

Dietary Intervention to Increase Fruit and Vegetable Consumption in Breastfeeding Women: A Pilot Randomized Trial Measuring Inflammatory Markers in Breast Milk

By: Shoshanah Ehrenreich, Penina Langer, and Miriam Samuels

Angela R. Essa; Eva P. Browne, MS; Elizabeth C. Punska; Katelyn Perkins; Emily Boudreau, RD, LDN; Hilary Wiggins, MS; Douglas L. Anderton, PhD; Lindiwe Sibeko, PhD*; Susan R. Sturgeon, DrPH*; Kathleen F. Arcaro, PhD. Dietary Intervention to Increase Fruit and Vegetable Consumption in Breastfeeding Women: A Pilot Randomized Trial Measuring Inflammatory Markers in Breast Milk. *Journal of the Academy of Nutrition and Dietetics*. 2018 December. 2287-2295.

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Background

- Diets rich in fruits and vegetables (F/V) are associated with reduced inflammatory cytokine profiles as well as increasing the circulating levels of adiponectin, and decreasing circulating levels of interleukin and C-reactive protein (CRP)
- What is a cytokine?
 - A small protein released by cells that has a specific effect on the interactions between cells, on communications between cells or on the behavior of cells. The cytokines includes the interleukins, lymphokines and cell signal molecules, such as tumor necrosis factor and the interferons, which trigger inflammation and respond to infections
- Recent reviews based on the relationship between circulating cytokines and healthy dietary pattern show a reduced level of CRP- a substance produced in the liver in response to high inflammation levels
- However, the extent to which CRP concentrations may reflect F/V-associated changes in other cytokines is poorly understood.

Background (con't)

- Inflammatory cytokines in the breast are directly related to the growth of premalignant cells. Therefore Fruit and vegetable consumption may play a role in breast cancer.
- Diets rich in F/V are frequently associated with reduced risk of breast cancer
- Breast milk reflects the breast micro-environment
- While there have been dietary supplementation studies that show altered levels of cytokines in breast milk the study of increased fruit and vegetable intake on the inflammatory profile has not been studied

Purpose

The purpose of this study is to investigate the feasibility of conducting a larger trial among breastfeeding women to test if increasing their fruit and vegetable intake reduces the inflammatory cytokine levels in breast milk.

Methods



Study Design

The study was conducted as a Randomized Control Trial

Study Population

- 10 Breastfeeding Women who lived within a 25 mile radius of Amhurst, MA
- Inclusion Criteria
 - Female
 - Ages 18 or older
 - Currently breastfeeding and planning to breastfeed for at least three more months
 - Live within a 25 mile radius of Amhurst, MA
- Exclusion Criteria
 - History of any cancer (except Melanoma)
 - Crohn's Disease, Celiac Disease, or any other malabsorption syndrome
 - Non-gestational diabetes
 - Currently eating more than five servings of fruits and vegetables a day on a regular basis

Recruitment and Sampling Method

- Advertisements placed in local businesses
- Referrals from Lactation Consultants
- Eligibility was initially determined by a screening phone call, and all potentially eligible women received a “Health and History” questionnaire to determine if they were actually eligible
 - All those who were determined eligible at this point had their answers to the questionnaire used as part of their baseline data
 - The questionnaire is a 10 question (two parts each) screener used to determine daily intake and serving sizes of fruits and vegetables

Procedures

- Participants had an introductory session at their home at the onset of the study (week 0)
 - Participants signed consent forms, gave basic information about themselves and were given information on who to be in contact with if they start having adverse effects from the study
 - Participants were also given instructions on how to collect breast milk, and given the materials needed to properly store it and submit it to the trial
- After initial visit, participants were randomized into groups by a random number generating system
- Visits to the home by trained research assistants occurred on week 1 and week 13

Procedures Cont'd.

- Participants were asked to express all their breast milk from both breasts into separate containers within a half hour to an hour of their child's first feeding of the day
 - They also completed forms daily about their medication intake for 24 hours prior to nursing
- During the week 1 and week 13 visits the assistant measured both the mother and the infants height and weight, and collected the samples of breast milk the mothers had been expressing and freezing
- Participants received nutrition counseling and boxes of fruits and vegetables delivered to their houses, which were tailored to their likes and dislikes
 - Counseling and food plans were done by an RD or trained Nutrition graduate student
 - Both counseling and deliveries occurred on a weekly basis from weeks 2-12

Procedures Cont'd.

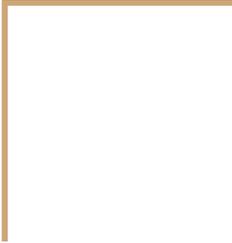
- Throughout the intervention, participants were asked to keep a food journal for five days each week to help with adherence to the plan
 - Three weekdays, one weekend, and one more day of their choice
 - Journals were collected weekly to help with counseling

Variables

- Independent Variable: Fruit and Vegetable intake of nursing mothers
 - Measured in servings per day
- Dependent Variable: Inflammation markers in breast milk
 - Measured in concentration of specific markers in breast milk in a specific testing format

Data Analysis

- Number of servings of F/V consumption were taken by screeners at weeks 1 and 13 from both the control and experimental groups using standardized coding systems
- BMI was recorded at both weeks 1 and 13
- t-Tests were conducted to compare week 1 and week 13 BMI's
 - Independent t-Tests were conducted to compare BMI's between groups
 - Dependent t-Tests were conducted to compare BMI's of week 1 and week 13 within the experimental group
 - P-Values presented had no set level of significance
- A mixed-effects repeated-measures model was used to determine the effect of diet on cytokines over an extended period of time
 - Two observations of cytokine levels were taken from each woman



Results



Participant Demographics

Characteristic	Control	Diet	P value
Age at donation (years)	33.2	33.8	0.545
Baby's age (days)	239.8	353.4	0.514
Age at menarche (years)	12.4	11.6	0.207
Age at first birth (years)	27.8	28.4	0.841
Number of children breastfed	2.4	2.4	0.514
Pre-pregnancy BMI	26.7	30.9	0.211
BMI at week 1	27.2	31.8	0.204
BMI at week 13	26.9	30.6	0.224

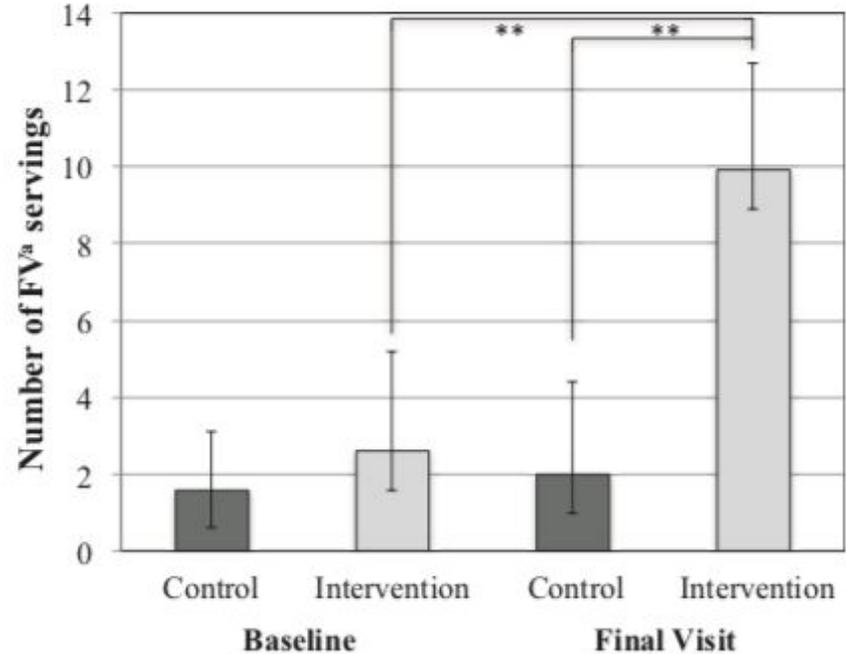
All values given are mean values.

Participant Demographics and Anthropometric Measures

- Most characteristics were similar between the two groups
- Compared to the control group, the women in the intervention group had a younger age at menarche and a higher BMI pre-pregnancy, at week 1, and at week 13
- The women in the intervention group lost an average of 2.91 kg by week 13
- The women in the control group gained an average of 1.12 kg by week 13

Behavioral Changes

- At baseline, both groups were consuming similar daily servings of fruits and vegetables
- At week 13:
 - The control group was consuming an average of two F/V servings a day
 - The intervention group was consuming an average of 9.9 F/V servings a day
- The daily average increase in servings in the intervention group is very significant ($P=0.004$)



Cytokine Levels

- 14 cytokines and growth factors were measured from the left and right breasts of 10 women collected at week 1 and week 13
- 2 cytokines, tyrosine kinase with immunoglobulin-like and endothelial growth factor indicated limited assay precision
 - There was no further examination of these 2 analytes
- The other 12 cytokines indicated excellent to good assay reliability for all analytes
 - At week 1, higher concentrations of 10 analytes were found in the control group (compared to the intervention group)
 - Concentration of all 10 analytes decreased by week 13
- Adiponectin, a hormone usually associated with a healthy diet, increased 27% in the intervention group by week 13 (13% higher than the control group)
- VEGF-D increased by 43% in the intervention group (35% higher than the control group)

Cytokines and Growth Factor Analytes Measured

Analytes ^b	Group	Week 1			Week 13			Change ^d		Model (P value ^e)
		Mean	Range	SD ^c	Mean	Range	SD	Mean	SD	
Singleplex analytes										
Adiponectin (ng/mL)	Control	30.1	18.8-43.4	11.2	27.9	13.5-41.9	11.1	-2.2	4.3	0.156
	Diet	23.2	8.8-41.9	12.9	31.6	10.6-58.7	20.4	8.4	12.1	
Leptin (pg/mL)	Control	1,812	1,093-3,015	910.4	1,160	682.8-1,890	535.3	-651.6	476.3	0.045
	Diet	1,457	530.4-2,278	670.7	1,279	566.5-2,160	732.3	-177.7	458.6	
CRP ^f (mg/L) ^g	Control	0.13	0.01-0.49	0.21	0.04	0.01-0.12	0.040	-0.09	0.167	0.033
	Diet	0.03	0.01-0.06	0.02	0.03	0.01-0.07	0.030	-0.00	0.015	
Pro-inflammatory panel										
IFN- γ ^h (pg/mL)	Control	5.7	1.0-13	5.4	1.7	0.5-2.8	0.97	-4.0	5.1	0.048
	Diet	2.3	1.3-4.5	1.3	2.1	0.5-4.0	1.4	-0.2	1.5	
IL-1 β ⁱ (pg/mL)	Control	3.2	1.2-7.5	2.6	2.3	0.2-5.4	2.24	-0.9	3.8	0.553
	Diet	1.2	0.4-2.7	0.9	1.3	0.9-2.5	0.7	0.1	0.3	
IL-6 (pg/mL)	Control	3.5	0.6-8.6	3.2	2.9	0.5-8.5	3.44	-0.7	1.2	0.368
	Diet	1.9	0.5-2.9	1.0	3.9	0.4-12.4	5.0	2.0	4.4	
IL-8 (pg/mL)	Control	752.8	32.4-1,511	603.6	586.6	0.6-1,188	558.2	-166.2	772.2	0.268
	Diet	290.4	123.8-428	110.8	551.3	186.5-914.8	303	260.87	224.6	
TNF- α ^j (pg/mL)	Control	2.8	0.4-5.2	2.1	2.2	0.5-5.8	2.2	-0.6	1.7	0.459
	Diet	0.9	0.4-1.3	0.4	1.2	0.4-2.3	0.76	0.4	0.4	
Angiogenesis panel										
bFGF ^k (pg/mL)	Control	3.1	0.9-5.5	2.2	2.4	0.8-6.9	2.6	-0.7	2.0	0.485
	Diet	5.7	0.7-17	6.6	7.5	0.7-18.3	7.9	1.8	6.22	
FLT-1 ^l (pg/mL)	Control	4,196	1,772-9,255	2,979	3,107	1,524-7,355	2,415	-1,089	865.6	0.044
	Diet	5,142	2,010-8,002	2,396	6,798	2,432-10,758	3,633	1,656	1,859	
PIGF ^m (pg/mL)	Control	229	50.6-644.3	249.4	147	44.2-420.9	154.9	-82	110.2	0.121
	Diet	175.9	19.2-284.6	118.5	467.5	18.3-1,505	595.4	291.5	524.3	
VEGF ⁿ -D (pg/mL)	Control	723.2	367.1-1,238	346.2	575.2	288.1-1,033	297.5	-148.0	52.1	0.022
	Diet	501.6	156.1-642.9	205.5	885.8	168.1-1,680	555.8	384.3	454.4	

Study Results

- The goal of this study was to change inflammatory markers to decrease risk of breast cancer by increasing fruit and vegetable intake in breastfeeding women
- Results show that it is possible to increase F/V intake, but it's unknown if the participants of this study had an easier time increasing their intake because of the weekly support, free box delivery, and food journals provided by this study
- It is unknown if the increase in adiponectin levels in the intervention group is related to the increase in F/V consumption, or if it is because they had lower baseline values than the control group. Weight loss generally increases adiponectin levels
 - In a previous study of 40 postmenopausal women, dietary induced weight loss did not increase adiponectin levels
 - The higher adiponectin levels in this study suggests that F/V consumption (directly/indirectly related to weight loss) may decrease the risk of breast cancer



Discussion



In Relation to Other Studies

- This is a pilot study, so it's the first study done on this topic
- This study was very small scale- there were only 10 participants and limited funding
- This study adds to literature on this topic by proving that it is worth doing this study on a larger scale
- In the future, a larger study would have similar BMI between groups which would allow them to determine if F/V intake alone results in weight loss in breastfeeding women, and if F/V consumption reduces inflammation (independent of weight loss)

Strengths

- Study shows that it is possible for breastfeeding women to increase F/V intake to 8-10 servings a day when receiving free weekly deliveries, weekly nutrition counseling, and practicing self monitoring by keeping a journal

Limitations

- Only 10 participants, so the results are preliminary
- F/V screener used doesn't measure the exact intake levels
 - For more accurate results, biomarkers or 24-hour recalls are recommended
 - Future studies should include analyses of carotenoid levels in skin, blood, or milk because they are correlated with dietary intake
- Changes in physical activity were not accounted for
 - Physical activity affects weight loss, cytokines, and breast cancer risk
- No theoretical framework was used
- Participants did not have similar characteristics. Varying factors included the infants age, whether the infant was only breastfeeding, and time to weaning variables
- It was hard to get conclusive results because of the difference in BMI between the intervention group and control group
- Can't be determined if this study would work for everyone- participants were receiving free F/V deliveries

Recommendations for Future Research

- Larger number of participants
- More commonalities between participants, such as:
 - Number of children
 - Age of infant
 - Whether the infant is solely breastfeeding
 - BMI prior to study
- Physical activity should be monitored
- Participants should not receive free F/V deliveries for the entire duration of the study, in order to see if it's possible to do independently

Citation

Definition of cytokine:

<https://www.medicinenet.com/script/main/art.asp?articlekey=11937>