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Background: During lactation, there is an increased maternal need for almost all nutrients. It has been reported that maternal status of some nutrients (i.e. vitamin A, C and DHA) can affect breast milk composition. Data about dietary patterns of lactating women in the United States are scarce and only a small number of studies evaluated micronutrient intake. Objectives: The primary objective of this pilot study is to describe the dietary patterns of lactating women in central North Carolina using established and emerging dietary assessment tools, with a particular focus on fruits and vegetables (F&V). A secondary objective is to explore the relationship between maternal intake of fruits, vegetables, and vitamin A, with breast milk vitamin A and carotenoid concentrations. Methods: In this cross-sectional study, 40 lactating women residing in central North Carolina were recruited between July 2018 and April 2019. We collected dietary information using three assessment methods: 24-hour Food Record, REAP dietary screener, and Veggie Meter™, a non-invasive biomarker of F&V intake that has been validated in non-lactating individuals. A single breast milk sample was also collected. Results: Thirty-one participants (78%) were flagged for referral to a Registered Dietitian based on their REAP scores. There was a significant correlation between the Food Record F&V servings and the Veggie Meter™ scores for subjects who reported that the 24-hour food record was reflective of their usual intake (n=9; p=0.031; R=0.71). The relationship of breast milk beta-carotene and the Veggie Meter™ scores was also statistically significant (n=12; p=0.022; R=0.65). Conclusion: F&V intake in lactating women is often below recommendations. The Veggie Meter™ scores correlated positively with breast milk beta-carotene and F&V intake which can provide an objective method of assessing F&V intake in lactating women in future studies.

DIETARY PATTERNS OF LACTATING WOMEN IN CENTRAL NORTH CAROLINA EVALUATED USING THREE VALIDATED ASSESSMENT TOOLS

by

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APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Background Information

Various health related organizations such as the World Health Organization (WHO) and American Academy of Pediatrics (AAP), and The Academy of Nutrition and Dietetics recommend exclusive breastfeeding during the first 6 months of life and partially until at least 12 months.[1] Breastfeeding is associated with several benefits for mother and the infant including: improved immunological functions; bonding between mother and infant; decreased risks of postpartum depression; and reduced health care costs for both infant and mother due to lower risk of developing certain diseases.[2]

Maternal nutritional needs significantly vary during non-pregnant, pregnant and lactation periods for both macro and micronutrients requirements. During lactation, there is an increased maternal need for almost all nutrients. It has been reported that maternal status of some nutrients (i.e. vitamin A, C and DHA) can affect breast milk composition.[9] Moreover, nutrition status can vary widely between mothers, due to biological, economic, cultural and geographical factors.[6-11] For this reason, an adequate maternal dietary intake is a key factor in providing proper nutrition to the infant through breast milk.

Data about dietary patterns of lactating women in the United States are scarce and only a small number of studies evaluated micronutrient intake.[6-9] Moreover, the collection method to obtain dietary intake information from lactating women varies to a great extent, causing the evaluation to be complex and not generalizable.[9] The most commonly used dietary collection methods are Food Frequency Questionnaires, 24-hour recalls and food records. Novel methods for assessing dietary intake using a biomarker are emerging but have not yet been used in lactating women. According to Kellie Casavale, Senior Nutrition Advisor in the FDA, pregnant and lactating women are considered a rare population in current national surveillance systems, thus obtaining more information about this population is essential to better understand the relationship between maternal dietary intake and breast milk composition.[10]

Some studies have reported low fruits and vegetables (F&V) intake in lactating women. F&V are excellent sources of vitamin A, such as sweet potatoes, carrots, mango and spinach, therefore a low consumption of F&V may result in inadequate vitamin A intake.[29] A recent study found that lactating women were below intake for vitamin A, though breast milk vitamin A concentration was not assessed.[6]

Objectives

The primary objective of this pilot study is to describe the dietary patterns of lactating women in central North Carolina using established and emerging dietary assessment tools, with a particular focus on F&V. A secondary objective is to explore the relationship between maternal intake of fruits, vegetables, and vitamin A, with breast milk vitamin A and carotenoid concentrations.

Study Aims

Specific aim 1: To describe the self-reported F&V intake of a convenience sample of lactating women in North Carolina using a 24-hour food record and a validated screener. Hypothesis 1: F&V intake will be lower than recommended for lactating women.

Specific aim 2: To evaluate relationships between self-reported F&V intake with a validated biomarker (Veggie Meter™) for F&V intake in lactating women. Hypothesis 2: There will be a positive relationship between Veggie Meter™ scores and self-reported F&V servings.

Specific aim 3: To evaluate the relationship between breast milk vitamin A concentration and F&V intake assessed using both a 24-hour food record and Veggie Meter™ scores. Hypothesis 3: Lactating women that consume more F&V will have higher concentrations of vitamin A in their breast milk.

CHAPTER II

REVIEW OF THE LITERATURE

Although lactation is a time of increased nutritional need for mothers, information on dietary patterns in lactating women and how this influences milk composition is scarce. This review will focus on what is known about fruits and vegetables (F&V) consumption in lactating women, and how maternal intake of F&Vs influences breast milk vitamin A.

What is Known About F&V Consumption in Lactating Women?

George et al.[12] examined the dietary patterns during pregnancy and the postpartum period of low-income women (n=149) in a longitudinal cohort study performed in 2005. The food groups recommendations were based on My Guide Pyramid. F&V consumption decreased from 3.4 to 1.7 servings of fruit/day and 2.5 to 2.0 vegetable servings/day when comparing pregnancy and 6 months postpartum. Fruit intake was higher during pregnancy among breastfeeding women when compared to women who were bottle feeding. Fruit consumption included fruit juices, which accounted for almost half of the total intake.

In a study conducted in North Carolina in 2006, overweight lactating women who were exclusively breastfeeding (n=35) were recruited to participate in an RCT and assigned to either a control or an intervention (diet + exercise) group. All the participants received a multivitamin supplement containing at least 50% of the RDA for lactating women. Dietary information was collected at baseline (4wk postpartum) and 14wk postpartum through a 3 consecutive day food record that was recorded by tape recorder. At baseline, the average servings of F&V consumed by the intervention and control group were 3 and 1.6 respectively. The vitamin A intake (RE) was ~1567. After 14 weeks, the average of F&V decreased to 1.75 and 1.45 respectively. However, vitamin A intake increased in the control group and decreased in the intervention group. The servings recommendations were based on the Food Guide Pyramid, which are 2-4 for fruits and 3-5 for vegetables. At 1 month postpartum, the recommendation for fruits was achieved, but not for vegetables. After 14 weeks, neither fruit nor vegetable intake reached the recommended amounts for lactating women.[13]

Østbye et al.[14] conducted a randomized control trial in 2009 to encourage long-term lifestyle changes in lactating women (n=450) in order to improve their health and reduce BMI. Over a period of 9 months, the intervention group was instructed to participate in eight nutrition education classes, ten physical activity classes and six counseling sessions by phone. Food patterns were analyzed through two 24-hour recall interviews at baseline (6 weeks postpartum) and 1 month after the intervention (12 months postpartum), as well as a brief FFQ that assessed, among other items, F&V consumption. Results showed that weight loss between groups was not significant, however attending classes had a positive association with weight loss. In the intervention group, F&V intake at baseline was 3.32 servings, compared to 3.38 servings post intervention. Changes in diet and higher activity levels were not observed between groups, and the authors attribute that to the fact that women during the postpartum period are very busy with their infant and that makes it even harder to target the population for interventions.

In a study conducted in North Carolina in 2011, Durham et al.[15] compared the differences in dietary intake among the 3 groups of women between 6 and 9 weeks postpartum (n=450) that were either fully breastfeeding (n=162), mixed feeding (n=126) or formula feeding (n=162), and compared to the DRIs and MyPyramid recommendations. Fully breastfeeding women consumed 2.7 less servings/day of fruits and 3.3 less servings/day of vegetables compared to recommendations. Women that were mixed feeding also did not achieve the recommendations and consumed 2.6 less serving/day of fruits and 3.4 of vegetables. All participants were not consuming adequate amounts of vitamin A, E, C, and folate and did not achieve the recommended amount of any food group servings.

Colleran et al.[16] evaluated the effectiveness of a meal plan based on MyPyramid for women in the lactation period from 4 to 20 weeks postpartum and compared with weight loss. The participants were randomly divided into two groups, the intervention and the minimal care groups. The 16-week intervention consisted of an intense exercise routine and reduction of calories intake by 500 kcal/day. The minimal care group was instructed to not exercise in a structured way and to not reduce the calories intake during the intervention period. In the minimal care group, fruit consumption decreased 19% and vegetable intake decreased 6% from baseline to endpoint. The intervention group increased the consumption of F&V by 21% and 17%, respectively. The consumption of

F&V was increased after the intervention, however the increase was not enough to reach the recommended amounts needed by lactating women.

Wiltheiss et al.[17] analyzed the diet quality of lactating women (n=392) that were either exclusively breastfeeding or mixed feeding their infants to determine alterations in dietary patterns and weight. The study was published in 2013 and diet and weight were assessed at five months postpartum (baseline) and after the intervention period (15 months postpartum) and compared to HEI-2005 scores. The participants were randomly assigned to an intervention and a control group. The intervention consisted of monthly kits received via mail with information about adequate portion sizes, strategies to increase F&V consumption, healthier recipes options and other relevant nutrition education information. The control group also received monthly kits but with general information about reading skills and how to motivate preschoolers instead. Results showed that pregnant women tend to be more aware of their diets, consuming higher amounts of vitamins and minerals through F&V during that period. However, after giving birth the trend was to consume less than the amount recommended for the lactation period. At baseline (2 to 7 months postpartum), approximately 91% of the participants did not achieve the recommended HEI score of 80 or higher. There were no changes in the total fruit scores at baseline and after intervention in any of the groups. Total vegetables scores showed a slight decrease, yet not significant, when comparing the control and intervention group after 15 months postpartum. However, the percentage of participants with a total HEI-2005 score over 80 points increased 50% in the intervention group compared to the control. Weight changes were not significant in either group.

In 2016, Stallings et al.[18] evaluated the variety of F&V intake among WIC participants during pregnancy (n=1085) and postpartum (n=1015) and compared to eligible and non-eligible women. Fruit intake decreased from 2.65 servings/day to 1.61 when comparing prenatal and postnatal periods. However, there was a slight increase in vegetable intake from 2.94 to 3.05 servings/day. F&V intake was significantly higher in breastfeeding women compared to non-breastfeeding. Moreover, those receiving the benefits from WIC had a higher consumption of F&V compared to those who were not receiving any benefits or were not eligible.

In a study conducted in 2018, Essa et al.[19] evaluated the possibility of increasing F&V consumption among lactating women (n=10) through an intervention design. Results showed that at baseline the average intake of F&V among a small sample of breastfeeding women was approximately 2 servings/day. After a 12-week intervention where the control group was instructed to verify the information provided by the "ChooseMyPlate" page for moms and moms-to-be, no significant changes in F&V consumption were observed. However, the intervention group received a box of fresh produce at home weekly and results showed a significant four-fold increase (2.6 to 9.9 servings) in the intake of F&V. While nutrients were not measured in breast milk, significant changes in breast milk cytokines, growth factors, and hormones were observed in the intervention group.

Based on several studies conducted over the past decades in the U.S., the diet patterns of lactating women appear insufficient when it comes to providing the recommended amount of F&V. Some interventions were shown to be effective in increasing the intake of F&V in lactating women including weekly produce boxes delivered at home, access to WIC benefits, and the use of a menu planner.

How Does Maternal Vitamin A Intake Impact Breast Milk Vitamin A Composition?

In 2001, Macias et al.[20] evaluated the levels of vitamin A in different stages of breast milk from 21 healthy women who delivered term infants. Samples were collected at birth (colostrum), 7d (transitory milk) and 15d (mature milk) postpartum. Results showed that colostrum samples had the highest amounts of beta-carotene (67.4 ng/ml) and total vitamin A (1.02 µg/ml) compared to the other two stages. After 15 days, the concentrations decreased 29 and 32%, respectively. The authors concluded that different carotenoids, based on polarity, are transferred to maternal milk through different mechanisms. Colostrum appears to contain the highest amount of beta-carotene and total vitamin A due to specific metabolic pathways utilized in colostrogenesis. Maternal intake presented a high variability, however analysis comparing dietary intake and breast milk composition were not performed.

Canfield et al.[21] conducted a cross-sectional study in 2003 that assessed the concentration of beta-carotene and retinol in breast milk from healthy lactating women (n=465). Samples were collected from women who lived in 9 different countries and compared to their dietary patterns. Participants were included in the study if they reported consuming at least 3 servings of F&V combined per day. Results showed that the mean concentration of beta-carotene in the milk was 0.045µmol/L among all the participants and retinol mean concentration was 1.233 µmol/L. Milk beta-carotene and retinol appear to be a good indicator of vitamin A status for both mother and infant in the majority of the countries evaluated. Dietary intake of vitamin A also showed a correlation to breast milk concentrations, given that levels mimicked changes in serum levels.

In 2007, Khan et al.[22] evaluated the effect of increasing the consumption of F&V in relation to vitamin A levels in serum and breast milk in anemic Vietnamese women. The intervention was divided into 4 different groups, which had an increased consumption of vegetables (n=73), fruit (n=69), retinol rich foods (n=70), and the control group (n=68). After 10 weeks of intervention, serum retinol levels were higher in the retinol-rich, vegetables and fruit groups compared to the control. Serum total beta-carotene levels were increased in all intervention groups when compared to the control. In breast milk, retinol levels were also higher in the intervention groups compared to the control. Beta-carotene in breast milk had the lowest levels after 10 weeks in the control group compared to the intervention groups. The most significant changes were observed in the retinol serum levels and beta-carotene levels were more evident in serum than in breast milk. In sum, although studies have suggested that serum levels are a better indicator of vitamin A status, there is also a strong connection between vitamin A intake and breast milk levels.

In 2009, Mello-Neto et al.[23] analyzed the composition of breast milk from Brazilian mothers (n=136) who had donated their milk to a human milk bank. They evaluated vitamin A levels in breast milk and compared to maternal characteristics. Results showed that 68% of the participants who had lower iron levels also presented a lower concentration of vitamin A in breast milk. Mothers who worked outside the home presented higher levels of milk vitamin A compared to those who stayed at home.

Moreover, maternal age appears to have an influence in vitamin A levels where older mothers had higher levels compared to younger mothers. Excessive body fat was negatively associated with vitamin A concentration in breast milk. In conclusion, several factors can influence vitamin A levels even in apparently healthy lactating women.

In 2019, Zielinska et al.[24] evaluated the relationship between carotenoids in breast milk and dietary intake of vitamin A in Polish lactating women (n=53). Composition was measured at 3 and 6 months of lactation and results showed a positive relationship between diet and breast milk carotenoids content. Beta-carotene levels stayed consistent from 3 to 6 months. Higher maternal BMI was associated with lower levels of carotenoids in breast milk. In conclusion, maternal dietary intake of vitamin A is important to maintain levels in breast milk adequate and provide proper nutrition to infants.

CHAPTER III

FRUIT AND VEGETABLE INTAKE OF LACTATING WOMEN IN CENTRAL NORTH CAROLINA AND RELATIONSHIPS WITH BREAST MILK VITAMIN A CONCENTRATIONS

A version of this article will be submitted to *The Journal of the Academy of Nutrition and Dietetics.*

Introduction

Various health related organizations such as the World Health Organization (WHO) and American Academy of Pediatrics (AAP), and The Academy of Nutrition and Dietetics recommend exclusive breastfeeding during the first 6 months of life and partially until at least 12 months.[1] Breastfeeding is associated with several benefits for mother and the infant including: improved immunological functions; bonding between mother and infant; decreased risks of postpartum depression; and reduced health care costs for both infant and mother due to lower risk of developing certain diseases.[2]

Maternal nutritional needs significantly vary during non-pregnant, pregnant and lactation periods for both macro and micronutrients requirements. During lactation, there is an increased maternal need for almost all micronutrients. It has been reported that maternal status of some nutrients (i.e vitamin A, C and DHA) can affect breast milk composition.[9] Moreover, nutrition status can vary widely between mothers, due to biological, economic, cultural and geographical factors.[6-11] For this reason, an adequate maternal dietary intake is a key factor in providing proper nutrition to the infant through breast milk.

Data about dietary patterns of lactating women in the United States are scarce and only a small number of studies evaluated micronutrient intake.[6-9] Moreover, the collection method to obtain dietary intake information from lactating women varies to a great extent, causing the evaluation to be complex and not generalizable.[9] The most commonly used dietary collection methods are Food Frequency Questionnaires, 24-hour recalls and food records. Novel methods for assessing dietary intake using a biomarker are emerging but have not yet been used in lactating women. According to Kellie Casavale, Senior Nutrition Advisor in the FDA, pregnant and lactating women are considered a rare population in current national surveillance systems, thus obtaining more information about this population is essential to better understand the relationship between maternal dietary intake and breast milk composition.[10]

Some studies have reported low fruits and vegetables (F&V) intake in lactating women. F&V are excellent sources of vitamin A, such as sweet potatoes, carrots, mango and spinach, therefore a low consumption of F&V may result in inadequate vitamin A intake. [29] A recent study found that lactating women were below intake for vitamin A, though breast milk vitamin A concentration was not assessed.[6] Vitamin A requirements for non-lactating women are 700µg of Retinol Activity Equivalents (RAE) per day and for lactating women they are 1300µg RAE/day. [33] For infants the AI are 400µg and is based on the assumption that breast milk contains on average 485µg/L. Overall, vitamin

A is important to growth, cell differentiation, immune system functions, vision and other developmental functions to the infant.[34]

Given the limited dietary data on lactating women, the primary objective of this pilot study is to describe the dietary patterns of lactating women in central North Carolina, and compare findings using established and emerging dietary assessment tools, with a particular focus on F&V. A secondary objective is to explore the relationship between maternal intake of fruits, vegetables, and vitamin A, with breast milk vitamin A and carotenoid concentrations.

Methods

Study Design and Participants

The primary purpose for the milk samples collected in this study was to determine how donations of human milk to a milk bank are influenced by milk banking processes. A secondary purpose was to assess potential relationships between maternal dietary patterns and breast milk Vitamin A, which is the focus of this research.

In this cross-sectional study, 40 lactating women residing in central North Carolina were recruited between July 2018 and April 2019 through flyers that were published online in social media platforms. Participation criteria were based on the ability to donate a relatively large volume of milk without impacting infant feeding routines, and included: gave birth to healthy term infants (>37 weeks gestation); currently breastfeeding an infant 4 to 11 months old ; able to typically pump at least 3 ounces of breast milk during one pumping session; and willing to complete a 24-hour food record and visit the research lab at the University of North Carolina Greensboro to provide a single breast milk sample. At the time participants visited our lab, written informed consent was obtained. Upon study completion, participants received a $40 gift card and a manual breast pump. This research was reviewed and approved by the University of North Carolina Greensboro Institutional Review Board.

Data Collection

Participants were asked to record all the foods and beverages consumed 24-hours prior to the visit to the research lab at the University of North Carolina Greensboro. They were provided a food model booklet, to help with portion sizes when completing the food record, and a food tracking log. During the lab visit, a researcher verified the food records with participants to ensure that any foods or beverages were properly recorded.

During the study visit, the Rapid Eating Assessment for Patients (REAP) screener was also administered.[25] It is a quick (10-15 minutes) 31-item validated screener that measures food intake, shopping and cooking patterns, as well as physical activity. The purpose of this screener is to help healthcare professionals to identify if a patient is at nutritional risk and if referral to a dietitian is necessary.[25]

The Veggie Meter™ is a validated non-invasive tool that is used as a biomarker for F&V intake. It measures skin carotenoids through finger scans using pressure-mediated reflection spectroscopy. [28] It has been used in food insecure populations, children, and adults, but it has not yet been tested in lactating women. [28] The tool became available part-way through the study; hence, the readings are only available for a subset of the study (n=12). Participants were asked to wash their hands with soap and water and dry them well before placing their right index finger in the device. Three Veggie Meter™ readings were obtained for each participant and the average score was computed.

Maternal weight was recorded using a Tanita BWB-800 digital scale and height was assessed using a Seca 216 Wall Mounted stadiometer. Participants were asked to use a new, manual breast pump (Lansinoh) to collect at least 3 ounces of breast milk, if possible. The expressed milk was divided into two equal portions and transferred to breast milk storage bags and frozen at -20C to mimic home storage conditions. One mL aliquots were also set aside from each sample for measurement of milk total vitamin A, beta-carotene and retinol. Aliquots were stored at -80C until analysis.

Dietary Intake Analysis

A single researcher entered 24-hour food record data into the Nutrition Data System for Research (NDSR) (software version 2017, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN). Data obtained from the 24-hour food records were converted to F&V servings using NDSR. Total vitamin A, retinol and beta-carotene intake, reported as µg of RAE,[35] were also obtained from the 24-hour food records. Participants who consumed 2.2 or more fruit servings were categorized as Meets Fruit MyPlate Recommendations = Yes. Participants who consumed 3.2 or more vegetable servings were categorized as Meets Vegetable MyPlate Recommendations = Yes. The cutoffs were established based on MyPlate recommendations for F&V servings using study population characteristics.[27] The food record also included a question to determine if the data reflected the participants usual intake which were coded as a binary variable (Y/N).

Answers from the REAP screener were evaluated using the scoring tool provided by the screener developers and further analyzed using NDSR to compute F&V servings. Individuals who identify 5 or more dietary patterns that are considered high risk (e.g. frequently consuming less than 2-3 servings of fruit daily; frequently consuming less than 3-4 servings of vegetables daily), are considered in need of referral to a dietitian, and were categorized at nutritional risk (Y/N). Specific questions about fruits ("Eat less than 2-3 servings of fruit a day?") and vegetables ("Eat less than 3-4 servings of vegetables/potatoes a day?") were converted to the affirmative (e.g. Meets Fruit and Vegetable Requirements) to allow comparison to 24-hour food record data as follows: Rarely/never (Yes); Sometimes /Usually/Often (No).

Veggie Meter™ results can range from 0 to 800, with higher values associated with greater F&V intake.[32] While no cut-off values are currently recommended for how to interpret Veggie Meter™ readings, a recent study of adults in New Zealand suggested that scores <250 warranted active encouragement of F&V intake, while scores >530 warranted endorsement of current intake patterns.[32] Therefore, for this study we classified F&V intake by Veggie Meter™ scores as Inadequate ($\leq$ 250), Appropriate ($\geq$ 530), and Indeterminant (>250 and < 530).

Breast Milk Analysis

Breast milk samples that had a corresponding Veggie Meter™ reading for the donor (n=12) were analyzed for total vitamin A, retinol and beta-carotene levels by the USDA Western Human Nutrition Research Center at the University of California, Davis using high performance liquid chromatography. Total RAE was calculated using a conversion of 1:1 for retinol and 1:12 for beta-carotene.[35]

Statistical Analyses

Descriptive statistics for F&V servings were computed from food records and the prevalence of participants not meeting MyPlate F&V recommendations were reported. The prevalence of participants with agreement regarding self-reported F&V intake using the 24-hour food record and REAP was also reported.

Pearson correlation coefficients were calculated using IBM SPSS Statistics Version 26 to probe for bi-variate relationships between numerical data including: F&V servings, total vitamin A, retinol and beta-carotene intakes (from the 24-hour food record); Veggie Meter™ scores; and breast milk vitamin A metabolites.

Results

A total of 40 women were recruited for the study and Veggie Meter™ and breast milk vitamin A measurements were obtained for a subset of 12 women. A summary of participants' demographics is shown in Table 1.

Table 1

Demographics of Study Participants (n=40)

	Mean ± SD	**Range**
Maternal age	29.6 ± 5.3	18-41
Weight (kg)	73.4 ± 18.2	46.5-129.4
Height (cm)	164.2 ± 9.4	149.5-179.5
BMI	27.2 ± 6	19.6-42.3
Infant Age (Months)	6.5 ± 2.3	4-11

Fruit and Vegetable Intake

A summary of numerical and categorical dietary data obtained from the Food Record, the REAP screener, and the Veggie Meter™ are shown in Table 2. Sixty-seven percent of participants (26/39) reported that their Food Record represented a typical day of intake. The percentage of participants self-reporting adequate intake of F&V was similar between the Food Record and REAP (30% and 33% respectively for fruit; 40% and 43% for vegetables). The within-subject agreement between the Food Record and REAP was 73% for fruit intake, and 48% for vegetable intake. When limiting comparison to just subjects reporting that the Food Record was a typical day (n=26), the within-subject agreement between the Food Record and REAP was 82% for fruit intake and 50% for vegetable intake. Seventy-eight percent of participants (31/40) were flagged for referral to a Registered Dietitian based on their REAP scores. Forty-two percent of participants (5/12) had Veggie Meter scores categorized as Inadequate, and 0% (0/12) had scores categorized as Adequate.

Retinol Activity Equivalents

The mean (range) percentage of RAE in maternal diets from retinol was 70% (1% to 99%), while 30% (1% to 99%) was from beta-carotene. Mean total breast milk RAE was 595 µg/L (332 to 1087 µg/L) with 98.9% (97.5% to 99.7%) of the RAE in milk coming from retinol and 1.1% (0.3% to 2.5%) coming from beta-carotene.

Table 2

Summary of Dietary Data Information Collected Using Three Different Dietary Assessment Methods

Summary of Numerical Dietary Data		
Food Record (n=40)	**Mean ± SD**	**Range**
Fruit Servings	1.8 ± 2.5	0-11
Vegetable Servings	3.2 ± 2.6	0.3-12.5
Retinol (RAE)	773 ± 586	25-2,361
Beta-Carotene (RAE)	475 ± 810	4-3241
Total Vitamin A (RAE)	1,248 ± 954	128-3,772
Veggie Meter (n=12)	**Mean ± SD**	**Range**
Veggie Meter Scores	263 ± 97	99 – 440

Summary of Categorical Dietary Data

Food Record (n=40)	Yes (%)		No (%)
Meets Fruit MyPlate Recommendation	30		70
Meets Vegetable MyPlate Recommendation	40		60
REAP Screener (n=40)	**Yes (%)**	**Sometimes (%)**	**No (%)**
Meets Fruit Intake Requirements of 2-3 Servings per Day	32.5	50	17.5
Meets Vegetable Intake Requirements of 3-4 Servings per Day	42.5	47.5	10

Relationships Between Measurements

There was a significant correlation between the Food Record F&V servings and the Veggie Meter™ scores (Figure 1), but only for subjects who reported that the 24-hour food record was reflective of their usual intake (n=9; p=0.031; R=0.71). The relationship of breast milk beta-carotene and the Veggie Meter™ scores (Figure 2) were also statistically significant (n=12; p=0.022; R=0.65). There was no significant relationship (p>0.05) between breast milk total vitamin A and retinol compared to the Veggie Meter™ scores or 24-hour food record data (total F&V servings, total Vitamin A intake, total retinol intake, and total beta-carotene intake). Similarly, there were no significant relationships between total dietary intake of vitamin A, retinol, or beta-carotene and Veggie Meter™ scores.

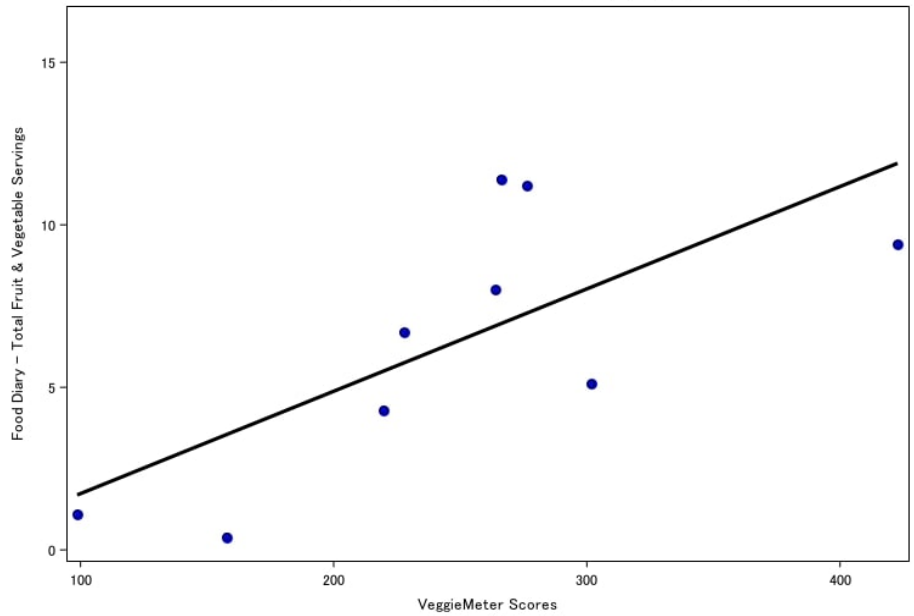

Figure 1. Relationship Between 24-hour Food Record Fruit and Vegetable Servings and Veggie Meter™ Scores.

Notes: Relationships were assessed using linear regression (p=0.031; R=0.71).

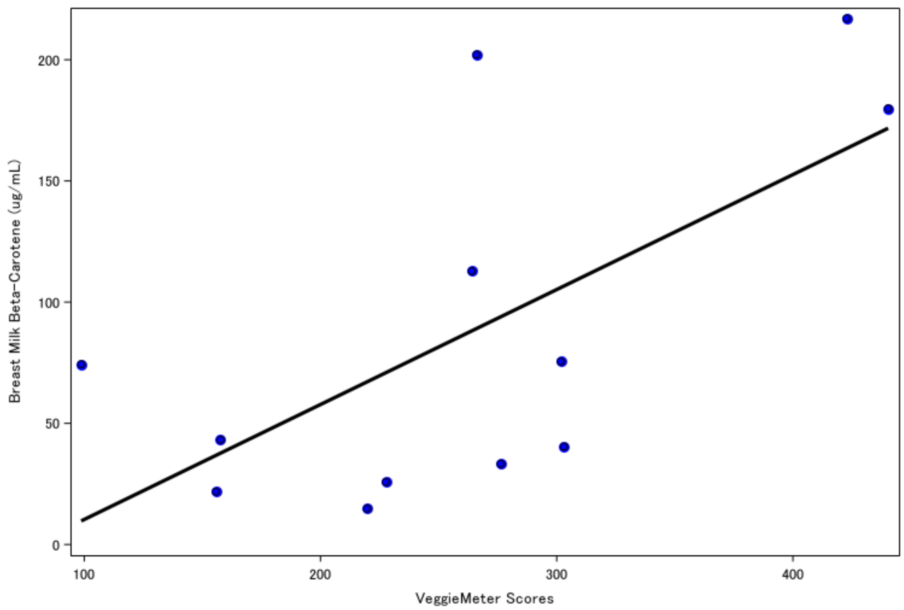

Figure 2. Relationship Between Breast Milk Beta-Carotene and Veggie Meter™ Scores.

Notes: Relationships were assessed using linear regression ($p=0.022$; $R=0.65$).

Discussion

In this study, we report poor overall diet quality of lactating women in central North Carolina, with 78% of them flagged for referral to a Registered Dietitian using a validated screener. The majority of lactating women who participated in the study consumed less F&V servings than recommended, according to three separate assessment instruments – a 24-hour Food Record, a validated screener, and a carotenoid skin scan. We found that there was a significant positive relationship between Veggie Meter™ scores and F&V servings in women whose 24-hour food record represented a typical day. Moreover, we found a significant positive relationship between Veggie Meter™ scores and breast milk beta-carotene values.

Fruits and Vegetables Intake in Lactating Women

Although dietary patterns of lactating women have not been widely explored, our findings of inadequate F&V consumption compared to recommendations are consistent with other authors.

Similar to our findings, five authors studied lactating women's consumption of F&V in the U.S. and found that their intake was below recommendations. The studies were performed between 2005 and 2018, and the number of participants included in the studies ranged from 10 to 1,015. The dietary assessment methods used included 24-hour recalls, food frequency questionnaires, and 3-day food records. [12,13,17,18,19]

Others have reported mixed results regarding F&V intake in lactating women. A study conducted in 2011 (N = 450) and a study conducted in 2012 (N = 27), both using 24-hour recalls as the dietary assessment method, reported that lactating women were consuming an inadequate amount of fruit servings and an adequate amount of vegetable servings. [15,16]

Contrary to our findings, Ostbye et al. conducted an RCT study in 2009 and found that a group of 450 lactating women were consuming an adequate amount of F&V. Over a period of 9 months, the intervention group was instructed to participate in eight nutrition education classes, ten physical activity classes and six counseling sessions by phone. In the intervention group, F&V intake at baseline was 3.3 servings, compared to 3.4 servings post intervention. However, the servings for F&V were combined instead of measured individually, which does not necessarily reflect an adequate amount for both F&V.[14]

The majority of contemporary evidence suggests that lactating women in the United States do not meet recommendations regarding F&V intake. Understanding the dietary beliefs of lactating women and barriers to F&V consumption is an important area of future research.

Veggie Meter™ as a Biomarker of Fruits and Vegetables Intake in Lactating Women

The Veggie Meter™ has been validated as a non-invasive biomarker of fruit and vegetable intake in children and adults, with different ages and ethnicities. Correlation coefficients ranged from R ~ 0.80 and R ~ 0.96 among the groups evaluated. [28] Compared to our findings, other studies found lower mean values of 175 ± 77 for food insecure populations,[30] similar values of 296.4 ±110.3 for African American and non-Hispanic White participants, [31] and higher values of 342 ± 116 for a mixed population.[32] Our study is the first that we are aware of to evaluate the Veggie Meter™ as a biomarker of F&V consumption in lactating women with a mean score of 263 ± 97 and a correlation coefficient of 0.71. While there is limited information regarding how to interpret scores, over 40% of our participants had scores below 250, which is the measure that Rush et al. identified as poor intake based on plasma carotenoid measurements.[32]

We were surprised to find no significant relationship between Food Record measurements (F&V servings, retinol intake, and beta-carotene intake) and breast milk beta-carotene concentrations (R=0.51, p=0.162), given the significant relationships between F&V servings and Veggie Meter™ scores, and Veggie Meter™ scores and breast milk beta-carotene. Potential explanations for this unexpected finding include the small sample size of our study and the fact that one participant reported F&V intake in excess of 11 servings/day, but consumed limited dark-green vegetables, deep-yellow vegetables or tomatoes. The subject's 24h-Food Record beta-carotene intakes were below average, reflecting the low intake of beta-carotene rich F&Vs. When this subject was excluded from analysis, the relationship between F&V servings and milk beta-carotene was stronger, and significant ($R=0.74$; $p=0.037$).

Given the validity and ease of the Veggie Meter™ in assessing F&V intake in lactating women in our pilot study, future research could explore the use of the Veggie Meter™ as a potential counseling tool to motivate dietary changes in lactating women.

Breast Milk Vitamin A and Maternal Intake

A relationship between Vitamin A intake in lactating women and breast milk vitamin A composition has been previously reported in the literature.[21-24] In contrast to the findings of others, our study did not show a relationship between maternal intake (total vitamin A, retinol, beta-carotene and F&V servings) assessed using a single 24-hour Food Record, and breast milk retinol, beta-carotene, and total vitamin A. Lack of significance in our study may be related to the small number of breast milk samples that were assessed for vitamin A, as well as the use of a single 24-hour Food Record. While we did not demonstrate a relationship with intake assessed using a 24-hour Food Record and breast milk vitamin A, we did find that the Veggie Meter™ assessment was significantly associated with both breast milk beta-carotene and 24-hour Food Record F&V servings. This suggests that larger studies might be able to reflect more accurately how maternal intake is associated with breast milk composition.

Limitations of the study included a small sample size, the Veggie Meter™ and breast milk analyses were only done in a subset of the participants. Only one 24-hour Food Record was collected, which may not have accurately captured typical intake. Advanced instructions to record intake may have influenced participants actually eating behaviors. Further, demographic data (education, race, income) were not collected.

Conclusion

The majority of lactating women who participated in the study were identified as candidates for nutritional counseling using a validated health care screener and were not consuming adequate amounts of F&V based on the current recommendations. For women who reported a typical day in the 24-hour food record, Veggie Meter™ scores and F&V servings were significant and positively correlated, suggesting that the Veggie Meter is a valid assessment tool for F&V consumption in lactating women. A significant positive relationship was also demonstrated between Veggie Meter™ scores and breast milk beta-carotene values. Future research is needed to confirm our findings of an association between Veggie Meter™, F&V intake, and breast milk beta-carotene. Additionally, research is needed to address beliefs and barriers regarding F&V consumption in lactating women.

CHAPTER IV

EPILOGUE

Conclusion

Given that lactating women are considered a rare population in current national surveillance systems, our study was conducted with the objective of closing the gap about dietary patterns of lactating women, more specifically F&V. It is important to better understand what they are eating because they are providing the nutrition necessary to the early development of their infants, a crucial time that might impact the infants' health in the future. For this reason, even a small study can help close the gap about lactating women F&V intake. Lactating women in central North Carolina who participated in the study were not consuming adequate amounts of fruits and vegetables (F&V) based on the current recommendations. For women who reported a typical day in the 24-hour food record, Veggie Meter™ scores and F&V servings were significant and positively correlated. A significant positive relationship was also demonstrated between Veggie Meter™ scores and breast milk beta-carotene values.

We did not find a relationship between maternal intake of Vitamin A, assessed using a 24-hour Food Record, and breast milk Vitamin A, which is in contrast to what others have reported. The divergent results could be based on our study being a small study with one day of dietary information, whereas the other studies had a larger number of participants, more and different dietary assessment methods.

Challenges

Since this project used secondary data from another research project, I did not participate in collecting the participants dietary information, which did not allow me to obtain more details by talking to the participants. For example, the 24-hour Food Record, even if double checked with the participant for missing information, was not as detailed as the NDSR program requires. I had to create rules for the size of foods (i.e. medium eggs), food preparation (i.e. unknown preparation), types of food (i.e. 2% milk), besides adapting some dishes when they were not available in the program. I tried to be as consistent as possible, but if more details were available in the 24-hour Food Record or if I had the opportunity to interact with participant during data collection periods, entering the information in the NDSR program would have been easier.

Moreover, there was only a limited number of articles available in the literature about lactating women intake and very few recent ones. It was difficult to find enough current information to be able to better understand the background about their consumption of F&V and how that specifically affects breast milk composition.

Lastly, the study methods changed mid-way through the project when we had the opportunity to add the Veggie Meter™ to our data collection process. The focus of the study slightly changed, and we had to adapt the methods to verify relationships with dietary intake and breast milk composition.

Overall, working on this project was a very enriching experience. I had the opportunity to learn how to perform a literature review with little current information available, how to manipulate the Veggie Meter™ and how it works, how to use the

NDSR program, learn about different dietary assessment methods like the REAP screener, as well as how to use the information available and transform it into a study to fill a gap in the literature.

Future Implications

Future research is needed to verify the associations between the Veggie Meter™ with diet and breast milk, as well as address the beliefs and barriers behind F&V consumption in this population. Moreover, using dietary assessment methods and obtaining a bigger sample size might provide more accurate results about lactating women F&V intake and how that affects their milk composition.

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APPENDIX A

CONSENT FORM

UNIVERSITY OF NORTH CAROLINA AT GREENSBORO

CONSENT TO ACT AS A HUMAN PARTICIPANT

Project Title: Analysis of nutrients and bacteria in donor human milk

Principal Investigator and Faculty Advisor (if applicable): Linda Friend & Dr. Maryanne Perrin

Participant's Name:

What are some general things you should know about research studies?
You are being asked to take part in a research study. Your participation in the study is voluntary. You may choose not to join, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. There may not be any direct benefit to you for being in the research study. There also may be risks to being in research studies. If you choose not to be in the study or leave the study before it is done, it will not affect your relationship with the researcher or the University of North Carolina at Greensboro.

Details about this study are discussed in this consent form. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. If you have any questions about this study at any time, you should ask the researchers named in this consent form. Their contact information is below.

What is the study about?
This is a research project. Your participation is voluntary. The purpose of this research is to evaluate the steps in human milk banking that can affect the amount of nutrients, health-promoting factors, and bacteria in breast milk.

Why are you asking me?
This study is seeking freshly expressed breast milk samples. The breast milk samples that you donate to this research will be used to simulate processes that occur in donor milk banking. In order to participate, you must be a lactating female who birthed a healthy, term infant who is currently 4-11 months of age.

What will you ask me to do if I agree to be in the study?
If you choose to participate in this study, a researcher will schedule a time for you to come to the Cemela Foundation Human Nutrition Research laboratory at the University of North Carolina Greensboro in Greensboro, North Carolina. Prior to your scheduled appointment, you will be asked to watch a 1-minute instructional video for sample collection using a manual breast pump and record food intake for 24 hours prior to the appointment at the Cemala Foundation Human Nutrition Research Laboratory at UNCG (341 Stone Building). On the day of the appointment, we ask that you ideally refrain from feeding/pumping from one breast at least 2 hours before your appointment to ensure adequate milk volumes for donation. The researcher will briefly go over your food journal, administer a short questionnaire about your eating and exercise habits, and will collect some basic information about you, including measuring and recording your height and weight. The researcher will also perform a brief, non-invasive scan of your finger using a machine that measures dietary factors in your skin. You will then be provided with a manual breast pump. If you are unfamiliar with how to use the manual pump, an instructional video will be available. In a private room that only contains you and the researcher, you will use the pump to provide a human milk sample from one or both breasts. After pumping, all parts of the breast pump will be returned to you, and you will also receive a $40 gift card at the completion of this study. The appointment should take no more than 90 minutes, and there are no additional visits or follow-up questions.

Is there any audio/video recording?
No.

What are the risks to me?
Taking the time to travel to campus to collect a milk sample and complete a survey about your diet may be inconvenient and may interfere with your daily activities. The estimated time to complete these activities is 90 minutes. The collected information will be kept strictly confidential (see "How will you keep my information confidential?"). You may experience some discomfort pumping breast milk. You may also experience embarrassment since exposure of the breast will be necessary to obtain a human milk sample. This risk will be mitigated by using a private, locked room with a 'do not disturb' sign posted on the door. There are no foreseeable risks.

Are there any benefits to society as a result of me taking part in this research?
This research has the potential to help us understand how to retain more nutrients in donor HM.

Are there any benefits to *me* for taking part in this research study?
There are no direct benefits to participants in the study.

Will I get paid for being in the study? Will it cost me anything?
Upon successful completion of the study, you will be compensated with a $40 gift card and manual breast pump. The cost to you is your time and travel to come to the study site.

How will you keep my information confidential?
All information obtained in this study is strictly confidential unless disclosure is required by law. The confidentiality of the data will be maintained according to UNCG's Policy on access to and retention of research data (https://policy.uncg.edu/university-policies/research_data/). You will be assigned a unique identification number that will be used instead of your name.

Identifying information will be kept in a password-protected, digital file that is separate from any data collected. The signed consent form and all other paper-based data (24-hour food journal, etc.) will be scanned into a password-protected digital format and kept in a locked drawer in the Cemala Lab for at least five years. Only the PI and Faculty Advisor will have access to the information, which will be shared using password-protected files on Box. Breast milk samples will be labeled according to your unique identification number, stored in the Perrin Lab at UNCG (Stone Building) in a -80C freezer for up to 10 years for additional analyses.

What if I want to leave the study?
You have the right to refuse to participate or to withdraw at any time, without penalty. If you do withdraw, it will not affect you in any way. If you choose to withdraw, you may request that any of your data which has been collected be destroyed unless it is in a de-identifiable state. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

What about new information/changes in the study?
If significant new information relating to the study becomes available which may relate to your willingness to continue to participate, this information will be provided to you.

Voluntary Consent by Participant:
By signing this consent form you are agreeing that you read, or it has been read to you, and you fully understand the contents of this document and are openly willing consent to take part in this study. All of your questions concerning this study have been answered. By signing this form, you are agreeing that you are 18 years of age or older and are agreeing to participate, in this study.

If you have questions, want more information or have suggestions, please contact the Principal Investigator, Linda Friend, who may be reached by phone at [number redacted] and by email at llfriend@uncg.edu; and the Faculty Advisor, Dr. Maryanne Perrin, who may be reached by phone at (336) 334-3397 and by email at mtperrin@uncg.edu.

If you have any concerns about your rights, how you are being treated, concerns or complaints about this project or benefits or risks associated with being in this study, please contact the Office of Research Integrity at UNCG toll-free at (855)-251-2351.

Signature: ________________________ Date: ________________

APPENDIX B

24-HOUR FOOD RECORD INSTRUCTIONS

Please keep a record of everything you eat and drink for 24 hours prior to your appointment. Include all meals, snacks, and beverages, and the time of day you are eating or drinking.
Please also record the supplements (i.e. vitamins, minerals, protein powders, sport supplements, shakes, etc.). Please be as honest and accurate as you can.

Instructions
1. Record all food and beverages consumed for the entire 24-hour period, one full day before the appointment. Provide the following:
- Type of Food Eaten: e.g. chicken noodle soup
- Brand Name: e.g. Campbell's, Lipton, Weight Watchers
- Food or Beverage Characteristics:
  - Color: e.g. green vs. yellow beans; white vs. whole wheat bread
  - Fat Content: % fat (e.g. skim, 1%, 2% or whole milk), leanness of meat (e.g. extra lean ground beef), fat claims (e.g. "light", "low-fat"), was skin removed from poultry?
  - Freshness: e.g. fresh, frozen, canned, or dried?
  - Other Details: e.g. 25% reduced sodium, "diet" products, etc.
- Time of Day you ate or drank

2. Estimate the amount of food eaten as best as possible.
- Always estimate portion sizes of food after cooking.
- Use household measures to specify serving sizes, such as cups, tablespoons, teaspoons, ounces.
- Count the number of food items if practical: e.g.: 20 grapes, 15 baby carrots, 8 medium-sized shrimp, etc.
- Use food labels to estimate quantities.
- Use your hand to estimate portion sizes quickly:

3. Record if anything was added when preparing the food, such as oil (list specific kind), sauce, butter, margarine, or other condiments or seasonings.
4. For combination dishes such as lasagna, casseroles, chili, soups, or stews include a description of the main ingredients. E.g. Lasagna: lean ground beef (1⁄4 cup per piece), mozzarella cheese (1 oz per piece), cottage cheese (1 oz per piece), 1⁄2 cup tomato sauce, 2 noodles, 1⁄4 cup spinach.
5. Include snack foods eaten. Don't forget to include candy, chips, cookies, popcorn, ice cream, and beverages such as soft drinks, juice, coffee, or tea.
6. Use the "notes" column to record any additional product information if available (e.g. 6 crackers – 80 calories, 2.5g fat, 1g fiber, 210 mg sodium).

7. Don’t forget to write down any alcoholic beverages consumed and how much you drank. This includes all wine, beer, and liquor.
8. When in doubt, include more details!

Daily Food Record

Participant ID: ______________________________ Date: ______________________

Please list all food/beverages/water/supplements. Estimate all food/drink amounts accurately.

Time	**Amount**	**Description**	**Notes**

Was this a typical day? If not, why?

Adapted from STAR Family Health Team 2013: http://www.starfht.ca/3_Day_Food_Intake_Record_STARFHT.pdf