A Comparison of Mediterranean-Style and MyPyramid Diets on Weight Loss and Inflammatory Biomarkers in Postpartum Breastfeeding Women

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Abstract

Background: Of postpartum women, 15%–20% retain ≥ 5 kg of their gestational weight gain, increasing risk for adult weight gain. Postpartum women are also in a persistent elevated inflammatory state. Both factors could increase the risk of obesity-related chronic disease. We hypothesized that breastfeeding women randomized to a Mediterranean-style (MED) diet for 4 months would demonstrate significantly greater reductions in body weight, body fat, and inflammation than women randomized to the U.S. Department of Agriculture's (USDA) MyPyramid diet for Pregnancy and Breastfeeding (comparison diet).

Methods: A randomized, controlled dietary intervention trial was conducted in 129 overweight (body mass index [BMI] 27.2 ± 4.9 kg/m²), mostly exclusively breastfeeding (73.6%) women who were a mean 17.5 weeks postpartum. Dietary change was assessed using a validated Food Frequency Questionnaire (FFQ) before and after intervention as well as plasma fatty acid measures (gas chromatography/flame ionization detector [GC/FID]). Anthropometric measurements and biomarkers of inflammation, tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), also were assessed at baseline and 4 months via enzyme-linked immunosorbent assay (ELISA).

Results: Participants in both diet groups demonstrated significant ($p < 0.001$) reductions in body weight (-2.3 ± 3.4 kg and -3.1 ± 3.4 kg for the MED and comparison diets, respectively) and significant ($p \leq 0.002$) reductions in all other anthropometric measurements; no significant between-group differences were shown as hypothesized. A significant decrease in TNF- α but not IL-6 was also demonstrated in both diet groups, with no significant between-group difference.

Conclusions: Both diets support the promotion of postpartum weight loss and reduction in inflammation (TNF- α) in breastfeeding women.

Introduction

EXCESS ADIPOSITY IS ASSOCIATED with increased risk of obesity-related chronic diseases,¹ such as cardiovascular disease (CVD), diabetes (DM), stroke, and select cancers^{2–4}. Additional risk factors for obesity-related chronic disease include hypertension (HTN), hyperlipidemia, and hyperinsulinemia,^{5,6} all of which are associated with a persistent elevated inflammatory state.^{2,4,7}

An estimated 34% of women are overweight/obese before pregnancy,⁸ and 15%–20% of these women will retain ≥ 5 kg of their gestational weight gain at 12 months postpartum.⁹ Increases in prepregnancy weight from one

pregnancy to the next are associated with greater weight retention,¹⁰ an important predictor of long-term obesity.⁹ Additionally, the early postpartum period has been associated with an upregulation of inflammatory cytokines,^{11,12} likely as a well-controlled, protective, biologic response to the stress of parturition¹³ and uterine involution.¹⁴ Although the exact magnitude and length of this upregulated state are unclear, previous research examining lymphocytes from breastfeeding mothers in *ex vivo* cell cultures demonstrated an increased production of proinflammatory Th1 cytokines in breastfeeding women compared with formula-feeding women and nonpostpartum controls.^{11,15} This state of modest inflammatory response persisted up to 12 months

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postpartum. This is probably a normal, protective response to parturition, although it is possible that a prolonged and excessive inflammatory state after pregnancy may increase a woman's risk of developing chronic diseases later in life. Few studies exist targeting a reduction in body weight and chronic inflammation during the postpartum period. Identification of effective dietary interventions during this period that promote appropriate weight loss, maintenance of a healthy weight thereafter, and a reduction in inflammation is an important clinical goal for optimal long-term health.

The traditional Mediterranean-style (MED) diet, rich in whole grains, fruits and vegetables, legumes and nuts, fish, olive oil, and low-fat dairy products and a high monounsaturated/saturated (MUFA/SAT) fatty acid ratio, has been promoted as an effective diet for the promotion of weight reduction/control.^{16,17} Additionally, a study by Salas-Salvadó et al.¹⁸ demonstrated that regular consumption of foods common to the MED diet was associated with a lower risk of inflammation, as measured by the biomarker interleukin-6 (IL-6) ($p=0.005$).¹⁸ This finding is supported by several other studies that demonstrated beneficial modification of biomarkers of inflammation by MED diets or the food components therein.^{19,20}

The objective of this research was to assess the effects of a MED diet and the United States Department of Agriculture's (USDA) MyPyramid diet for Pregnancy and Lactation²¹ on body weight, adiposity, and biomarkers of inflammation, tumor necrosis factor- α (TNF- α) and IL-6, in breastfeeding women. The biomarkers of inflammation, IL-6 and TNF- α , were chosen because they have been shown to be more consistently elevated in postpartum women^{11,22} as well as favorably modulated by a MED diet.^{23,24}

Materials and Methods

Research design

This randomized, controlled dietary intervention trial (ClinicalTrials.gov#NCT01459991) tested the hypothesis that breastfeeding women randomized to a MED diet will show significant reductions in body weight and body fat, as well as significant reductions in biomarkers of inflammation, compared to breastfeeding women randomized to the USDA MyPyramid diet for Pregnancy and Breastfeeding (comparison diet). Participants completed a 1-week run-in period during which they were advised to consume a diet that targeted habitual intake of select foods daily (i.e., 170 g of orange juice and 28 g of cheese consumed twice daily) along with their regular diet to evaluate the likelihood for ongoing adherence and retention to a diet study requiring specific daily dietary behavioral changes. Randomization was performed using a table of random numbers, independent of study personnel, at the Behavioral Measurement Shared Service (BMSS) at the Arizona Cancer Center. After randomization, women completed a 4-week washout period consisting of usual diet plus 5 or fewer servings of fruits and vegetables daily and elimination of the intake of nuts (Fig. 1). The 4-week washout period was designed to standardize diet before study entry and identify participants who may require additional support to maintain study participation. All participants provided written informed consent before study enrollment. The University of Arizona Internal Review Board

Human Subjects Committee approved the study protocol before study initiation.

Study population and eligibility criteria

This research was conducted among breastfeeding women residing in the greater Tucson, Arizona, metropolitan area who were between 18 and 40 years of age, in general good health, and without a diagnosis or history of DM, liver or kidney disease, or cancer (other than nonmelanoma skin cancer). Women were eligible for study enrollment if their infants were between 2 weeks and 6 months of age and the mothers were willing to maintain the following eligibility criteria: breastfeed ≥ 3 times per day for a minimum of 6 additional months, refrain from estrogen-containing contraceptives, and discontinue use of all vitamins/supplements for the duration of the study, with the exception of the study-provided prenatal vitamins (One-A-Day Women's Prenatal, Bayer HealthCare, Morristown, NJ). The decision to include postpartum women with infants between 2 weeks and 6 months of age who were breastfeeding at least 3 times per day was made for the following reasons: (1) the probability of continued breastfeeding ≥ 3 times daily decreases with increasing infants' age, especially beyond 6 months,²⁵ (2) the majority of postpartum weight loss occurs within the first 6 weeks,^{26,27} increasing the likelihood of weight loss occurring thereafter to be a direct result of the diet intervention, and (3) recruitment and retention of postpartum women into a clinical trial is challenging, as documented by previous research studies.^{28–30} There were no restrictions on body mass index (BMI). Women were asked to be available for clinic visits and telephone contact throughout the 4-month study period and to complete study questionnaires. Women were ineligible if they used tobacco products or had a personal/family history of food allergies.

Recruitment and retention strategies have been described in detail previously.³¹ Briefly, recruitment strategies included the distribution of brochures and fliers at local hospitals, obstetric/gynecologic and pediatric offices, The Special Supplemental Nutrition Program for Women, Infants, and Children offices, La Leche League meetings, local libraries, and health fairs. Print advertisements were also used and included local newspapers, a University of Arizona employee ListServ, Craig's List, and a local parenting magazine, *The Tucson Parent*. In addition, study staff conducted on-site recruitment visits to newly postpartum women at local hospitals, obstetrics/gynecology clinics, breastfeeding and parent education classes, and breastfeeding support groups. Retention strategies included the provision of gift cards upon completion of study visits, a manual breast pump (Medela, Inc., McHenry, IL), and lactation support from a Certified Lactation Consultant as needed throughout the study. Incentive gifts were also provided: a picture album at the baseline visit, a coffee mug at the 2-month visit, and a first-aid kit at the 4-month visit.

Study diets

After completion of the 5-week run-in/washout period, study participants received dietary education specific to the MED diet or normal diet recommended for lactation as described by the USDA MyPyramid for Pregnancy and Breastfeeding. Participants received one-on-one diet counseling

with a registered dietitian about the assigned diet and target dietary behaviors. Counseling methods incorporated common behavioral change techniques, including self-efficacy promotion, goal setting, and self-monitoring, in order to promote adoption and adherence to the assigned study diet. Individual counseling sessions consisted of 24-hour dietary recalls assessing participant adherence to the assigned diet. Dietary recommendations were individualized to meet the participant's personal dietary goals as well as to meet the goals of the assigned diet. Counseling visits were repeated on-site at the study clinic at 2 weeks and 2 months and were complemented with written materials (diet education notebook specific to study diet randomization, including serving size recommendations, tips to follow the assigned diet, and recipes) as well as telephone consultations with a registered dietitian twice monthly for the first 2 months and once during the third month. Two registered dietitians were assigned to meet with study participants throughout the duration of the study.

Participants randomized to the intervention diet were provided with nutrition education in order to follow a MED pattern of eating emphasizing a plant-based diet with whole

grains, fresh fruits and vegetables, legumes and nuts, fish and poultry, olive oil, and low-fat dairy products, while limiting the intake of red meat and processed meats/foods (no more than two servings per month).³² Specifically, participants in the intervention group were instructed to consume study-provided walnuts (28 g/day), 1–2 tablespoons/day of olive oil (refined or virgin), and ≥7 servings/day of fruits and vegetables for the duration of the diet intervention. Although no other specific dietary goals were set, participants were encouraged to consume ≥6 servings of whole grains per day and ≥2 servings of fish per week and to increase consumption of legumes while limiting the intake of whole fat dairy products, red meats, processed foods, desserts, and sources of fat in the diet other than olive oil. Walnuts were included in the diet because they are a common component of the traditional MED diet,³² have proposed beneficial effects on weight control through increased satiation,³³ and have anti-inflammatory properties.³⁴

Participants randomized to the comparison diet were provided with general nutrition education guidelines based on the USDA MyPyramid diet for Pregnancy and Breastfeeding,

emphasizing healthy eating choices. Intake of nuts, the use of olive oil, and an increase in fruits and vegetables were deemphasized in order to differentiate the diet from the MED diet. Participants in both groups were instructed to consume the study-provided prenatal vitamin daily. Frequency of contact with study personnel was consistent across study groups.

Demographics and breastfeeding habits

Participants completed study questionnaires related to demographic and lifestyle characteristics upon study enrollment. Assessment of breastfeeding patterns was completed with each clinic visit or telephone follow-up call and included frequency and duration of breastfeeding, assessment of supplemental formula use (type, frequency, and amount), and any possible problems/concerns related to breastfeeding or infant feeding.

Dietary intake

Change in dietary intake was estimated using repeated administrations of the validated Arizona Food Frequency Questionnaire (AFFQ)³⁵ at baseline and 4 months. The AFFQ, a scannable 153 food/beverage item questionnaire, is a regionally appropriate modification of the food frequency component of the validated Block National Cancer Institute (NCI) Health Habits and History Daily Eating Pattern Assessment³⁶ and includes responses on serving sizes and frequency of intake. Study personnel blinded to study arm assignment reviewed questionnaires for completeness, and participants were contacted by telephone to ascertain missing data. Using this approach, there were no AFFQs with more than 5 missing items; however, 7 women did not return either the baseline or 4-month AFFQ, resulting in 95 AFFQs included in this analysis. The BMSS at the Arizona Cancer Center used Metabolize Software[®] to complete the nutrient analyses of the AFFQ. Metabolize is a 4-module system of programs that reduces data from scanned questionnaires to individual nutrients per day. The database used to quantify nutrient intake from the AFFQ was derived from the Continuing Survey of Food Intakes of Individuals (CSFII) 1994–1996 and 1998 and the Nutrition Data System for Research (NDS-R) (versions 11–13).^{37,38}

MED scores were also calculated from the AFFQ data using the following method. The MED score is the sum of scores from 9 different food groups: vegetables, legumes, fruits and nuts, whole grain cereals, fish, meat/poultry, dairy, MUFA/SAT ratio, and ethanol.³⁹ A score of 1 is awarded for each food group in which the target grams (median from the total population) are met or exceeded in the case of vegetables, legumes, fruits and nuts, whole grain cereals, fish, and the MUFA/SAT ratio; or in which the target grams are not exceeded in the case of meat/poultry and dairy; or if the target grams are within a specified range in the case of ethanol. The MED score results in a range of scores of 0–9, with 9 indicating the best adherence.

Body weight and body composition measures

Body weight, height, and waist and hip circumference were measured at baseline and 4 months, following standardized protocols.^{40,41} Body composition was measured using bio-

electric impedance analysis (BIA) (OMRON Body Fat Analyzer, OMRON Healthcare, Inc., Vernon Hills, IL). This method has been validated previously for use in postpartum and breastfeeding women.^{42,43}

Analysis of plasma fatty acids

Plasma fatty acid analyses were completed as a measure of adherence to the MED diet. Specifically, oleic acid as a biomarker of olive oil consumption,⁴⁴ α -linolenic acid as biomarker of walnut intake,³⁴ and linoleic acid as a biomarker of increased fish consumption.⁴⁵ Fatty acid compositions of plasma were determined by capillary gas-liquid chromatography (GC) using a flame ionization detector (FID) (Lipid Analytical Laboratories, Guelph, ON, Canada). Lipids were extracted from plasma samples according to the method of Bligh and Dyer.⁴⁶ The fatty acid methyl esters were prepared using boron trichloride in methanol and heating the methylation tubes in a boiling water bath. The resulting fatty acid methyl esters were analyzed on a Varian 3400 gas-liquid chromatograph (Palo Alto, CA) with a 60-m DB-23 capillary column (0.32 mm internal diameter).

Analysis of inflammatory biomarkers

Eight-hour fasting venous blood samples were collected at baseline and at 4 months to assess biomarkers of inflammation (IL-6 and TNF- α). Blood was collected by venipuncture into two 9-mL tubes, centrifuged at 4°C and 1500g for 15 minutes, with serum aliquoted into 1.5-mL cryovials and stored at –80°C.

IL-6 was analyzed by the Quantikine High Sensitivity Human IL-6 Immunoassay, a 5.5-hour solid-phase enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). Serum samples were analyzed in duplicate according to the manufacturer's instructions. Briefly, a monoclonal antibody specific for IL-6 is precoated onto a microplate. Standards and serum samples are pipetted into the wells, and the immobilized antibody binds any IL-6 present. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 is added to the wells. After a second wash step, a substrate solution is added to the wells. After an incubation period, an amplifier solution is added to the wells, and the color develops in proportion to the amount of IL-6 bound in the initial step. The color development is stopped, and the intensity of the color is determined by dual wavelength absorbance measurement at 490 nm. The intraassay and interassay coefficients of variation (CV) were 11.7% and 15.2%, respectively.

The Quantikine High Sensitivity Human TNF- α Immunoassay is a 6.5-hour solid-phase ELISA (R&D Systems). Serum samples were analyzed in duplicate following the manufacturer's instructions. This assay also employs the quantitative sandwich enzyme immunoassay technique described above using monoclonal and polyclonal antibodies specific to TNF- α . The intraassay and interassay CVs were 10.9% and 13.9%, respectively.

Statistical analysis

The power analysis for sample size was conducted using nQuery Advisor (Statistical Solutions, Saugus, MA), comparing sample sizes of similar diet intervention studies from

published literature. Two-sample, 2-tailed *t* tests of mean changes at 0.05 significance, 95% power, varying effect sizes (ranging from 0.75 to 2.75), and standard deviations (SD) (ranging from 7.8 to 8.0) were calculated based on analysis of similar research designs. The calculated sample sizes ranged from 29 to 106; thus, a sample size of 138 subjects was identified as providing sufficient statistical power to test our study hypothesis^{23,47} with consideration for an estimated 25% attrition rate, slightly greater than the 10%–15% attrition rate commonly seen with clinical diet intervention studies conducted in adults,⁴⁸ as this trial targeted new mothers.

Differences between diet groups were tested using Fisher's exact tests for categorical variables and two-sample *t* tests for continuous variables. Changes in dietary (or body composition) measures between baseline and 4 months (end of study) were tested using paired *t* tests. Differences between the two diet groups in changes in dietary measures over time were tested using linear regression models, adjusted for the baseline dietary measure and baseline energy intake. Differences between groups defined by formula supplement use (yes vs. no) in changes in body size measures over time were tested using two-sample *t* tests. Such changes in body size measurements were further tested using linear regression models, adjusted for the baseline body size measurement, but the results were not substantially different (data not shown). Changes in biomarker levels were tested similarly, using paired *t* tests (between baseline and 4 months) and linear regression models (difference in change between diet or weight loss groups), adjusted for baseline biomarker level and, in the case of weight loss stratification, baseline weight. Weight loss groups were defined according to whether or not each participant lost at least 10% of total body weight between baseline and 4 months. Effect of supplemental formula use on biomarkers of inflammation was also assessed for potential confounding, and no significant differences within or between groups were noted. Linear regression models were also conducted to assess change in waist circumference and change in biomarkers, adjusted for baseline values. All outcome variables were assessed for normality. If a participant had missing

data for any outcome variable, she was excluded from that analysis. All two-sample *t* tests were calculated assuming unequal variance using Welch's formula. All tests were two-sided and conducted using Stata 11.1 (StataCorp, College Station, TX).

Results

One hundred thirty-eight women were consented and enrolled into the study. Of these, 9 discontinued study participation before the baseline visit: 2 reported cessation of breastfeeding, 4 were lost to follow-up, and 3 discontinued the study on their own volition. Of the 129 women who initiated the study diets, 102 women completed the 4-month dietary intervention and final study visit. Among the 27 women who discontinued study participation after initiation of the study diet, 11 reported cessation of breastfeeding, 7 were lost to follow-up, and 9 reported other reasons, resulting in a final attrition rate of 20.9% among women who initiated the study diet. There was no statistically significant difference in attrition rates between diet groups; these data have been reported in detail previously.³¹

At baseline, women were an average age of 29.7 years and 17.5 weeks postpartum (Table 1). Participants were, on average, overweight, with a mean BMI of 27.2 kg/m² and a self-reported average prepregnancy BMI of 25.5 kg/m². The women were predominantly non-Hispanic white (75.2%), and 26.8% reported supplementation with infant formula at baseline. There were no significant differences in baseline characteristics between women in assigned diet groups.

Participants in the MED group demonstrated significant decreases in total energy intake (−251.2 kcal/day, *p*=0.045) and significant increases in legumes (7.2 g/day, *p*=0.044), whole grains (30.8 g/day, *p*=0.011), and dairy (132.8 g/day, *p*=0.009) (Table 2). Participants in the MyPyramid group demonstrated significant decreases in total energy (−437.5 kcal/day, *p*=0.003) and significant increases in vegetables (63.8 g/day, *p*=0.033). The only significant between-group difference was in fish intake (*p*=0.001). Women

TABLE 1. BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS

Characteristic	Total (n=129)	Mediterranean (n=65)	MyPyramid (n=64)
Age, years, mean ± SD	29.7 ± 4.6	30.1 ± 4.5	29.4 ± 4.7
Hispanic ethnicity, <i>n</i> (%)	32 (24.8)	15 (23.1)	17 (26.6)
College degree, <i>n</i> (%)	89 (69.0)	42 (64.6)	47 (73.4)
Infant's age, weeks, mean ± SD	17.5 ± 8.2	17.5 ± 8.0	17.5 ± 8.5
Formula supplementation use, ^a <i>n</i> (%)	34 (26.8)	18 (28.2)	16 (25.4)
Prepregnancy weight (kg), mean ± SD	69.4 ± 13.4	70.1 ± 14.4	68.8 ± 12.3
Prepregnancy BMI (kg/m ²), mean ± SD	25.5 ± 4.7	25.4 ± 4.7	25.6 ± 4.6
Prepregnancy BMI ≥ 25.0 kg/m ² , <i>n</i> (%)	64 (49.6)	30 (46.2)	34 (53.1)
Weight (kg), mean ± SD	74.1 ± 14.3	75.0 ± 16.1	73.2 ± 12.3
BMI (kg/m ²), mean ± SD	27.2 ± 4.9	27.1 ± 5.2	27.2 ± 4.6
Body fat (%), ^b mean ± SD	30.8 ± 6.6	30.8 ± 7.0	30.8 ± 6.2
Waist circumference (cm), mean ± SD	91.9 ± 12.8	91.4 ± 12.6	92.5 ± 13.0
Hip circumference (cm), mean ± SD	108.7 ± 10.4	109.1 ± 11.6	108.2 ± 9.2
Waist/hip ratio, mean ± SD	0.84 ± 0.07	0.84 ± 0.05	0.85 ± 0.08

There are no significant differences between diet groups.

^a*n*=127.

^b*n*=109.

BMI, body mass index; SD, standard deviation.

TABLE 2. CHANGE IN DIETARY INTAKE AMONG STUDY PARTICIPANTS

	<i>Mediterranean diet (n=50)</i>				<i>MyPyramid diet (n=44)</i>			
	<i>Baseline</i>	<i>4 months</i>	Δ	P ^a	<i>Baseline</i>	<i>4 months</i>	Δ	P ^a
Energy (kcal/day)	2711 (1305) ^b	2460 (1072)	−251.2 (865.3)	0.045	2950 (1363)	2513 (1128)	−437.5 (1331)	0.035
Vegetables	0.54 (0.503)	0.64 (0.485)	0.10 (0.647)	0.275	0.47 (0.505)	0.58 (0.499)	0.11 (0.532)	0.166
Legumes	0.48 (0.505)	0.60 (0.495)	0.12 (0.521)	0.109	0.53 (0.505)	0.60 (0.495)	0.07 (0.495)	0.366
Fruits and nuts	0.52 (0.505)	0.60 (0.495)	0.08 (0.601)	0.346	0.49 (0.506)	0.49 (0.506)	0.00 (0.640)	1.000
Whole grains	0.48 (0.505)	0.62 (0.490)	0.14 (0.670)	0.144	0.53 (0.505)	0.76 (0.435)	0.22 (0.560)	0.012
Fish	0.54 (0.503)	0.66 (0.479)	0.12 (0.659)	0.201	0.47 (0.505)	0.38 (0.490)	−0.09 (0.514)*	0.248
Meat/poultry	0.48 (0.505)	0.54 (0.503)	0.06 (0.620)	0.491	0.49 (0.506)	0.38 (0.490)	−0.11 (0.573)	0.197
Dairy	0.48 (0.505)	0.42 (0.499)	−0.06 (0.512)	0.405	0.51 (0.506)	0.49 (0.506)	−0.02 (0.499)	0.763
Monounsaturated/ saturated fat ratio	0.5 (0.505)	0.64 (0.485)	0.14 (0.700)	0.162	0.49 (0.506)	0.53 (0.505)	0.04 (0.673)	0.655
Alcohol	0.06 (0.240)	0.04 (0.198)	−0.02 (0.247)	0.564	0.02 (0.151)	0.07 (0.252)	0.04 (0.208)	0.157
Total MED diet score*	4.08 (2.117)	4.76 (2.036)	0.68 (2.736)	0.083	4.00 (1.508)	4.27 (1.684)	0.27 (1.572)	0.252

^aWilcoxon signed-rank test used for within group changes; Mann-Whitney U test used for between group changes.

^bMean (SD).

*Significant difference between group scores at 4 months ($p=0.006$).
MED, Mediterranean diet.

randomized to the MED group increased fish intake by 4.6 ± 22.2 g/day, and women in the MyPyramid group decreased fish intake by -1.3 ± 12.0 .

Women randomized to either diet group demonstrated significant ($p \leq 0.002$) reductions from baseline in all anthropometric measurements at 4 months (Table 3). There were no statistically significant differences in the change in any of the anthropometric measures over time between diet groups.

Significant increases in plasma linoleic acid (1.44 ± 2.94 , $p=0.001$), linolenic acid (0.18 ± 0.30 , $p<0.001$), total n-3 fatty acids (0.29 ± 0.82 , $p=0.013$), and the n3/n6 fatty acid ratio (0.01 ± 0.02 , $p=0.035$) and a significant decrease in the n6/n3 fatty acid ratio (-0.79 ± 2.59 ; $P=0.032$) were noted in the MED diet group (Table 4). There were no significant changes in fatty acid levels in the comparison group. These changes were significantly different between groups, with the exception of the n6/n3 fatty acid ratio, even when adjusted for baseline body weight and fatty acid level.

IL-6 did not significantly change over time within groups, and no between-group difference in change in IL-6 was noted (Table 5). Study participants in both diet groups demonstrated significant reductions in TNF- α between baseline and

4 months (MED -0.89 , $p=0.021$) vs. comparison -0.53 , $P<0.001$); however, these differences were not significantly different across groups. When all study participants were combined as one cohort to evaluate change in inflammatory biomarkers, results were similar.

Discussion

To our knowledge, no MED diet interventions for the promotion of weight loss/control and the reduction of inflammation have been conducted previously in breastfeeding women. In our study, women randomized to the MED diet and the MyPyramid diet demonstrated significant reductions in anthropometric measurements and TNF- α , a biomarker of inflammation, but changes were not significantly different between groups as hypothesized.

Epidemiologic and clinical research suggests that inflammation can be modified by MED-related dietary constituents, including alpha-linolenic acid (ALA) found in walnuts; MUFAs found in olive oil; polyunsaturated fatty acids (PUFA); fiber found in whole grains, lentils, and beans; and antioxidants found in vegetables, fruit, wine, nuts, and seeds.^{34,49–51}

TABLE 3. CHANGE IN ANTHROPOMETRICS BETWEEN BASELINE AND 4 MONTHS, BY DIET GROUP

	<i>Mediterranean diet (n=53)</i>				<i>MyPyramid diet (n=49)</i>				
	<i>Baseline</i>	<i>4 months</i>	<i>Change</i>	P ^a	<i>Baseline</i>	<i>4 months</i>	<i>Change</i>	P ^a	P ^b
Weight (kg)	74.7 $\pm$ 16.8 ^c	72.4 $\pm$ 17.6	−2.31 $\pm$ 3.42	<0.001	72.7 $\pm$ 12.9	69.6 $\pm$ 13.8	−3.11 $\pm$ 3.35	<0.001	0.581
BMI (kg/m ²)	27.1 $\pm$ 5.29	26.2 $\pm$ 5.58	−0.85 $\pm$ 1.24	<0.001	26.7 $\pm$ 4.82	25.6 $\pm$ 5.24	−1.13 $\pm$ 1.22	<0.001	0.594
Waist (cm)	91.1 $\pm$ 13.3	87.6 $\pm$ 14.0	−3.47 $\pm$ 4.46	<0.001	92.2 $\pm$ 13.9	87.6 $\pm$ 14.1	−4.59 $\pm$ 4.34	<0.001	0.968
Hip (cm)	109.2 $\pm$ 12.0	107.0 $\pm$ 11.7	−2.19 $\pm$ 3.97	<0.001	107.8 $\pm$ 9.52	104.9 $\pm$ 10.7	−2.90 $\pm$ 5.48	0.001	0.520
Waist/hip ratio	0.83 $\pm$ 0.06	0.82 $\pm$ 0.07	−0.02 $\pm$ 0.04	0.001	0.85 $\pm$ 0.08	0.83 $\pm$ 0.07	−0.02 $\pm$ 0.04	0.001	0.237
Body fat (%) ^d	30.9 $\pm$ 7.20	29.7 $\pm$ 7.79	−1.19 $\pm$ 2.43	0.002	30.6 $\pm$ 6.47	28.4 $\pm$ 7.13	−2.20 $\pm$ 2.89	<0.001	0.252

There were no significant differences between diet groups at baseline.

^aPaired t test for within-group change between baseline and 4 months.

^bMann-Whitney U-test for difference between diet groups at 4 months.

^cMean $\pm$ SD.

^dMissing body fat data for 13 participants (7 in Mediterranean group, 6 in MyPyramid group).

TABLE 4. CHANGE IN FATTY ACID LEVELS AMONG STUDY PARTICIPANTS, BY DIET GROUP

	Mediterranean diet (n=53)				MyPyramid diet (n=49)					
	Baseline	4 months	Change	P ^a	Baseline	4 months	Change	P ^a	P ^b	P ^c
Oleic acid	17.9 (1.86) ^d	17.7 (1.86)	-0.22 (2.05)	0.444	17.8 (1.79)	18.2 (2.33)	0.45 (2.07)	0.130	0.101	0.105
Linoleic acid	33.5 (3.49)	35.0 (3.44)	1.44 (2.94)	0.001	34.2 (3.49)	34.1 (3.61)	-0.13 (2.71)	0.746	0.006	0.012
Linolenic acid	0.67 (0.20)	0.85 (0.28)	0.18 (0.30)	<0.001	0.64 (0.18)	0.68 (0.21)	0.04 (0.26)	0.261	0.014	0.001
n3	3.38 (0.99)	3.67 (1.35)	0.29 (0.82)	0.013	3.30 (0.80)	3.18 (0.78)	-0.13 (0.78)	0.261	0.010	0.008
n6	45.0 (3.01)	45.8 (3.07)	0.75 (2.75)	0.051	45.4 (2.81)	45.7 (3.07)	0.23 (2.58)	0.542	0.319	0.443
n3/n6 ratio	0.08 (0.02)	0.08 (0.03)	0.01 (0.02)	0.035	0.07 (0.02)	0.07 (0.02)	-0.00 (0.02)	0.168	0.015	0.015
n6/n3 ratio	14.2 (3.44)	13.4 (3.05)	-0.79 (2.59)	0.032	14.7 (4.61)	15.2 (4.19)	0.55 (2.99)	0.201	0.457	0.404

^aPaired *t* test for within-group change between baseline and 4 months.

^bTwo-sample *t* test for unadjusted between-group change.

^cWald statistic from linear regression model for between-group change, adjusted for baseline weight and fatty acid level.

^dMean (SD).

One cross-sectional study examining the association between specific components in the MED diet and circulating biomarkers of inflammation found that the consumption of fruits, cereals, virgin olive oil, and nuts was associated with lower inflammatory biomarkers—high-sensitivity C-reactive protein (hsCRP), IL-6, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1)—in subjects with increased risk for CVD.¹⁸ Our results indicate an inflammatory-modulating effect of both diets, however, suggesting that both diets promoted healthier food selections. It is possible that the women randomized to the MED diet group may not have consistently met the dietary behavioral targets, which may explain the lack of a statistically significant difference in the biomarkers of inflammation between diet groups. Although positive changes were demonstrated in the fatty acid profiles of the women randomized to the MED diet group, changes not shown in the comparison group, there was a nonsignificant decrease in plasma oleic acid concentrations in the MED diet group, suggesting the women may have been compliant with the daily walnut intake goal but not with the daily olive oil intake goal. Other factors may also have influenced this lack of a significant difference. For example, both diet groups lost a similar amount of weight, possibly reducing inflammation. Additionally, participants demonstrated relatively low levels of IL-6 at baseline (2.1 ± 4.8 and 1.0 ± 0.7 pg/mL for the MED and comparison diet groups, respectively) compared with studies in high-risk individuals who demonstrated higher baseline IL-6 levels ranging typically from 4.1 to 6.8 pg/mL.^{18,52,53} This may have made it more difficult to detect significant change.

The amount of time required to return to prepregnancy weight is unclear. It could be argued that the amount of weight loss attained in both groups was related to continuous, gradual postpartum weight loss, given that diet assignment did not cause significant between-group reductions. Two studies reported that 75%–80% of gestational weight gain is lost by 2–6 weeks postpartum.^{26,27} Women in our study demonstrated a mean postpartum time of 17.5 ± 8.2 weeks at baseline and, therefore, entered the study well after the period of maximum weight loss.

Prepregnancy BMI is one factor known to influence postpartum weight retention.⁵⁴ In our study, women with lower prepregnancy BMI demonstrated greater reductions in body

weight over time regardless of diet assignment (data not shown), in contrast to results from a prospective cohort study of 405 women examining factors associated with postpartum weight retention. In that study, the authors found that for every 1-unit increase in prepregnancy BMI, there was a 0.51-kg decrease in postpartum weight retention.⁵⁵ Breastfeeding may also contribute to postpartum weight loss.⁵⁶ When all study participants were stratified by breastfeeding status (exclusive breastfeeding or breastfeeding $\geq 3 \times$ /day + formula supplementation) in a *post-hoc* analysis, significant reductions were demonstrated in all anthropometric measurements among the exclusively breastfeeding women (data not shown). Women who used supplemental formula also showed reductions in most anthropometric measures over time, but only select measurements reached statistical significance, suggesting that the added energy requirements of lactation likely account for the energy deficit and the majority of weight loss demonstrated. Additional evidence also has suggested that exclusive breastfeeding promotes greater postpartum weight loss than breastfeeding with formula supplementation,^{57,58} although this has not been demonstrated consistently.^{59,60}

A second *post-hoc* analysis combined all study participants into one cohort and then stratified the group by amount of weight loss ($< 10\%$ or $\geq 10\%$) to assess change in biomarkers of inflammation. This weight loss cutoff point was chosen based on results from a randomized controlled trial (RCT) in 93 obese subjects who maintained a very low energy diet for 8 weeks, followed by randomization to orlistat or placebo and lifestyle intervention for 3 years.⁶¹ Results from this study indicated that 10% body weight loss was the minimum amount required to effectively reduce biomarkers of inflammation as measured by hsCRP, adiponectin, and fibrinogen. In the present study, no significant within-group difference was noted for IL-6 in the adjusted model regardless of weight loss amount. It is possible that the lack of a significant difference in TNF- α , as well as IL-6, in the greater weight loss category is related to the very small number of women who actually lost $\geq 10\%$ of their total baseline body weight ($n=8$) (data not shown). Further, Although some previous studies examining the effects of weight loss on biomarkers of systemic inflammation (hsCRP, IL-6, and TNF- α) have shown significant decreases in these biomarkers in combination with weight loss, others have found conflicting results,^{62,63} possibly due to varying methods of

TABLE 5. CHANGE IN BIOMARKERS OF INFLAMMATION AMONG STUDY PARTICIPANTS, BY TREATMENT GROUP

	Mediterranean diet (n=52)			Control (n=49)		
	Baseline	4 months	Change	Baseline	4 months	Change
IL-6 (pg/mL)	1.977 (0.68-3.27) ^a	1.585 (0.84-2.33)	-0.392 (-1.08-0.30)	0.916 (0.71-1.13)	0.888 (0.66-1.11)	-0.028 (-0.21-0.16)
TNF- α (pg/mL)	4.189 (1.30-7.08)	3.301 (1.10-5.50)	-0.888 (-1.64-0.14)	2.475 (1.48-3.47)	1.940 (1.18-2.70)	-0.535 (-0.82- -0.25)
p			0.258			0.761
			0.021			<0.001

No significant differences between groups.

^aMean (95% Confidence interval).

IL-6, interleukin-6; TNF- α , tumor necrosis factor- α .

assessment or differing baseline biomarker concentrations between studies.

Several limitations to this research exist, some resulting from the need to reduce participant burden to promote adherence and reduce recidivism. Participants were not asked to maintain food diaries for self-monitoring or to attend group counseling sessions, methods proven to be effective for adherence to dietary interventions.^{64,65} Second, we used the FFQ to estimate diet change, and FFQs are known to have significant measurement error.⁶⁶ The FFQ was unable to quantify olive oil intake separate from total fat intake, an important component of the MED diet; we were, however, able to capture change in oleic acid intake through the assessment of plasma fatty acids. We did not collect data in regard to total gestational weight gain (only prepregnancy BMI and BMI at time of study entry), a factor that may have influenced the weight loss demonstrated.⁵⁵ Finally, the attrition rate of 20.9% may have introduced sampling bias. The literature suggests that retention is particularly challenging in this study population and that our attrition rates were below expected rates. Of importance, our attrition rates were well below rates in previous trials involving postpartum breastfeeding women, which have been reported to range from 31% to 43%.^{28-30,67}

The strengths of this research include the RCT design, the use of registered dietitians to provide nutritional counseling, the use of lactation consultants to provide breastfeeding assistance to support continued study participation, and the provision of guidance about dietary recommendations for the reduction of body weight and biomarkers of inflammation among breastfeeding mothers. Additionally, the study had an ample sample size to test our *a priori* hypothesis and blinded inflammatory biomarker analyses.

Conclusions

Results from this RCT in breastfeeding women suggest that both the MED diet and USDA's MyPyramid diet can effectively promote weight loss/control, favorable change in body fat, and a reduction in the biomarker of inflammation, TNF- α , over a 4-month period. The authors recognize that the USDA MyPyramid dietary recommendations have been replaced by the MyPlate recommendations⁶⁸; however, the underlying dietary recommendations remain sound. Future research should continue to examine intervention strategies to promote successful weight loss/control and to reduce persistent inflammation during the postpartum period to decrease the risk of obesity-related chronic disease. Specifically, future diet interventions should aim for a minimum of 10% weight loss, more robust changes in oleic acid intake, and increased differential in dietary changes between the MED and MyPyramid diets in order to positively modulate biomarkers associated with inflammation in postpartum breastfeeding women.

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Disclosure Statement

The authors have no conflict of interests to report.

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