked to take part in a research study to assess the effect of maternal diet on colic and allergy symptoms of exclusively breastfed infants

Why is this research study being done?

The purpose of this study is to investigate whether following a specific diet by the mother would reduce colic and allergy symptoms in exclusively breastfed infants

What is the duration of the research study?

The study will take 5 months to be completed. Your participation will require one visit from you to Sophia Maternity Center after delivery of your baby.

What will happen during the study?

If you take part in this study, you will receive one lactation consultation. You will also be required to fill up a questionnaire and provide a stool sample of your baby on one day of week 2, 4 and 7 after delivery of your baby. You will also be contacted 3 times per week during week 2, 4 and 7 postnatal to collect information about your daily food intake (24-hour recall). Once the stool sample tests are completed, you will have the option to receive the results. However, no counseling regarding the results of the test will be provided.

What are other alternatives?

You have the alternative to choose not to participate in this research study.

What are the potential benefits if you decide to take part in this study?

There are no direct benefits to you. However, the results of this study may ultimately benefit future research aiming to mitigate infant colic and allergy through dietary measures, thus improving the overall health of the mother and child.

What are the potential risks and discomfort if you decide to take part in this study?

This research study is considered to be of minimal risk. That means that the risks associated with this study are the same as what you face every day. There are no additional risks to those who take part in the study.

What will it cost you if you take part in this study?

There will be no cost to you from taking part in this study, except for the time involved in participating in the study. The stool test to be conducted and lactation consultation to be provided will be free of charge.

Will you be paid for taking part in this study?

You will not be paid to participate in this study.

How will you keep my information confidential?

We will keep your study records as confidential as possible. We will carefully protect the information you provide about yourself and your child. What we learn from your surveys will be described only in a way that does not identify you. To protect your privacy, the surveys will be linked to a secret code. Your name will only be recorded on the informed consent form. We will keep the secret code carefully protected in a locked file. The surveys will be stored unless you ask to have them destroyed after the study is completed. Access is only by the principal investigator of the study and authorized personnel. In addition, study records may be reviewed by the Institutional Review Board at the Lebanese American University. Your records will be monitored and may be audited without violating confidentiality. We may publish what we learn from this study. If we do, any published information resulting from the study will not mention the names of the people who participated in this study.

Voluntary Participation or Withdrawal

You should only take part in this study if you want to volunteer. You are free to participate in this study or withdraw at any time. If you choose not to be in the study or to withdraw later on from the study, this will not affect you in any way. There will be no penalty or loss of benefits you are entitled to receive if you stop taking part in the study. If you wish to withdraw from the study, you can contact Dr. Maya Bassil by phone Tel: 01786456 Ext, 2469 or by email: mbassil@lau.edu.lb. The researcher may decide to discontinue your participation in this study without your permission if they feel it may be bad for you.

Whom to contact to get answers to your questions, concerns and complaints.

If you have any questions, concerns or complaints, please call the Principal Investigator / researcher of the study, listed on the first page of this informed consent document

If you have any questions about your rights or welfare as a participant in this study, or you want to talk to someone outside the research group, please contact the Institutional Review Board Office at the Lebanese American University at (01-786456 ext. 2546).

If you have any questions regarding the study, please contact any of the doctors listed on the first page of this informed consent document

CONSENT TO TAKE PART IN THE STUDY

I have carefully read the above information about this study. All of my questions have been answered to my satisfaction. I know that I may refuse to take part in or withdraw from the study at any time. **I freely give my consent to take part in this study.** I understand that by signing this form I am agreeing to take part in the study. I have received a copy of this form to take with me

____

Name of Subject

____________________________________ _______________

Signature of Subject Date

STATEMENT OF PERSON OBTAINING CONSENT

I certify that I have fully explained to the person taking part in the study the nature of the above research study, the potential risks and benefits and I have offered to answer any question that he/she may have.

__________________________________ _______________

Signature of Principal Investigator/Designate Date

____

Name of Principal Investigator/Designate

Appendix C: Questionnaire

The effect of a maternal dairy-free Mediterranean diet on colic and allergy symptoms in exclusively breastfed infants

Code: ______________________

<u>Week 2 postnatal</u>

1. **Mother's Age (years):**
2. **Mother's Pre-pregnancy weight (Kg):**
3. **Mother's current weight (Kg):**
4. **Mother's height (cm):**
5. **Baby's birthweight (g):**
6. **Baby's current weight (g):**
7. **Is the mother exclusively breastfeeding?** □ YES □NO
8. **Place of residence :**
   □ North of Lebanon □ Mount Lebanon

   □ Beirut □ South of Lebanon

   □ Beqaa □ Nabatieh

9. **Mother's Education:**
   - □ < 7 years of schooling
   - □ High School Education
   - □ College Education
   - □ Technical Education
   - □ Graduate Education

10. Which of the following problems did your baby have during the past 2 weeks? (please "x" all that apply)

- □ Fever
- □ Diarrhea
- □ Vomiting
- □ Ear Infection
- □ Colic (Excessive straining and body tension)
- □ Fussy or irritable
- □ Runny nose or cold
- □ Cough or wheeze
- □ Asthma
- □ Eczema
- □ Reflux
- □ None of these

11. Did your baby receive any of the following medicines in the past 2 weeks? (Please do not include vitamins or minerals.)

	YES	NO
Antibiotics		
Other medicines		

12. How many stools (dirty diapers) does your baby usually have in a 24-hour period? If less than one a day, how many days usually pass between stools?

___________ NUMBER OF STOOLS IN 24 HOURS

OR

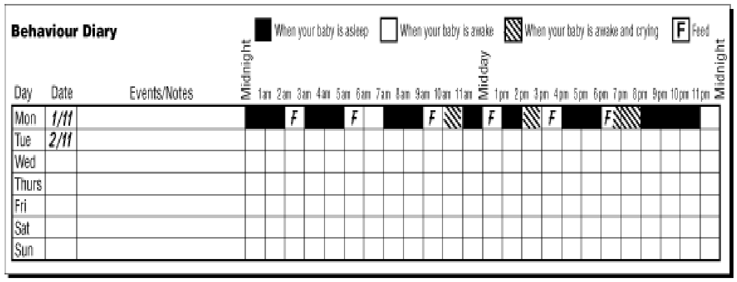
ONE STOOL EVERY ____________ DAYS

13. How would you describe your baby's stool in the past 7 days? (please "x" all that apply)

- □ Hard
- □ Formed
- □ Soft
- □ Semi-watery
- □ Watery

14. Baby Behavior Diary (Please complete on the last page of this questionnaire)

Example:



Day	Mon	Tue	Wed	Thurs	Fri	Sat	Sun
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Notes							
Mid-night							
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5 am							
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Mid-day							
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5 pm							
6 pm							
7 pm							
8 pm							
9 pm							
10 pm							
11 pm							

Week 4 postnatal

1. **Mother's current weight (Kg):**
2. **Baby's current weight (g):**
3. **Is the mother exclusively breastfeeding?** □ YES □NO
4. Which of the following problems did your baby have during the past 2 weeks? (please "x" all that apply)
   - □ Fever
   - □ Diarrhea
   - □ Vomiting
   - □ Ear Infection
   - □ Colic (Excessive straining and body tension)
   - □ Fussy or irritable
   - □ Runny nose or cold
   - □ Cough or wheeze
   - □ Asthma
   - □ Eczema
   - □ Reflux
   - □ None of these

5. Did your baby receive any of the following medicines in the past 2 weeks? (Please do not include vitamins or minerals.)

	YES	NO
Antibiotics		
Other medicines		

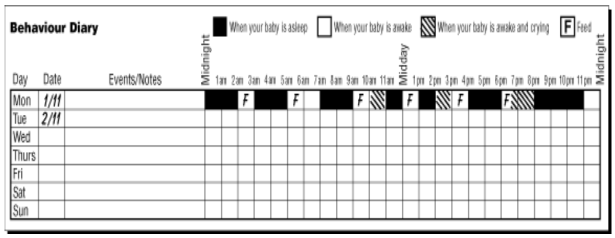
6. How many stools (dirty diapers) does your baby usually have in a 24-hour period? If less than one a day, how many days usually pass between stools?

   ___________ NUMBER OF STOOLS IN 24 HOURS
   OR
   ONE STOOL EVERY ____________ DAYS

7. How would you describe your baby's stool in the past 7 days? (please "x" all that apply)
   - □ Hard
   - □ Formed
   - □ Soft
   - □ Semi-watery
   - □ Watery

8. Baby Behavior Diary (Please complete on the last page of this questionnaire)

Example:



Day	Mon	Tue	Wed	Thurs	Fri	Sat	Sun
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Week 7 postnatal

1. **Mother's current weight (Kg):**
2. **Baby's current weight (g):**
3. **Is the mother exclusively breastfeeding?** □ YES □NO
4. Which of the following problems did your baby have during the past 2 weeks? (please "x" all that apply)

   □ Fever □ Runny nose or cold
   □ Diarrhea □ Cough or wheeze
   □ Vomiting □ Asthma
   □ Ear Infection □ Eczema
   □ Colic (Excessive straining and body tension) □ Reflux
   □ Fussy or irritable □ None of these

5. Did your baby receive any of the following medicines in the past 2 weeks? (Please do not include vitamins or minerals.)

	YES	NO
Antibiotics		
Other medicines		

6. How many stools (dirty diapers) does your baby usually have in a 24-hour period? If less than one a day, how many days usually pass between stools?

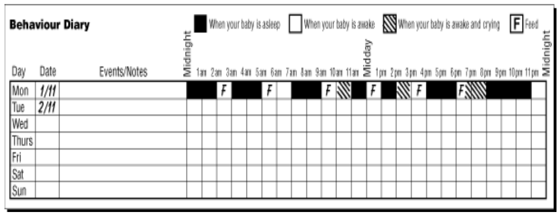
   ___________ NUMBER OF STOOLS IN 24 HOURS
   OR
   ONE STOOL EVERY ____________ DAYS

7. How would you describe your baby's stool in the past 7 days? (please "x" all that apply)

   □ Hard
   □ Formed
   □ Soft
   □ Semi-watery
   □ Watery

8. Baby Behavior Diary (Please complete on the last page of this questionnaire)

Example:



Day	Mon	Tue	Wed	Thurs	Fri	Sat	Sun
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Appendix D: Advertisements

You are Invited to a Research Study on
“Maternal Diet and the exclusively breastfed baby”

The Department of Natural Sciences, Nutrition program is conducting a research study to evaluate the effect of maternal diet on colic and allergy symptoms of exclusively breastfed babies

If you or your family members are pregnant in their first or second trimester, with their first child and plan to exclusively breastfeed, please contact:

Marya Hanna, MS Student, Lebanese American University, at *“70/234344”* or e-mail at *marya.hanna@lau.edu*

If you are found eligible and choose to participate, you will receive one free breastfeeding consultation.

أنت مدعوة لدراسة بحثية
"تأثير النظام الغذائي للأمهات على أعراض المغص والحساسية من الأطفال الذين يرضعون حصريا الرضاعة الطبيعية"

يقوم قسم العلوم الطبيعية في برنامج التغذية بإجراء دراسة بحثية لتقييم تأثير النظام الغذائي للأم على أعراض المغص والحساسية لدى الأطفال الذين يرضعون من الثدي بشكل حصري.

إذا كنت أنت أو أفراد عائلتك حاملًا في المرحلة الأولى أو الثانية من الحمل ، مع طفلك الأول وتخططين للرضاعة الطبيعية حصراً ، يرجى الاتصال بـ:

Marya Hanna، Lebanese American University "70/234344" أو البريد الإلكتروني على
marya.hanna@lau.edu

إذا كنت مؤهلة وترغبين المشاركة ، ستحصلي على جلسة تعليم الرضاعة واحدة مجانية.