RESEARCH
Original Research: Brief

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

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*

ARTICLE INFORMATION

Article history:
Submitted 21 October 2017
Accepted 19 June 2018
Available online 10 September 2018

Keywords:
Breast milk
Inflammation
Diet intervention
Cytokines
Fruits and vegetables

https://doi.org/10.1016/j.jand.2018.06.015

*L. Sibeko, S. R. Sturgeon, and K. F. Arcaro contributed equally to this work.

ABSTRACT

Background Diets rich in fruits and vegetables (F/V) can reduce the inflammatory profile of circulating cytokines and potentially decrease the risk of breast cancer. However, the extent to which a diet rich in F/V alters cytokine levels in breast tissue remains largely unknown. Breast milk provides a means of assessing concentrations of secreted cytokines in the breast microenvironment and is a potential tool for studying the effects of diet on inflammation in breast tissue and breast cancer risk.

Objective The aim of this pilot randomized trial was to test the feasibility of increasing F/V intake in breastfeeding women and of measuring changes in markers of inflammation in breast milk.

Design and intervention Participants randomized to the intervention (n=5) were provided weekly boxes of F/V, along with dietary counseling, to increase consumption of F/V to 8 to 10 daily servings for 12 consecutive weeks. Controls (n=5) were directed to the US Department of Agriculture's "ChooseMyPlate" diet for pregnancy and breastfeeding.

Participants/setting Ten breastfeeding women consuming fewer than five servings of F/V per day, as estimated by the National Institutes of Health "All-Day" Fruit and Vegetable Screener (F/V Screener), were recruited through flyers and a lactation consultant between February and May 2016 in the Western Massachusetts area.

Main outcome measures Baseline demographic and F/V intake data were collected during enrollment. At week 1 and week 13 (final) home visits, participants provided milk samples and anthropometric measurements were recorded. Participants completed F/V screeners at baseline and at study end. Adiponectin, leptin, C-reactive protein, and 11 additional cytokines were measured in breast milk collected at weeks 1 and 13.

Statistical analyses F/V consumption at baseline and after the final visit, and between controls and intervention groups, was compared with dependent and independent *t* tests, respectively. Differences between cytokine levels at weeks 1 and 13 were assessed with a mixed-effects repeated-measures model.

Results All women in the intervention increased F/V intake and were consuming more servings than controls by week 13; daily serving of F/V at baseline and final visit: controls=1.6 and 2.0, diet=2.6 and 9.9. Most cytokines were detected in the majority of milk samples: 12 were detected in 90% to 100% of samples, one was detected in 75% of samples, and one was detected in 7.5% of samples; coefficients of variation were below 14% for 11 of the cytokines.

Conclusions These preliminary findings indicate that it is feasible to significantly increase F/V intake in breastfeeding women and provide support for conducting a larger diet intervention study in breastfeeding women, in which longer-term benefits of the intervention are assessed.
J Acad Nutr Diet. 2018;118(12):2287-2295.

DIETS RICH IN FRUITS AND VEGETABLES (F/V) ARE associated with reduced inflammatory cytokine profiles, including increased circulating levels of adiponectin and decreased circulating levels of interleukin (IL)-6 and C-reactive protein (CRP).[1] Three recent reviews examining the relationship between dietary patterns and circulating cytokines reported healthy dietary patterns to be associated with significantly lower levels of CRP, with limited evidence for other biomarkers.[1-3] CRP is an acute-phase protein synthesized in the liver in response to

circulating inflammatory biomarkers; however, the extent to which CRP concentrations may reflect F/V-associated changes in other cytokines is poorly understood.

The effects of F/V consumption on inflammatory markers in the breast microenvironment may play an important role in the underlying biology of breast cancer, because inflammatory cytokines in the breast are directly related to the growth and proliferation of early premalignant cells,[4-6] and diets rich in F/V are frequently associated with reduced breast cancer risk.[7-10] Breast milk reflects the breast microenvironment and may provide a tool to assess the effects of diet on the inflammatory status of the breast. Indeed, levels of cytokines in breast milk reflect local secretion from multiple cell types, as well as peripheral production, and are not always correlated with blood levels.[11-13] Importantly, markers of inflammation and other proteins in breast milk have been associated with breast cancer risk.[14-18] Although dietary supplementation studies focused on infant nutrition have been shown to alter concentrations of select cytokines in breast milk,[19-21] the effects of increased F/V consumption on the inflammatory profile of breast milk has not been investigated. The goal of the present pilot study was to determine the feasibility of conducting a larger trial among breastfeeding women in which the hypothesis, that increased F/V consumption decreases the inflammatory profile in the breast, could be tested.

RESEARCH SNAPSHOT

Research Questions: Is it feasible to conduct a diet intervention study among breastfeeding women to increase their consumption of fruits and vegetables? Does increased fruit and vegetable intake (8 to 10 servings per day for 12 consecutive weeks) alter the inflammatory cytokine profile of breast milk?

Key Findings: Conducting a diet intervention study among breastfeeding women is feasible; weekly home delivery of fruits and vegetables and supportive nutrition counseling resulted in an increase in fruit and vegetable intake among breastfeeding women from a mean of 2.6 daily servings at week 1 to a mean of 9.9 daily servings at week 13 (t=5.48; P=0.003). After 12 weeks, levels of adiponectin in breast milk increased in the intervention group but not the control group.

METHODS

Participant Recruitment and Study Design

An overview of the study, approved by the University of Massachusetts (UMass) Amherst Institutional Review Board, is shown in Figure 1. The intervention was characterized by a combination of behavioral intervention and F/V supplementation. The behavioral intervention employed strategies shown to be successful in previous dietary modification studies.[22-24] The F/V supplementation strategy was based on previous research demonstrating both short- and long-term increases in F/V consumption in intervention studies that included food provision.[25-27]

Ten breastfeeding women living within a 25-mile radius of Amherst, MA were recruited between February and May 2016 through advertisements placed in local businesses and referral by lactation consultant. Enrollment was limited to 10 women due to minimal financial support. Preliminary eligibility was determined via telephone screening. Potentially eligible women were mailed a Health and History Questionnaire and the National Institutes of Health publically available All-Day Fruit and Vegetable Screener: Eating at America's Table Study, Quick Food Scan 2000 (F/V Screener)[28] to determine full eligibility and collect baseline data. The F/V Screener is a short dietary assessment instrument used to calculate daily serving intake of F/V based on answers to 10 two-part questions about portion size and frequency of consumption over the past month. Compared with 24-hour dietary recalls, F/V Screeners similar to the one we used either slightly underestimate F/V intake for women[29] or provide similar results.[30]

Inclusion criteria comprised: (1) being female and ≥18 years of age, (2) presently breastfeeding and planning to continue for at least 3 months following enrollment, and (3) living within 25 miles of UMass Amherst. Exclusion criteria comprised having a history of: (1) invasive breast cancer; (2) any other cancer, except nonmelanoma skin cancer, in the past 5 years; (3) Crohn's disease, celiac sprue, or other malabsorption syndrome; (4) nongestational diabetes; and (5) presently eating more than five servings of F/V per day. Eligible women (n=10) were scheduled for an introductory (week 0) visit at their home, during which participants provided written informed consent and were given telephone numbers and advised to call either study or nonstudy UMass personnel to report any adverse effects of participating in the study. Women also were provided instructions for collecting breast milk, along with prelabeled storage containers (Milk Storage Bags, Lansinoh) and plastic freezer bags as secondary containment for samples, a small cooler, and a Polar Pak icepack (ULINE). After the week 0 visit, participants were randomized to the control or intervention group via a random number-generating system.

Teams consisting of two trained research assistants conducted week 1 and week 13 study visits. Morning week 1 visits at participants' homes were scheduled for 1 week after week 0 visits. Women were previously asked to pump or express all of the milk from both breasts (into separate labeled containers) within 30 minutes to 1 hour after their baby had nursed for the first time that morning and to complete a breast milk collection form, which included questions regarding medications (eg, nonsteroidal anti-inflammatory drugs) taken within the 24 hours prior to donation. During the visit, research staff measured participant height and weight using a standard protocol (ie, no shoes, light weight clothing, replicate measurements) with research equipment (Leicester Height Measure; BC544 Inner Scan, Tanita) and the infant's length and weight using a standard protocol (ie, stretching the heel, dry diaper only, replicate measurements) and research equipment (Measurement Mat II, Hopkins Medical Products; 553KL Professional Digital Pediatric Scale, Health o meter Professional). Samples were transported to the laboratory in coolers with ice packs and processed upon arrival. Participants in the intervention received weekly nutrition counseling and a box of F/V (described later) at the week 1 visit and for each of the next 11 weekly visits (weeks 2 to 12). Weekly F/V boxes were delivered directly to women's homes.

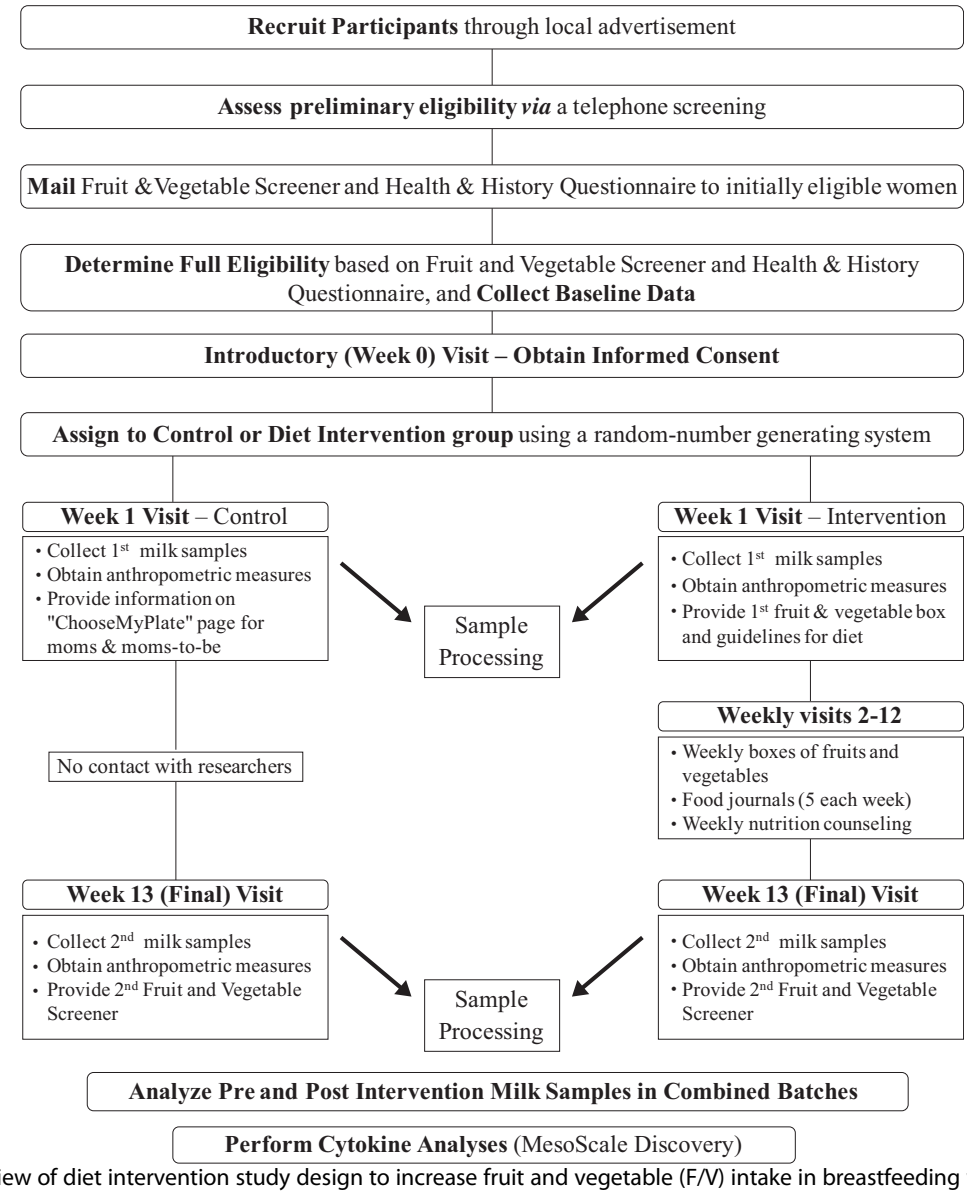

Figure 1. Overview of diet intervention study design to increase fruit and vegetable (F/V) intake in breastfeeding women to at least 8 to 10 nutrient-dense servings of F/V for 12 consecutive weeks. A total of 14 home visits were conducted for participants in the intervention arm, and two home visits were conducted for participants in the control arm. Each participant provided milk from the left and right breasts at two time points (weeks 1 and 13) for a total collection of 40 milk samples. The National Institutes of Health, All-Day Fruit and Vegetable Screener[22] was completed by all participants to provide baseline and end of study F/V consumption.

Week 13 (final) visits at participants' homes were scheduled for 1 week after the delivery of the 12th box of F/V (13 weeks after week 0 visit) to collect second breast milk samples, collection forms, and anthropometric measurements. At this visit, women were provided with a second F/V Screener to complete immediately and return in a provided postage-paid envelope. F/V Screeners collected at baseline and week 13 were used to compare pre- and postintervention F/V serving consumption among women in both the control and intervention groups.

Diet Intervention Arm

The goal of the intervention was to increase participants' consumption of nutrient dense, darkly pigmented F/V to 8 to 10 daily servings. Although the US Department of Agriculture (USDA) recommends daily consumption of 2.5 servings of vegetables and two servings fruit per day for an individual consuming 2,000 calories,[31] research has shown that consuming greater than 7.5 to 8 daily F/V servings may be more effective at lowering overall disease risk.[25,32] This provided the rationale for increasing participants' consumption of F/V to at least 8 daily servings.

At week 1 visits, a registered dietitian or trained graduate nutrition student met with study participants in the intervention group to establish a counseling plan based on that participant's characteristics, including her work schedule, time available for food preparation, F/V likes and dislikes, and other diet-related lifestyle factors. Individualized counseling

plans were aimed at engaging participants and increasing adherence to the dietary guidelines of the study. Participants also received the first weekly box of fresh, darkly pigmented F/V, information outlining the guidelines of the intervention, and "quick sheets" containing suggestions on how to increase daily F/V intake. To boost adherence to the intervention, participants were asked to self-monitor total dietary intake five times each week with food journals: three times during the week, once on the weekend, and once more on a day of their choice. Food journals were collected weekly and used as an aid during counseling sessions. For each of the next 11 visits, participants in the intervention were provided with weekly boxes of F/V, food journals, and nutrition counseling. Food boxes were provided to facilitate increased F/V intake by supplementing each family's groceries; however, 32 servings were provided regardless of family size. Counseling focused on helping participants identify and address barriers to achieving the goal of 8 to 10 F/V servings per day (eg, recipe modification, food preparation) and also served as an opportunity for participants to report any challenges of the diet, such as trouble incorporating vegetables or digestive issues.

Weekly F/V boxes contained approximately 32 servings of fresh, darkly pigmented F/V. Boxes were prepared at UMass Dining Services (except for boxes needed during weeks when Dining Services was closed) according to the following guidelines: box must contain 32 servings of F/V, must comprise 75% vegetables (24 servings) and 25% fruit (8 servings), and must contain at least six servings of leafy greens. Food items were defined by culinary definitions (eg, tomato=a vegetable), and there must be at least one item in the box from each of the eight defined categories (dark leafy green vegetables; deep orange or yellow fruits, roots, and tubers; red fruits or vegetables; citrus fruits; cabbage family vegetables; lettuces; allium vegetables; red, purple, or blue berries; and other, as described by Pennington and Fisher).[33] One serving was defined as: 1 cup of cut-up, raw, or cooked vegetables, fruit, or 100% fresh vegetable juice or 2 cups of raw dark leafy greens. Serving size was defined in accordance with USDA recommendations.[34] Dried fruit, white potatoes, and iceberg lettuce were not counted toward the daily goal, but consumption of these foods was not discouraged. Weekly box contents depended on availability of certain foods at Dining Services, as well as availability and pricing of certain foods at local grocery stores.

Control Arm

Participants randomized to the control arm were not asked to adhere to specific dietary guidelines. At week 1 visits, members of this group were directed to the USDA's "ChooseMyPlate" webpage for "Moms/Moms-to-Be"[35] to review healthy eating plans for pregnant and breastfeeding women, but had no contact with the researchers during the intervention period. As noted previously, control participants also completed the F/V Screener at baseline and week 13.

Cytokine and Growth Factor Measurements

Five 96-well plates of antibody sandwich assays with electrochemiluminescent detection (Mesoscale Discovery) were used to measure cytokines and growth factors. A custom-ordered Human V-PLEX Pro-Inflammatory panel included assays for five cytokines: interferon-γ, IL-1β, IL-6, IL-8, and tumor necrosis factor-α, and a custom-ordered Human V-PLEX Angiogenesis panel included assays for six growth factors: basic fibroblast growth factor, fms-related tyrosine kinase 1, placental growth factor, tyrosine kinase with immunoglobulin-like and endothelial growth factor−like domains 2, vascular endothelial growth factor C (VEGF-C), and vascular endothelial growth factor D (VEGF-D). Three single-analyte assays were used for leptin, adiponectin, and CRP. Milk was diluted with phosphate-buffered saline (1:5 for proinflammatory and angiogenesis panels; 1:3 for leptin, CRP; and 1:2.5 for adiponectin). Forty diluted milk samples (from left and right breasts of 10 women at weeks 1 and 13) were tested in duplicate in each of the five 96-well plates. Assays were performed according to manufacturer's instructions with 8-point standard curves (in duplicate) on each plate and read on a SECTOR Imager 2400A (Mesoscale Discovery), which provided signal intensity and calculated concentrations using a four-parameter logistic regression as well as lower and upper limits of detection. Samples below the detection range were still within the fit curve range, and concentrations were generated based on standard curve.

Data Analysis

The number of F/V servings consumed by participants in the control and intervention groups was extracted from F/V Screeners collected at baseline and week 13 using a standardized coding system provided by National Institutes of Health.[36] With this standardized coding system, responses to each of the 10 two-part questions in the F/V Screener correspond to a Pyramid or MyPyramid cup equivalent portion size. Fraction or mixed number values corresponding to each survey response are summed to estimate the total number of F/V servings consumed per day by respondents.

Descriptive statistics were prepared with Stata.[37] Within- and between-group comparisons of body mass index (BMI) and F/V intake were conducted with dependent and independent t tests, respectively, using Excel.[38] Given the pilot nature of this study, we emphasize the substantive significance of differences reported. We also present P values for comparisons, however, with no set level of significance. To test the hypothesis that diet affected levels of cytokines over time, we fit mixed-effects repeated-measures models for each cytokine with two observations per woman (random effect), diet vs control, time 1 and 2, and the interaction of diet and time, using the REML procedure within Stata.[37] The interactions thus test the significance of the effect of diet over time controlling for other fixed effects and random between-woman variation.

RESULTS

Participant Demographics, Anthropometric Measures, and Behavioral Changes

Table 1 shows demographic parameters collected for all 10 participants. Most characteristics were similar between groups. Women in the intervention had a younger age at menarche compared with controls at menarche (11.6 vs 12.4 years; P=0.207) and had a higher prepregnancy BMI (30. 9 vs 26.7; P=0.211), week 1 BMI (31.8 vs 27.2; P=0.204), and week 13 BMI (30.6 vs 26.9; P=0.224). Participants in intervention group lost weight by week 13, with average loss of 2.91 kg,

Table 1. Participant demographic information for women (n=5) in the control group (referred to US Department of Agriculture's "ChooseMyPlate" diet for pregnancy and breastfeeding) and women (n=5) in the diet intervention group (instructed to eat eight to 10 daily servings of fruits and vegetables for 12 consecutive weeks), collected at baseline and weeks 1 and 13 of the intervention period

Characteristic	Control	Diet	*P* value[e]
	←——mean (range)——→		
Age at donation (years)[a]	33.2 (31-35)	33.8 (26-37)	0.545
Baby's age (days)[a]	239.8 (30-561)	353.4 (171-810)	0.514
Age at menarche (years)[a]	12.4 (12-13)	11.6 (10-13)	0.207
Age at first birth (years)[a]	27.8 (19-33)	28.4 (25-33)	0.841
Number children breastfed[a]	2.4 (1-5)	2.4 (1-4)	0.514
Participant			
Prepregnancy BMI[ab]	26.7 (22.0-29.1)	30.9 (23.0-36.8)	0.211
BMI at week 1[c]	27.2 (22.0-29.3)	31.8 (23.0-37.9)	0.204
BMI at week 13[d]	26.9 (21.3-30.5)	30.6 (23.6-36.3)	0.224

[a]Self-reported demographic collected or calculated at baseline using Health and History Questionnaire that was mailed to participants to determine eligibility.
[b]BMI=body mass index; calculated as kg/m^2.
[c]Calculated from the average of duplicate measurements obtained at week 1 visit.
[d]Calculated from the average of duplicate measurements obtained at week 13 visit.
[e]*P* values based on independent two-tailed *t* tests comparing groups.

and participants in the control had an average weight gain of 1.12 kg.

Women in the intervention group increased consumption of nutrient-dense F/V over the course of the 12-week intervention period (Figure 2). At baseline, women in the control and intervention groups were consuming similar daily servings of F/V (mean daily servings=1.6 and 2.6, respectively; t=0.79; P=0.455). At week 13, the control group was still consuming a daily average of two F/V servings, and the intervention group was consuming a daily average of 9.9 F/V servings (t=5.48; P=0.003). This represents an average increase in F/V serving consumption in the intervention participants between baseline and week 13 (t=5.88; P=0.004). All five participants in the intervention increased their consumption of F/V, starting on week 1 and continuing through week 13 (data extracted from weekly food journals [not shown]). No adverse effects (eg, changes in gastrointestinal health or reduced total milk volume) were reported throughout the study.

Cytokine Levels

Fourteen cytokines and growth factors were measured in breast milk from the left and right breasts of 10 women collected at week 1 and week 13. As shown in Table 2, two

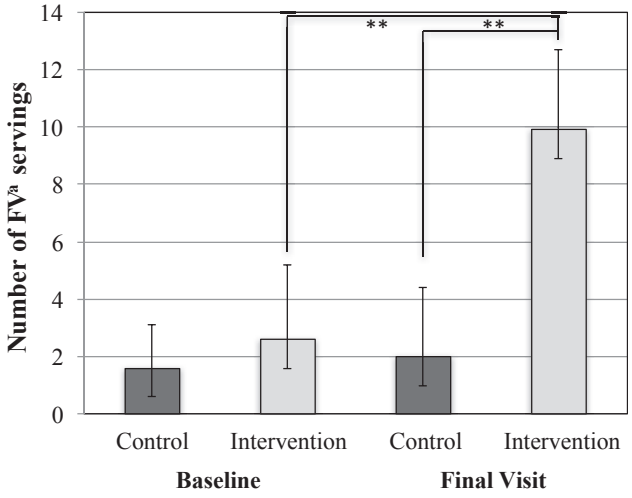

Figure 2. Comparison of mean fruit and vegetable (F/V) intake in the control (n=5) and diet intervention (n=5) groups at baseline and week 13 (final visit). F/V consumption was estimated using the National Institutes of Health publically available All-Day Fruit and Vegetable Screener: Eating at America's Table Study, Quick Food Scan 2000. F/V consumption increased among women in the intervention group between baseline and the final visit, and at the final visit, F/V intake was higher among than intervention than the control group. [a]FV=fruit and vegetable. **P<0.01.

cytokines, tyrosine kinase with immunoglobulin-like and endothelial growth factor–like domains 2 and VEGF-C, had 7.5% and 75% of the samples below the limit of detection and high (79.1 and 32.4) coefficient of variations (CVs; CV=standard deviation/mean) of the duplicate measures, indicating limited assay precision. These two analytes are not further examined. The remaining 12 analytes had 90% to 100% of the samples within the detection range and CVs ranging from 1.6% (CRP) to 39.4% (IL-1β), with eight analytes having CVs below 10% and 11 below 14%, indicating excellent to good assay reliability for all analytes except IL-1β.

Visual inspection of analyte concentrations at week 1 (Table 3) shows higher concentrations in the control as compared with the diet intervention group for 10 of the 12 analytes: adiponectin (1.3-fold), leptin (1.2-fold), CRP (3.8-fold), interferon-γ (2.5-fold), IL-1β (2.7-fold), IL-6 (1.8-fold), IL-8 (2.6-fold), tumor necrosis factor-α (3.1), placental growth factor (1.3-fold), and VEGF-D (1.4-fold). The higher concentrations at week 1 among the control group for 10 analytes were not due to single outliers, and we found no justification for removing any of the high values. Concentrations of all 10 analytes decreased in the control group by week 13. Although baseline differences between groups are to be expected with small sample sizes, the decrease among the control group in 10 of 12 analytes appears to drive several of the models. For example, the P value of 0.033 obtained for the CRP interaction term is due primarily to the high week 1 control mean of 0.13 mg/L, and the other three means are very close to each other (0.03, 0.03, and 0.04 mg/L).

Despite the small sample size and high baseline control values, some results are worth noting. For example, adiponectin, a hormone previously shown to be associated with a healthy diet, increased 27% among women in the

Table 2. Assay parameters for the 14 analytes measured in breast milk from the left and right breasts of women (n=5) in the control group (referred to US Department of Agriculture's "ChooseMyPlate" diet for pregnancy and breastfeeding) and women (n=5) in the diet intervention group (instructed to eat 8 to 10 daily servings of fruits and vegetables for 12 consecutive weeks) at weeks 1 and 13 of the intervention study[a]

Analyte	CV[bc]	%[d]	LLOD[e]	ULOD[f]
Pro-inflammatory panel				
IFN-γ[g]	13.7	92.5	0.12	1,410
IL[h]-1β	39.4	100	0.01	510
IL-6	12.7	90	0.09	769
IL-8	3.79	97.5	0.03	507
TNF-α[i]	10.9	100	0.03	316
Angiogenesis panel				
bFGF[j]	9.5	100	0.10	1,840
FLT-1[k]	5.8	100	1.05	8,300
PlGF[l]	4.6	100	0.45	3,455
Tie-2[m]	79.1	7.5	64.14	79,000
VEGF[n]-C	32.4	75	15.25	23,500
VEGF-D	2.2	100	4.06	22,850
Singleplex analytes				
CRP[o]	1.6	100	2.8	195,000
Adiponectin	5	100	0.0072	1,000
Leptin	6.9	100	109.5	100,000

[a]Analytes were measured with electrochemiluminescent assays (MesoScale Discovery).
[b]CV=coefficient of variation.
[c]CV based on the mean of the calculated concentrations of the technical duplicates from 40 breastmilk samples [left and right breasts of 10 women (5 control and 5 diet intervention) at two time periods (weeks 1 and 13)].
[d]Percent of samples within the detection range.
[e]LLOD=lower limit of detection.
[f]ULOD=upper limit of detection.
[g]IFN-γ=interferon-γ.
[h]IL=interleukin.
[i]TNF-α=tumor necrosis factor-α.
[j]bFGF=basic fibroblast growth factor.
[k]FLT-1=fms-related tyrosine kinase 1.
[l]PlGF=placental growth factor.
[m]Tie-2=tyrosine kinase with immunoglobulin-like and endothelial growth factor–like domains 2.
[n]VEGF=vascular endothelial growth factor.
[o]CRP=C-reactive protein.

intervention group to a week 13 level that is 13% above the control group at week 13. Similarly, VEGF-D increased 43% among women in the intervention group, and by week 13 was 35% above the control value.

DISCUSSION

The objective of this pilot study was to increase F/V consumption in breastfeeding women with the goal of altering inflammatory markers in a manner consistent with decreased breast cancer risk. Results demonstrate that it is feasible to increase F/V intake in breastfeeding women to 8 to 10 daily servings. Although F/V intake has been increasing in the United States, most Americans still fail to reach the recommended number of daily servings (2.5 servings of vegetables and two servings of fruit).[31,39] The extent to which the high F/V consumption attained here was due to weekly supportive counseling, free box delivery, use of weekly food journals as a self-monitoring tool, or a strong motivation among breastfeeding mothers to eat foods perceived as highly nutritious and beneficial to their breastfeeding child is unknown.

After 12 weeks, breast milk adiponectin levels increased in the intervention group but not the control group. The extent to which this increase is due to F/V consumption is secondary to weight loss, or is an artifact due to the lower baseline levels of adiponectin in the intervention group, remains to be determined. Adiponectin levels are, in general, inversely associated with obesity, and weight loss tends to lead to increases in circulating levels.[40] However, in a study of 40 postmenopausal women, moderate dietary-induced weight loss did not result in an increase in adiponectin.[41] Furthermore, weight loss had no effect on breast or adipose mRNA levels of the adiponectin gene in postmenopausal women.[42] Still, the higher adiponectin levels, together with research showing a significant inverse association between circulating adiponectin levels and breast cancer risk,[43] suggest a mechanism by which F/V consumption, either directly or indirectly through weight loss, may decrease breast cancer risk.

There was an intriguing observation for weight loss in the intervention but not the control group. However, this is confounded by the unequal BMIs at baseline: control BMI=27.2 and intervention BMI=31.8. Interestingly, in a recent meta-analysis, increased consumption of F/V was not associated with a change in total energy intake,[44] and therefore would not be expected to result in weight loss. However, no studies of breastfeeding women were included in this meta-analysis. Although obesity is associated with inflammation and weight loss with a reduction in inflammation, increased consumption of cruciferous and apiaceous vegetables can alter levels of circulating inflammatory markers even in the absence of weight change.[45] Regardless, our pilot findings remain tempered by the weight loss observed among the women in the intervention group. In a larger study, the randomization of participants should lead to similar BMIs between groups and the ability to determine if increased F/V intake alone results in weight loss in breastfeeding women, as well as the extent to which F/V consumption reduces inflammation independent of weight loss.

There are several limitations of this study. First, this pilot study is restricted to 10 women and therefore all results are strictly preliminary. Second, although the F/V Screener used in the present study is appropriate for studying the relationship between intake and disease and for obtaining gross estimates of intake, it is considered suboptimal for assessing precise intake levels.[29,30] Rather, biomarkers or 24-hour recalls are recommended.[46] To confirm the reported increases in F/V consumption, future studies should include analyses of carotenoid levels in blood, milk, or skin, because these levels are correlated with dietary intake.[47] Third, there was no consideration of changes in physical activity, which is known to affect weight loss, circulating cytokines, and breast cancer risk.[48-50] Another limitation of this diet intervention is that a theoretical framework was not used. However, participant

Table 3. Cytokines and growth factor analytes were measured with electrochemiluminescent sandwich assays (MesoScale Discovery) in left and right breast milk samples from women (n=5) in the control group (referred to US Department of Agriculture's "ChooseMyPlate" diet for pregnancy and breastfeeding) and women in the (n=5) diet intervention group (instructed to eat eight to 10 daily servings of fruits and vegetables for 12 consecutive weeks) at week 1 and week 13[a]

		Week 1			Week 13			Change[d]		Model
Analytes[b]	Group	Mean	Range	SD[c]	Mean	Range	SD	Mean	SD	(*P* value[e])
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-1β[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	
PlGF[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	

[a]Mean values of the left and right breast milk samples were used to calculate group mean, range, and SD.
[b]Data are presented for the 12 analytes with >75% of the samples within the detection range.
[c]SD=standard deviation.
[d]Change=difference between week 13 and week 1 levels.
[e]*P*=probability value of the interaction term testing diet and time interaction from a mixed effects repeated measures model controlling for random effect of woman and fixed effects of constant, diet and time.
[f]CRP=C-reactive protein.
[g]To convert mg/L CRP to nmol/L, multiply mg/L by 9.524. To convert nmol/L CRP to mg/L, multiply nmol/L by 0.1049. CRP of 0.13 mg/L=1.238 nmol/L.
[h]IFN-γ=interferon-γ.
[i]IL=interleukin.
[j]TNF-α=tumor necrosis factor-α.
[k]bFGF=basic fibroblast growth factor.
[l]FLT-1=fms-related tyrosine kinase 1.
[m]PlGF=placental growth factor.
[n]VEGF=vascular endothelial growth factor.

barriers, motivations, and issues of self-management were identified and addressed, which does provide insight into important intervention components. The testing of a theoretical framework would have provided guidance on how to make the intervention more effective or on what the most effective parts of the intervention were.[51] Finally, participants varied with respect to the length of time breastfeeding their infant (infant's age), whether the infant was exclusively

breastfeeding, and time to weaning—variables that could impact both weight loss and breast inflammatory profiles.[52,53]

CONCLUSION

This pilot study demonstrates that is it feasible to increase F/V intake in breastfeeding women to 8 to 10 daily servings when they receive weekly deliveries of F/V at no cost, weekly nutrition counseling, and complete daily food journals to practice self-monitoring. The extent to which the observed increase in breast milk adiponectin is due to increased intake of F/V, or is secondary to weight loss or changes in physical activity, remains to be determined.

References

1. Barbaresko J, Koch M, Schulze M, Nöthlings U. Dietary pattern analysis and biomarkers of low-grade inflammation: A systematic literature review. *Nutr Rev*. 2013;71(8):511-527.
2. Neale EP, Batterham MJ, Tapsell LC. Consumption of a healthy dietary pattern results in significant reductions in C-reactive protein levels in adults: A meta-analysis. *Nutr Res*. 2016;36(5):391-401.
3. Chan DSM, Bandera E, Greenwood D, Norat T. Circulating C-reactive protein and breast cancer risk-systematic literature review and meta-analysis of prospective cohort studies. *Cancer Epidemiol Biomarkers Prev*. 2015;24(10):1439-1449.
4. Hugo H, Saunders C, Ramsay RG, Thompson EW. New insights on COX-2 in chronic inflammation driving breast cancer growth and metastasis. *J Mammary Gland Biol Neoplasia*. 2015;20(3-4):109-119.
5. Delort L, Rossary A, Farges M, Vasson M, Caldefie-Chézet F. Leptin, adipocytes and breast cancer: Focus on inflammation and anti-tumor immunity. *Life Sci*. 2015;140:37-48.
6. Galdiero M, Marone G, Mantovani A. Cancer inflammation and cytokines. *Cold Spring Harb Perspect Biol*. 2018;10:a028662.
7. Rice M, Eliassen AH, Hankinson S, Lenart E, Willett W, Tamimi R. Breast cancer research in the nurses' health studies: Exposures across the life course. *Am J Public Health*. 2016;106(9):1592-1598.
8. Eliassen AH, Hendrickson S, Brinton L, et al. Circulating carotenoids and risk of breast cancer: Pooled analysis of eight prospective studies. *J Natl Cancer Inst*. 2012;104(24):1905-1916.
9. Jung S, Spiegelman D, Baglietto L, et al. Fruit and vegetable intake and risk of breast cancer by hormone receptor status. *J Natl Cancer Inst*. 2013;105(3):219-236.
10. Boggs D, Palmer J, Wise L, et al. Fruit and vegetable intake in relation to risk of breast cancer in the black women's health study. *Am J Epidemiol*. 2010;172(11):1268-1279.
11. Hawkes J, Bryan D, Gibson R. Cytokine production by human milk cells and peripheral blood mononuclear cells from the same mothers. *J Clin Immunol*. 2002;22(6):338-344.
12. Hines E, Rayner J, Barbee R, et al. Assays for endogenous components of human milk: Comparison of fresh and frozen samples and corresponding analytes in serum. *J Hum Lact*. 2007;23(2):144-156.
13. Brunner S, Schmid D, Zang K, et al. Breast milk leptin and adiponectin in relation to infant body composition up to 2 years. *Pediatr Obes*. 2014;10(1):67-73.
14. Arcaro K, Browne E, Qin W, Zhang K, Anderton D, Sauter E. Differential expression of cancer-related proteins in paired breast milk samples from women with breast cancer. *J Hum Lact*. 2012;28(4):543-546.
15. Qin W, Zhang K, Kliethermes B, Amjad R, Clarke K, Sauter E. Differential expression of cancer-associated proteins in breastmilk. *Breastfeed Med*. 2013;8(1):120-126.
16. Qin W, Zhang K, Kliethermes B, et al. Differential expression of cancer associated proteins in breast milk based on age at first full term pregnancy. *BMC Cancer*. 2012;12:100.
17. Qin W, Zhang K, Clarke K, Sauter E. Family history of breast cancer predicts breastmilk protein expression. *Breastfeed Med*. 2014;9(4):231-232.
18. Yang H, Schneider S, Chisholm C, et al. Association of TGF-β2 levels in breast milk with severity of breast biopsy diagnosis. *Cancer Causes Control*. 2015;26(3):345-354.
19. Hawkes J, Bryan D, Makrides M, Neumann M, Gibson R. A randomized trial of supplementation with docosahexaenoic acid-rich tuna oil and its effects on the human milk cytokines interleukin 1 beta, interleukin 6, and tumor necrosis factor alpha. *Am J Clin Nutr*. 2002;75(4):754-760.
20. Hawkes JS, Bryan DL, Neumann MA, Makrides M, Gibson RA. Transforming growth factor beta in human milk does not change in response to modest intakes of docosahexaenoic acid. *Lipids*. 2001;36(10):1179-1181.
21. Linnamaa P, Nieminen K, Koulu L, et al. Black currant seed oil supplementation of mothers enhances IFN-γ and suppresses IL-4 production in breast milk. *Pediatr Allergy Immunol*. 2013;24(6):562-566.
22. Parsons J, Pierce J, Natarajan L, et al. A randomized pilot trial of dietary modification for the chemoprevention of noninvasive bladder cancer: The dietary intervention in bladder cancer study. *Cancer Prev Res (Phila)*. 2013;6(9):971-978.
23. Parsons J, Pierce J, Mohler J, et al. A randomized trial of diet in men with early stage prostate cancer on active surveillance: Rationale and design of the men's eating and living (MEAL) study (CALGB 70807 [alliance]). *Contemp Clin Trials*. 2014;38(2):198-203.
24. Thomson C, Ravia J. A systematic review of behavioral interventions to promote intake of fruit and vegetables. *J Am Diet Assoc*. 2011;111(10):1523-1535.
25. Watzl B, Kulling S, Möseneder J, Barth S, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. *Am J Clin Nutr*. 2005;82(5):1052-1058.
26. Neville C, McKinley M, Draffin C, et al. Participating in a fruit and vegetable intervention trial improves longer term fruit and vegetable consumption and barriers to fruit and vegetable consumption: A follow-up of the ADIT study. *Int J Behav Nutr Phys Act*. 2015;12:158.
27. Pomerleau J, Lock K, Knai C, McKee M. Interventions designed to increase adult fruit and vegetable intake can be effective: A systematic review of the literature. *J Nutr*. 2005;135(10):2486-2495.
28. National Cancer Institute, Division of Cancer Control & Population Sciences, Epidemiology and Genomics Research Program. Fruit & vegetable screeners in the eating at America's table study (EATS): Instruments. https://epi.grants.cancer.gov/diet/screeners/fruitveg/instrument.html. Updated 2015. Accessed April 11, 2018.
29. Thompson FE, Kipnis V, Subar AF, et al. Evaluation of 2 brief instruments and a food-frequency questionnaire to estimate daily number of servings of fruit and vegetables. *Am J Clin Nutr*. 2000;71(6):1503-1510.
30. Yaroch A, Tooze J, Thompson F, et al. Evaluation of three short dietary instruments to assess fruit and vegetable intake: The national cancer institute's food attitudes and behaviors survey. *J Acad Nutr Diet*. 2012;112(10):1570-1577.
31. USDA Center for Nutrition Policy and Promotion. Dietary guidelines for Americans 2015-2020 eighth edition. https://www.cnpp.usda.gov/2015-2020-dietary-guidelines-americans. Accessed April 11, 2018.
32. Aune D, Giovannucci E, Boffetta P, et al. Fruit and vegetable intake and the risk of cardiovascular disease, total cancer and all-cause mortality-a systematic review and dose-response meta-analysis of prospective studies. *Int J Epidemiol*. 2017;46(3):1029-1056.
33. Pennington JA, Fisher RA. Food component profiles for fruit and vegetable subgroups. *J Food Compost Anal*. 2010;23(5):411-418.
34. USDA food patterns. http://riskfactor.cancer.gov/diet/screeners/fruitveg/allday.pdf. Accessed April 11, 2018.
35. USDA ChooseMyPlate.gov. Moms/moms-to-be. https://www.choosemyplate.gov/moms-pregnancy-breastfeeding. Updated 2017. Accessed January 10, 2015.
36. National Cancer Institute, Division of Cancer Control & Population Sciences, Epidemiology and Genomics Research Program. Scoring the all-day screener. https://epi.grants.cancer.gov/diet/screeners/fruitveg/scoring/allday.html. Updated 2016. Accessed April 11, 2018.
37. *Stata*. Version 13.1. College Station, TX: StataCorp; 2013.
38. *Excel 2013*. Redmond, WA: Microsoft Corp; 2013.

39. Pearson Stuttard J, Bandosz P, Rehm C, et al. Comparing effectiveness of mass media campaigns with price reductions targeting fruit and vegetable intake on US cardiovascular disease mortality and race disparities. *Am J Clin Nutr*. 2017;106(1):199-206.
40. Oller Moreno S, Cominetti O, Núñez Galindo A, et al. The differential plasma proteome of obese and overweight individuals undergoing a nutritional weight loss and maintenance intervention. *Proteomics Clin Appl*. 2018;12(1):1600150.
41. Ryan AS, Nicklas BJ, Berman DM, Elahi D. Adiponectin levels do not change with moderate dietary induced weight loss and exercise in obese postmenopausal women. *Int J Obes Relat Metab Disord*. 2003;27(9):1066-1071.
42. Mason C, Wang L, Duggan C, et al. Gene expression in breast and adipose tissue after 12 months of weight loss and vitamin D supplementation in postmenopausal women. *NPJ Breast Cancer*. 2017;3.15-15.
43. Macis D, Guerrieri Gonzaga A, Gandini S. Circulating adiponectin and breast cancer risk: A systematic review and meta-analysis. *Int J Epidemiol*. 2014;43(4):1226-1236.
44. Fulton S, McKinley M, Young I, Cardwell C, Woodside J. The effect of increasing fruit and vegetable consumption on overall diet: A systematic review and meta-analysis. *Crit Rev Food Sci Nutr*. 2016;56(5):802-816.
45. Navarro S, Schwarz Y, Song X, et al. Cruciferous vegetables have variable effects on biomarkers of systemic inflammation in a randomized controlled trial in healthy young adults. *J Nutr*. 2014;144(11):1850-1857.
46. Thompson F, Kirkpatrick S, Subar A, et al. The national cancer institute's dietary assessment primer: A resource for diet research. *J Acad Nutr Diet*. 2015;115(12):1986-1995.
47. Mayne S, Cartmel B, Scarmo S, Jahns L, Ermakov I, Gellermann W. Resonance Raman spectroscopic evaluation of skin carotenoids as a biomarker of carotenoid status for human studies. *Arch Biochem Biophys*. 2013;539(2):163-170.
48. Thomson C, McCullough M, Wertheim B, et al. Nutrition and physical activity cancer prevention guidelines, cancer risk, and mortality in the women's health initiative. *Cancer Prev Res (Phila)*. 2014;7(1):42-53.
49. Dieli Conwright C, Parmentier J, Sami N, et al. Adipose tissue inflammation in breast cancer survivors: Effects of a 16-week combined aerobic and resistance exercise training intervention. *Breast Cancer Res Treat*. 2017;168(1):147-157.
50. Picon Ruiz M, Morata Tarifa C, Valle Goffin J, Friedman E, Slingerland J. Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. *CA Cancer J Clin*. 2017;67(5):378-397.
51. Michie S, Jochelson K, Markham WA, Bridle C. Low-income groups and behaviour change interventions: A review of intervention content, effectiveness and theoretical frameworks. *J Epidemiol Community Health*. 2009;63(8):610-622.
52. Fornetti J, Martinson H, Betts C, et al. Mammary gland involution as an immunotherapeutic target for postpartum breast cancer. *J Mammary Gland Biol Neoplasia*. 2014;19(2):213-228.
53. Martinson H, Lyons T, Giles E, Borges V, Schedin P. Developmental windows of breast cancer risk provide opportunities for targeted chemoprevention. *Exp Cell Res*. 2013;319(11):1671-1678.

AUTHOR INFORMATION

A. R. Essa is a clinical research coordinator, Psychiatric and Neurodevelopmental Genetics Unit, Center for Genomic Medicine, Massachusetts General Hospital, Boston; at the time of the study, she was study coordinator, Department of Veterinary and Animal Sciences, University of Massachusetts Amherst. E. P. Browne is a research fellow and E. C. Punska is research technician, Department of Veterinary and Animal Sciences, University of Massachusetts Amherst, Life Sciences Laboratories, Amherst. K. Perkins is a physician assistant student, Massachusetts College of Pharmacy and Health Sciences, Boston; at the time of the study, she was an undergraduate student, University of Massachusetts Amherst. E. Boudreau is dining hall assistant manager, University of Massachusetts Amherst. H. Wiggins is a nutrition educator, University of New England, Biddeford, ME; at the time of the study, she was a graduate student and teaching assistant, Department of Nutrition, University of Massachusetts Amherst. D. L. Anderton is distinguished professor and department chair, Department of Sociology, University of South Carolina, Columbia. L. Sibeko is an assistant professor, Department of Nutrition, S. R. Sturgeon is a professor, Department of Public Health and Health Sciences, and K. F. Arcaro is a professor, Department of Veterinary and Animal Sciences, University of Massachusetts Amherst.

Address correspondence to: Kathleen F. Arcaro, PhD, Department of Veterinary and Animal Sciences, University of Massachusetts, 240 Thatcher Road, LSL 1 Room N529, Amherst, MA 01003-9298. E-mail: karcaro@umass.edu

STATEMENT OF POTENTIAL CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

FUNDING/SUPPORT

This work was supported by the National Institute of Food and Agriculture, US Department of Agriculture, the Center for Agriculture, Food and the Environment and the Department of Veterinary and Animal Sciences at University of Massachusetts Amherst, under project number MAS00512. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA or NIFA. Material support was provided in the form of fruits and vegetables donated by University of Massachusetts Amherst Dining Services, Big Y Amherst, and Peapod.

ACKNOWLEDGEMENTS

The authors thank Ken Toong, MBA, and his colleagues at UMass Dining Services, Big Y Amherst, and Peapod for their contributions to the weekly fruit and vegetable food boxes; and Tanya Lieberman, IBCLC, and Dawn Kennedy, CLC, for their assistance with recruitment.

AUTHOR CONTRIBUTIONS

K. F. Arcaro, S. R. Sturgeon, and L. Sibeko designed the study. L. Sibeko supervised the intervention counseling, K. F. Arcaro supervised the laboratory analysis, and A. R. Essa acted as study manager. A. R. Essa, E. P. Browne, E. C. Punska, K. Perkins, E. Boudreau, and H. Wiggins conducted the study. A. R. Essa and K. F. Arcaro prepared the manuscript. D. L. Anderton ran the statistical models and helped with data analysis. All authors read and edited the manuscript. All authors reviewed and approved the final version of the manuscript.