THE DIET STUDY IN LACTATING WOMEN:
A MEDITERRANEAN-STYLE DIET INTERVENTION AND ITS EFFECTS ON
POSTPARTUM WEIGHT LOSS, BODY COMPOSITION AND SELECT BIOMARKERS OF
INFLAMMATION

by
Nicole R. Stendell-Hollis

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF NUTRITIONAL SCIENCES
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA
2011
As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Nicole R. Stendell-Hollis entitled “THE DIET STUDY IN LACTATING WOMEN: A MEDITERRANEAN-STYLE DIET INTERVENTION AND ITS EFFECTS ON POSTPARTUM WEIGHT LOSS, BODY COMPOSITION AND SELECT BIOMARKERS OF INFLAMMATION” and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

________________________________________________________Date: 5/31/11
Cynthia A. Thomson, PhD

________________________________________________________Date: 5/31/11
Patricia A. Thompson, PhD

________________________________________________________Date: 5/31/11
Joy Winzerling, PhD, RD

________________________________________________________Date: 5/31/11
Michael Daines, MD

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copies of the dissertation to the Graduate College. I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

________________________________________________________________________Date: 5/31/11
Dissertation Director: Cynthia A. Thomson, PhD, RD
STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Request for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Nicole R. Stendell-Hollis
ACKNOWLEDGEMENTS

I would first and foremost like to acknowledge my primary advisor, Dr. Cynthia Thomson, who has guided, mentored, and supported me throughout my graduation and dissertation work. I would like to acknowledge the other members of my committee, Dr. Patricia Thompson, Dr. Joy Winzerling, and Dr. Michael Daines, who have also provided me with enormous support and guidance throughout my research project. I would also like to acknowledge all of the additional support I have received from members of Dr. Thomson’s and Dr. Thompson’s lab, as well as everyone who has assisted me throughout graduate school and the dissertation research process. I would like to acknowledge the financial support I have received from the California Walnut Commission, the Nutritional Sciences Department at the University of Arizona, and the American Dietetic Association Foundation. Finally, I would like to acknowledge my family who has been patient and supportive throughout graduate school, especially my parents, Rey and Marlynn Stendell, and my daughters, E’Lexis and Endya Hollis.
# TABLE OF CONTENTS

LIST OF FIGURES......................................................................................................................6

LIST OF TABLES........................................................................................................................7

ABSTRACT......................................................................................................................................8

CHAPTER 1: INTRODUCTION.......................................................................................................10

CHAPTER 2: RECRUITMENT OF LACTATING WOMEN INTO A RANDOMIZED DIETARY INTERVENTION: SUCCESSFUL STRATEGIES AND FACTORS PROMOTING ENROLLMENT AND RETENTION.................................................................17

CHAPTER 3: MEDITERRANEAN-STYLE AND MYPYRAMID DIETS ARE EFFECTIVE IN PROMOTING WEIGHT LOSS IN POSTPARTUM, BREASTFEEDING WOMEN.........................................................................................................................41

CHAPTER 4: CHANGE IN BIOMARKERS OF INFLAMMATION IN RESPONSE TO A MEDITERRANEAN-STYLE DIET INTERVENTION IN BREASTFEEDING WOMEN.................................................................................................................................67

CHAPTER 5: IMPLICATIONS AND FUTURE DIRECTIONS..........................................................94

APPENDIX A: STUDY TIMELINE FOR THE DIET STUDY IN LACTATING WOMEN.........................................................................................................................101

APPENDIX B: MEDITERRANEAN DIET SCORE..........................................................................103

APPENDIX C: LIST OF ABBREVIATIONS..................................................................................105

REFERENCES...............................................................................................................................107
LIST OF FIGURES

FIGURE 1. STUDY DESIGN FOR THE DIET STUDY IN LACTATING WOMEN......36

FIGURE 2. CONSORT DIAGRAM..............................................................................37

FIGURE 3. STUDY DESIGN.........................................................................................62
LIST OF TABLES

TABLE 1. COMPLETERS VERSUS NON-COMPLETERS FOR A MED STUDY IN LACTATING WOMEN BASED ON RECRUITMENT SOURCE..................................................38

TABLE 2. COMPARISON OF BASELINE CHARACTERISTICS OF WOMEN ENROLLED INTO THE DIET STUDY FOR LACTATING WOMEN: COMPLETERS VERSUS NON-COMPLETERS..........................................................................................39

TABLE 3. BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS ..................62

TABLE 4. CHANGE IN DIETARY MEASURES BETWEEN BASELINE AND 4 MONTHS, BY DIET GROUP..............................................................................................64

TABLE 5. CHANGE IN ANTHROPOMETRICS BETWEEN BASELINE AND 4 MONTHS, BY DIET GROUP..............................................................................................65

TABLE 6. CHANGE IN ANTHROPOMETRICS BETWEEN BASELINE AND 4 MONTHS, STRATIFIED BY FORMULA SUPPLEMENTATION USE........................................66

TABLE 7. BASELINE CHARACTERISTICS OF STUDY PARTICIPANTS...............89

TABLE 8. CHANGE IN DIETARY MEASURES BETWEEN BASELINE AND 4 MONTHS, BY DIET GROUP..............................................................................................90

TABLE 9. CHANGE IN ANTHROPOMETRICS BETWEEN BASELINE AND 4 MONTHS, BY DIET GROUP..............................................................................................91

TABLE 10. CHANGE IN BIOMARKERS OF INFLAMMATION AMONG STUDY PARTICIPANTS, BY DIET GROUP.................................................................................92

TABLE 11. CHANGE IN BIOMARKERS OF INFLAMMATION AMONG STUDY PARTICIPANTS, STRATIFIED BY AMOUNT OF WEIGHT LOSS.................................93
ABSTRACT

Obesity-related diseases account for the majority of morbidity and mortality in U.S. adults. An estimated 4 million women in the United States deliver an infant annually, of which approximately 34% are overweight/obese prior to pregnancy. More than 30% of these women gain weight that exceeds the IOM’s recommendations; increasing their risk of postpartum weight retention and possibly increasing their risk of greater weight gain and retention over time. This research sought to test the efficacy of a traditional MED diet for 4-months on weight loss/control and biomarkers of inflammation in breastfeeding women compared to women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet). At baseline, the women (N=129) were 29.7±4.6 years, overweight (BMI: 27.2±4.9 kg/m²), and primarily non-Hispanic white (75.2%). The majority of women were exclusively breastfeeding (73.6%) and a mean 17.5 weeks postpartum. Adherence to the MED diet was evaluated via calculation of the MED diet score from validated FFQs administered pre- and post- the diet intervention. Anthropometric measurements (body weight, body fat, and waist and hip circumference) and biosamples (blood, urine, and breast milk) were collected at baseline and 4-months (diet completion). Biomarkers of inflammation (IL-6 and TNF-α) were assessed via standard ELISA kits. The MED diet score was increased by 0.68±2.74 and 0.27±1.57 for the MED and control group, respectively. Increases in fish and dairy intake and a decrease in meat/poultry intake were significantly different between diet groups.
Participants in both diet groups demonstrated significant \( P=0.002 \) reductions in all anthropometric measurements; no significant between group differences were shown. A significant decrease in TNF-\( \alpha \), but not IL-6, was demonstrated in both diet groups. There were no significant between group differences. Both the MED diet and the USDA’s MyPyramid diet were effective in reducing anthropometric measurements and inflammation in postpartum breastfeeding women.
CHAPTER 1

INTRODUCTION
Introduction

An estimated 1 out of every 2 adults in the United States suffer from at least one type of chronic disease, such as CVD, stroke, DM, and select cancers [1]. For women of reproductive age (15 – 44 years), the most common chronic diseases are depression, HTN, and DM; all of which are associated with modifiable risk behaviors such as insufficient physical activity, smoking, excess saturated fat intake, as well as excess caloric intake and poor diet [2]. Of the numerous risk factors that are related to an increased risk of chronic disease, many are also associated with a persistent elevated inflammatory state [3-6]. While no direct evidence in humans exist showing repeated elevations in inflammation over time cause chronic disease, there is abundant evidence that biomarkers of these processes are increased in those with chronic disease [7].

Of the estimated 4 million women in the U.S. who deliver an infant annually [8], roughly 34% are overweight/obese prior to pregnancy [9], and more than 30% gain weight that exceeds the IOM’s recommendations [10]. While most women will return to their pre-pregnancy weight within one year of parturition if they do not exceed the IOM’s recommendations for gestational weight gain (GWG) [11], 15 – 20% of women will retain ≥ 5 kg [12]. Increases in pre-pregnancy weight from one pregnancy to the next are associated with greater weight retention over time [13, 14], an important predictor of long-term obesity [12, 15]. One approach that may assist the postpartum woman in lowering this risk is to choose to breastfeed her infant(s). Breastfeeding is associated with increased energy expenditure which may
lead to a faster return to pre-pregnancy weight [16]. However, changes in body weight and body composition in response to the metabolic load imposed by lactation are highly variable among and within diverse populations; and this effect is most strongly seen in women who exclusively breastfeed [17, 18].

The postpartum period has also been associated with an up-regulation of inflammatory cytokines [19-21]; most likely as a well-controlled biological response to the stress of parturition [22] and uterine involution [23]. While the exact extent and duration of this up-regulated state is unclear, previous research from ex vivo cell cultures suggest it persists through postpartum months 1 – 12 [19, 24]; and thus, it is possible that this prolonged elevated inflammatory state following pregnancy and continuing through lactation may increase the woman’s risk of developing chronic diseases later in life, particularly in women with chronic, low-grade, obesity-related inflammation. Thus, efforts to minimize inflammation during the postpartum lactation period coupled with reductions in body weight and body adiposity, may contribute to a decrease in risk of future obesity- and inflammation-associated chronic disease. It is feasible that effective diet interventions during the postpartum period that promote weight loss/weight control as well as positive modulation of biomarkers of inflammation may be one method of lowering the risk of chronic disease in the longer term.

**The Traditional Mediterranean-style Diet**
The traditional MED diet is characterized by a high MUFA:SAT fat ratio; high consumption of legumes, whole grain breads, fruits, vegetables, and nuts (including walnuts); moderate alcohol (wine) and dairy consumption; low consumption of meat products; and liberal use of olive oil. Together these foods provide a rich source of BAFCs, antioxidant vitamins and minerals, glutathione, and MUFA and PUFAs [25]. The MED diet is also significantly lower in both n-6 fatty acids and animal fats in comparison to the Western-style diet [26]. Numerous studies have shown an increased risk of chronic disease associated with a low MUFA:SAT fat ratio [27-30]. Accumulating evidence from several large epidemiological studies suggest that greater adherence to the MED diet confers a significant protection against total mortality, as well as the occurrence of CVD and other major chronic diseases [31-35].

Walnuts are a common component of the traditional MED diet and provide a unique combination of compounds identified as having potential anti-inflammatory properties, including BAFCs, ALAs, as well as protein, fiber, vitamins, tannins, folates, and polyphenols; thus increasing the potential to reduce risk of chronic disease with increased intake [36]. A study by Blomhoff et al investigated various tree nuts to determine total antioxidant content and found that of the nuts studied, walnuts were among those with the highest levels of total antioxidant content with more than 20 mmol of antioxidants per 100 g [37], illustrating the potential of walnuts to increase antioxidant concentrations and thus, provide increased protection against chronic disease.
The traditional MED diet has been promoted for weight loss/weight control [38, 39]. In a cross-sectional study examining the prevalence of obesity associated with adherence to a MED diet, researchers found a 51% reduced risk of obesity with greater adherence to the diet among free-living, healthy adults [40]; this is likely related to the healthier food composition found in the MED diet, including decreased animal fats and increased fruits and vegetables. Increased walnut intake, as a component of the MED diet, has also been shown to contribute to optimal weight control despite their energy-dense composition; possibly due to their proposed satiating effects [41, 42]. In a study by Canales et al examining the effects of the consumption of a walnut-enriched meat product in overweight/obese seniors, the addition of nuts to the intervention group did not affect weight status despite the fact that the control group’s diet was actually lower in total fat and energy compared to the intervention group [43]. Accordingly, a MED diet emphasizing walnuts is unlikely to result in weight gain, and in combination with a MED diet and breastfeeding, may actually support weight loss.

The traditional MED diet has also been associated with lowered risk of inflammation [44]. In a study by Salas-Salvadó et al, investigators demonstrated that regular consumption of some of the foods in the traditional MED diet (cereals and fruits) was significantly ($P=0.005$) associated with a lowered risk of inflammation as measured by the biomarker, IL-6 [45]. Of importance in Salas-Salvadó’s study, is that researchers found a significant ($P=0.003$) inverse association between inflammation, as measured by hsCRP, and the consumption of nuts. These
findings suggest the efficacy of nuts in the adjuvant treatment of inflammation. In support of this, a study by Zhao et al also demonstrated that a diet high in ALA, obtained from walnuts, walnut oil and flaxseed oil, was effective in lowering inflammation in a population of hypercholesterolemic subjects [46]. However, only a small number of studies have examined these relationships; thus, it is necessary to conduct additional clinical research trials to further investigate these associations.

In conclusion, because it has been demonstrated that a traditional MED diet, and likely walnut consumption, is effective in promoting weight loss/weight control [40], and is also inversely associated with inflammation [44]; a combination of the MED diet with daily walnut consumption holds strong potential to effectively modulate these conditions in the breastfeeding woman. In this research the efficacy of a traditional MED diet rich in walnuts to promote postpartum weight loss/weight control and positively modulate biomarkers of inflammation was evaluated in breastfeeding mothers. My role in this investigation was as a Co-Principle Investigator and I was involved in all aspects of the study including the study design conception and grant writing, the Human Subject’s approval process, recruitment, study implementation, data collection and analyses, and writing for publication.

STUDY HYPOTHESES
1. Women randomized to the Mediterranean diet will show a significant reduction in body weight, body fat and/or waist:hip circumference over time as compared to women randomized to usual diet.

2. Adherence to a Mediterranean diet rich in walnuts will decrease biomarkers of inflammation.

**STUDY AIMS**

1. Assess feasibility and optimal approaches for recruitment of lactating women to a dietary intervention trial.

2. Assess repeated measures of change in body weight and composition related to dietary group assignment.

3. Determine indirect/secondary effects of diet intervention on biomarkers of inflammation in serum as measured by IL-6 and TNF-α via ELISA kits.

4. Evaluate adherence and exposure to the diet intervention using the validated AFFQ and through calculation of the Mediterranean Diet Score.
CHAPTER 2

RECRUITMENT OF LACTATING WOMEN INTO A RANDOMIZED DIETARY INTERVENTION: SUCCESSFUL STRATEGIES AND FACTORS PROMOTING ENROLLMENT AND RETENTION

Nicole R. Stendell-Hollis*, Monica J. Laudermilk, Julie L. West, Patricia A. Thompson, Cynthia A. Thomson

aNutritional Sciences Department, University of Arizona, 1177 E. 4th St., Tucson, AZ, 85721, USA; bArizona Cancer Center, University of Arizona, 1501 N. Campbell Ave., Tucson, AZ, 85719, USA; c College of Public Health, University of Arizona, 1295 N. Martin Ave., Tucson, AZ, 85724, USA

Contemp Clin Trials. 2011 Mar 5 [epub ahead of print]
Abstract

*Introduction:* Recruitment and retention of lactating women requires unique strategies to prevent high attrition. The purpose of this report is to identify successful recruitment strategies and evaluate demographic and lifestyle characteristics associated with study completion.

*Methods:* A randomized, controlled trial was initiated to test the hypothesis that lactating women adhering to a MED diet will show a significant reduction in anthropometric measurements as compared to lactating women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet). Measurements were collected at baseline, 2 months, and 4 months. Recruitment methods and baseline characteristics of completers and non-completers are described.

*Results:* The largest percentage of women, 24.8%, were recruited from a local parenting magazine, 20.9% from Craig’s List, 20.2% from local hospitals, and 34.1% from various other sources. At baseline, women (n=129) were mostly Non-Hispanic (75.2%), average age 29.7 years, BMI averaged 27.2 kg/m2, waist:hip ratio 0.84 cm (SD: 0.07), and body fat averaged 30.8%. Approximately 72% were exclusively breastfeeding, a mean 17.5 weeks postpartum, and 69.0% had a college degree. Non-completers were more likely to have supplemented with formula at baseline as compared to completers (P<0.001). No other characteristics were significantly associated with attrition.
Conclusion: Researchers conducting studies with lactating women may consider “exclusive breastfeeding” as a study inclusion criterion to prevent high attrition rates or include additional breastfeeding support to study participants.
Introduction

Overweight/obesity, percent body fat, and adult weight gain have been consistently associated with risk of postmenopausal breast cancer with epidemiological studies demonstrating a positive relationship between BMI and breast cancer with numerous studies demonstrating a significant RR ranging from 1.27 to 2.52 [47-51]. A major period of vulnerability for adult weight gain in women occurs with pregnancy and retention of weight postpartum. Promotion of weight loss during lactation through modification of the maternal diet may offer one approach to lower risk of breast cancer during a period when mothers tend to be motivated for diet change in order to enhance the health of their newborns [52]. However, recruitment of a sufficient number of lactating women for a diet intervention poses a number of challenges and presents with several unique barriers specific to this population. These include identification of participants who are representative of the general target population for the intervention and who demonstrate sufficient commitment to breastfeeding to complete the study intervention. Lactating women and infants are a vulnerable population, and even in studies with few risks, potential participants may feel that the research protocol and requirements are too extensive and burdensome. This may be a particular concern for new mothers who are caring for newborn and possibly other children/relatives, and/or planning to return to work or school; limiting their availability for study participation.
Retention of study participants after consent is equally challenging and involves the development and maintenance of relationships that encourage participants to continue for the duration of the study [53, 54]. Attrition rates in previous trials of this study population range from 31 – 43% [8, 55-57], and while some attrition is bound to occur in any research, high attrition rates are likely to introduce sampling bias to study results. Thus, it is prudent to a priori identify contextual, and research- or researcher-related factors and/or personal demographic and lifestyle characteristics that may serve to predict attrition rates, either positively or negatively; especially as little is known about the factors associated with higher participant recruitment and retention success for this target population.

Here we report on the success of different recruitment strategies and the demographic and lifestyle characteristics in lactating women associated with completion of a randomized controlled dietary intervention designed to evaluate the postnatal weight loss efficacy of a MED diet rich in walnuts as compared to the USDA's MyPyramid diet for Pregnancy and Breastfeeding.

Materials and Methods

Study Design

This randomized, controlled dietary intervention trial was designed to test the hypothesis that lactating women adhering to a MED diet rich in walnuts will
show a significant reduction in body weight, body fat and/or waist:hip ratio as compared to lactating women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet). All participants completed a 1 week run-in diet which incorporated 6 oz of orange juice and 1 oz of cheese consumed twice a day into their regular diet, as part of the eligibility process to assess their ability to consume a specific food repetitively over time. After the successful completion of the 1 week run-in diet, randomization was performed using a table of random numbers, independent of study personnel, at the Biometry Shared Service at the Arizona Cancer Center. After randomization, participants completed a 4 week wash-out period consisting of 5 or less servings of fruits and vegetables daily and elimination of the intake of nuts prior to commencement of the 4 month study intervention or control diet (Figure 1). If participants were still breastfeeding at 6 and 12 months past the baseline visit, they were invited to provide additional biosamples at these time-points to assess potential long-term effects of the study diets. All subjects completed the consent process prior to study enrollment. This research was approved by the University of Arizona Human Subjects Committee prior to study initiation.

**Study Population**

This study was conducted among lactating women living in the greater Tucson, AZ area who were between 18 and 40 years of age and in general good health with no diagnosis or history of DM, liver or kidney disease, or cancer (other
than non-melanoma skin cancer). Primipara or multipara women were eligible if their infants were between the ages of 2 weeks and 6 months and met the following criteria: breastfeed for a minimum of 3 times per day for at least 6 additional months; use a non-soy based formula if planning to supplement; refrain from estrogen-containing contraceptives; avoid 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); maintain and follow the prescribed study diet for 4 months; be available for clinic visits and telephone contact; and be able to complete study questionnaires. Women were asked to use non-soy based formula, if supplementing daily breastfeeding, in order to reduce potential confounding in relation to estrogen exposure and infant health outcomes [58]. Women were ineligible if they used tobacco products or had a family history of food allergies. Study participants were categorized as “completers” if they fulfilled all study requirements for the 6 month diet study (5 week run-in/wash out period + 4 months of diet assignment).

**Recruitment and Retention**

Preliminary recruitment strategies included the distribution of brochures and flyers at local hospitals, OB/GYN and pediatric offices, W.I.C. offices, La Leche League meetings, local libraries, health fairs, and survivor fund-raiser “walks”. Brochures and flyers were made available for the duration of the recruitment period; approximately 15 months. Paid print advertisements were also utilized and
included publication in local newspapers and as an email distributed on the University of Arizona employee ListServ. The paid advertisements in local newspapers were posted in the “Lifestyles” section and ran for one-week on 2 separate occasions, approximately one month apart; and advertisement on an employee ListServ was run on 4 separate occasions approximately every 2.5 months. All brochures, flyers, and print advertisements were distributed in English only. In addition, study staff completed on-site recruitment visits to newly postpartum women at local hospitals, OB/GYN clinics, breastfeeding and parent education classes, and breastfeeding support groups weekly for approximately 15 months. This effort was supported as a 25% time staff recruitment position.

Initial retention strategies included the bestowment of gift cards upon completion of clinic visits when biosamples (blood, urine, and breast milk) were provided; and free lactation support from a Certified Lactation Consultant as needed throughout the study. A free Medela Manual Breast Pump (Medela, Inc., McHenry, IL) was also provided.

Data tracking systems were implemented to assess recruitment and retention. Data collected specific to recruitment included: recruitment source, eligibility status, reasons for ineligibility, and enrollment outcomes. Retention was tracked via participant appointment schedules. Recruitment during the first 3 months of the study was below estimates by approximately 66.0%. In response, recruitment efforts were expanded to include weekly advertisements posted on Craig’s List under either Childcare or For Sale – Baby and Kids, and an
advertisement was developed for publication in a local magazine, *The Tucson Parent*, which ran on the back page of the magazine for 4 months. Higher than anticipated attrition rates (45.5%) were also identified upon assessment of the 33 women enrolled into the study during the first 6 months of recruitment. Hence, retention strategies were expanded to provide additional gift cards at all clinic visits and 15 - 20 minute telephone contact follow-up calls. Various incentive gifts were also provided at numerous time-points throughout the study: 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 completion).

**Study Diets**

After completion of the 5 week run-in/wash-out period, study participants received dietary education specific to their randomized diet assignment, either MED diet emphasizing vegetables, fruits, MUFAs, whole grains and nuts/walnuts or the USDA's MyPyramid for Pregnancy and Breastfeeding [59]. All participants were provided nutrition education, lifestyle counseling, and support to adopt and adhere to the assigned study diet via one-on-one diet education with a Registered Dietitian at the baseline, 2 week, and 2 month clinic visits; written materials (diet education notebook specific to study diet randomization); as well as telephone consultations with a Registered Dietitian bi-monthly for the first 2 months on study and then once during the third month of the study. Participants in both groups were instructed to consume the study provided prenatal vitamin daily.
Study Assessments

Demographics, Breastfeeding and Dietary Intake Assessments

All participants completed questionnaires related to demographic and lifestyle characteristics upon study enrollment. Breastfeeding assessment was completed with each contact (clinic visit or telephone call) and included such items as frequency and duration of breastfeeding, formula usage (type, frequency and amount), and problems/concerns related to breastfeeding. Dietary intake was estimated using repeated administrations of the validated AFFQ at baseline and 4 months [60, 61]. Additionally, random telephone 24-hour recalls were completed by non-study personnel once per month for a total of 4 times per participant.

Power Analysis, Sample Size, and Statistical Analyses

The power analysis for sample size was calculated using nQuery Advisor software based on analysis of similar research designs. The calculated sample sizes ranged from 6 to 106, thus, a sample size of 100 subjects was identified as providing sufficient statistical power to test our study hypothesis [62, 63]. This estimated sample size (n=100) included the consideration of a likely 10 - 15% attrition rate commonly seen with weight loss diet intervention studies conducted in adults [64].

Fisher’s exact test was calculated to assess associations among recruitment sources and study completion. Summary statistics (mean, standard deviations, ranges, and proportions) were calculated for baseline characteristics including
demographic, lifestyle, and anthropometric factors of completers versus non-completers using 2-sample t-tests for continuous variables and Chi-square tests for categorical variables. All data were assessed for normalcy, outliers, and missing values. The significance level was set at $P=0.05$. All computations were conducted using the SPSS 18.0 statistical software package (Statistical Program for the Social Sciences, Version 18.0, Chicago, IL).

Results

The CONSORT diagram for the diet intervention trial is shown in Figure 2. The original study timeline of 6 months for recruitment was expanded by 9 months and the total study population was increased from 100 participants to 138 participants due to slower than estimated recruitment rates and higher than predicted attrition. This resulted in the subsequent extension of all other planned study activities, i.e. the compilation of baseline data, administration of the diet intervention, collection of repeat measurements and biosamples, and statistical analyses and publication. In all, 319 women were screened for eligibility, 69.6% met eligibility screening criteria and 138 (43.3%) women were enrolled into the study. Of those meeting eligibility criteria, 39.8% refused to adhere to study protocol. Nine participants discontinued the study or failed to meet protocol after the consent and run-in period but prior to the baseline visit; 2 reported no longer breastfeeding, 4 were lost to follow-up, and 3 for other reasons. Of these women, 2
were recruited from breastfeeding support classes, 2 from local hospitals, 2 from Craig’s List, 2 from a local parenting magazine, and 1 from a University of Arizona employee ListServ. There were no significant differences among these 9 participants and recruitment source as compared to the women randomized into the study. None of the participants enrolled into the study withdrew consent to participate. Thus, 129 women were randomized and started the intervention (n=65) or control (n=64) diet. Of those women, 27 discontinued the study intervention after the baseline visit for an attrition rate of 20.9%; 15 discontinued prior to their 2 month visit and 12 between the 2 month and 4 month visit. Of these women, 11 reported discontinuation of breastfeeding, 7 were lost to follow-up, 2 reported dislike of the MED diet/walnuts, 2 felt overwhelmed/too busy to continue study participation, 2 participants relocated, 2 women became pregnant, and 1 infant began soy (versus non-soy) formula supplementation.

Table 1 describes the differences between completers and non-completers based on recruitment source. Of the 129 women randomized into the study, the largest percentage, 24.8%, were recruited from The Tucson Parent magazine, and of those women, 26.5% completed all study requirements; thus, representing the largest percentage of completers based on recruitment source. The second largest recruitment source, representing 20.9% of the total women randomized, was from advertisement on Craig’s List, and of those women, 22.6% completed all study requirements. Twenty-six women (20.2%) were recruited from local hospitals with 18.6% completing all study requirements. The remaining 34.1% of the women
randomized into the study were recruited from other print advertisements, breastfeeding/parenting classes, breastfeeding support groups, word of mouth, or other/unknown sources.

**Table 2** summarizes the baseline characteristics for the overall study population as well as data stratified by study completers as compared to non-completers. Overall the women were predominantly non-Hispanic whites (75.2%), 29.7±4.6 years, had a college degree or higher (69.0%), and were overweight with a BMI of 27.2±4.9 kg/m² upon study commencement. On average, the women entered the study at 17.5±8.2 weeks postpartum and 26.8% were providing supplementation of formula. Non-completers were more likely to supplement with formula as compared to completers (57.7% and 18.8%, respectively; *P*<0.001). Of the 9 women who withdrew from study participation prior to the baseline visit, 77.8% were non-Hispanic, and 55.5% attained an undergraduate degree or higher. Although none of these women stated they were supplementing with formula at the initial consent visit, 55.6% were undecided about future use of formula feeding.

**Discussion**

The current attrition rate for those lactating women who initiated the intervention or control diet in this research is 20.9%. Attrition rates in weight loss trials involving non-lactating women and men have been estimated to be approximately 4 – 30% [65-67]; however, attrition rates for weight loss trials in
postpartum women are estimated to be as high as 31 - 43% [8]. In a meta-analysis by Kuhlmann et al of RCTs involving weight management interventions for postpartum women ranging from between 6 - 12 months in duration, all studies evaluated were found to have low retention rates ranging from 58 - 69%; however, specific reasons for attrition were not addressed with the exception of one correspondence weight-control intervention trial that noted that non-completers were significantly heavier at enrollment and had retained more weight after delivery [55-57], factors that were not significantly different between completers and non-completers in this analysis. Other reasons for high attrition rates in weight loss studies have been identified and include but are not limited to: loss of motivation; susceptibility to stress-related eating or eating out of boredom; and increased likelihood of being obese at study entry [65, 68]. Poor retention rates in these studies suggest that lifestyle interventions for postpartum women are particularly challenging.

The primary aspect effecting discontinuation of study participation as reported by the participants was an inability/unwillingness to continue breastfeeding ≥ 3 times per day, generally due to a reported loss of milk supply. Other factors effecting retention as cited by participants include: dislike of the intervention diet; feeling overwhelmed or too busy; becoming pregnant; and beginning soy formula supplementation. In this study, the only statistically significant attribute associated with discontinuation of study participation was formula supplementation upon study entry; a factor known to be closely associated
with the early cessation of breastfeeding [69-71]. Thus, we conducted a logistic regression model to examine the crude association between formula supplementation use at baseline and attrition during the study and found that the odds of attrition were 5.89 times higher (OR: 5.89, 95% CI: 2.34-14.80; \(P=0.001\)) among women who used some formula compared with those who breastfed exclusively. Additionally, while it appears as though a larger proportion of non-Hispanic women as compared to Hispanic women discontinued study participation before study completion; in fact, 78.4% of non-Hispanic and 81.3% of Hispanic women completed the diet intervention (not statistically significantly different).

While this study did not identify specific recruitment sources as a predictor of attrition, previous studies examining the challenges associated with recruitment suggest that the importance of the first contact is paramount regardless of whether participants respond to a passive recruitment method or are actively approached by research personnel [53, 54]. The individual responsible for making the first contact must be properly educated about the aim and process of the study, must possess an attitude that is sensitive to the cultural values of the population under study, and be prepared to skillfully answer all potential questions in order to elicit trust and genuine cooperation from the participant.

RCTs involving recruitment of women and infants are particularly challenging as newly postpartum women may have more difficulty making informed decisions and may feel vulnerable and possibly coerced at the time of consent [72]. A meta-analysis by Tooher et al identified several major themes that should be
addressed in order to enhance participant recruitment and retention in maternal and perinatal trials. Chiefly, the participant should have a clear understanding of the potential risks, the recruitment processes and procedures, and the overall research process [53]. The protocol for consent for this research involved mailing the consent packet to potential participants at least 1 week prior to the scheduled consent appointment to allow the women sufficient time to read through the study protocol and discuss with their family to assure their commitment and willingness to complete all study procedures. During the consent appointment, study personnel reviewed the consent packet again with the participant, allowing time for questions to assure comprehension of the required study procedures. Common reasons reported for refusing study participation specific to this investigation include: unwillingness to stop other nutritional vitamins/supplements, particularly omega-3 supplements; dissatisfaction with being randomized to the non-intervention arm; opposition to adhering to the 4 week wash-out diet (elimination of nuts and ≤ 5 servings of fruits and vegetables per day); and aversion to traveling to the clinic for research visits.

Recruitment and retention of minorities, such as Hispanic women, has proven to be particularly challenging in previous RCTs [73, 74]. Of interest in this research, is the relatively large percentage, 24.8%, of Hispanic women who were recruited and enrolled into the study, although this population was not specifically targeted. According to the 2009 U.S. Census data, Arizona’s Hispanic population is 30.8%, approximately double the U.S. Hispanic population of 15.8% [75]. Thus,
while recruitment in Arizona is likely to result in a higher percentage of Hispanic participants as compared to other states, this population remains difficult to recruit into clinical trials for numerous reasons. These include but are not limited to: a lack of trust and perceived ‘hassles’ of participation, lack of transportation, lack of time, and lack of culturally competent research [76-78]. It is imperative to identify successful recruitment and retention strategies for this vulnerable population as Hispanic women are more susceptible to pregnancy associated weight gain and retention of weight in the postpartum period [79, 80]. Of the Hispanic women enrolled in this study, 25.0% were recruited from local hospitals, 21.9% from a local parenting magazine, 12.5% from a University employee listserv, and 12.5% from advertisement on Craig’s List. Future researchers may consider these sources as primary areas to target for recruitment for RCTs in this hard to reach population.

**Conclusions**

In this study, we identified the use of advertisements specifically targeting new mothers – *i.e.* a local magazine, *The Tucson Parent*, and Craig’s List under either Childcare or For Sale – Baby and Kids, to be the most effective approach to identifying lactating women for enrollment. Additionally, recruitment of women from local hospitals immediately postpartum was highly successful. Of the women randomized into the study, 65.9% were recruited from these 3 sources. Further, we identified exclusive breastfeeding as an important predictive factor of retention.
when conducting clinical research in lactating women. Thus, future researchers may consider commitment to exclusive breastfeeding a study criterion for clinical research involving lactating women. However, this is likely to introduce bias into the study as women who exclusively breastfeed are more likely to have higher levels of education and income, live with a partner, have had previous pregnancies, are at an older age at pregnancy and present with lower pre-pregnancy BMIs [81, 82]. Hence, future research in this population should not only promote exclusive breastfeeding, but also consider an *a priori* subgroup analysis based on frequency and duration of breastfeeding; this of course would result in the need for a larger sample size. To promote breastfeeding, investigators could consider providing more intense and frequent breastfeeding support, particularly in the first month postpartum; the use of peer-counselors either in person, or via telephone or internet contact; and/or reaching mothers pre-parturition to provide antenatal breastfeeding education and counseling; all methods shown to be successful in the promotion of exclusive breastfeeding [83-85]. Finally, maintaining an ongoing dialogue between researchers and study participants in order to facilitate increased rapport, education and understanding may also augment continued study participation, adherence to study protocol, and completion of all aspects of the intervention.

**Acknowledgements**
The authors would like to acknowledge the California Walnut Commission for their financial support and for providing the walnuts for the intervention group, Bayer HealthCare for donating One-A-Day Women’s Prenatal vitamins, and Medela for donating the manual breast pumps. The authors would also like to acknowledge Jennifer Ravia and Amy Butalla for assistance with the recruitment process.
Figure 1. Study Design for the Diet Study in Lactating Women. Model of all study procedures, the intervention timeline, frequency of contact, and collection of biosamples and anthropometric data.
Figure 2. **CONSORT diagram.** Participant flow in “The Diet Study for Lactating Women".
<table>
<thead>
<tr>
<th>Recruitment Source</th>
<th>Randomized (n=129)</th>
<th>Completers (n=102)</th>
<th>Non-Completers (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Print Advertisements</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local Parenting Magazine</td>
<td>32 (24.8)</td>
<td>27 (26.5)</td>
<td>5 (18.5)</td>
</tr>
<tr>
<td>Craig’s List</td>
<td>27 (20.9)</td>
<td>23 (22.6)</td>
<td>4 (14.8)</td>
</tr>
<tr>
<td>University ListServ</td>
<td>6 (4.7)</td>
<td>4 (3.9)</td>
<td>2 (7.4)</td>
</tr>
<tr>
<td>Local Newspapers</td>
<td>5 (3.9)</td>
<td>4 (3.9)</td>
<td>1 (3.7)</td>
</tr>
<tr>
<td>Local Hospitals</td>
<td>26 (20.2)</td>
<td>19 (18.6)</td>
<td>7 (25.9)</td>
</tr>
<tr>
<td><strong>Word of Mouth</strong></td>
<td>21 (16.3)</td>
<td>17 (16.7)</td>
<td>4 (14.8)</td>
</tr>
<tr>
<td><strong>Breastfeeding/Parenting Classes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&amp; Support Groups</td>
<td>8 (6.2)</td>
<td>5 (4.9)</td>
<td>3 (11.1)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>4 (3.1)</td>
<td>3 (2.9)</td>
<td>1 (3.7)</td>
</tr>
</tbody>
</table>
Table 2. Comparison of baseline characteristics of women enrolled into The Diet Study for Lactating Women: completers versus non-completers

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 129)</th>
<th>Completers (n = 102)</th>
<th>Non-Completers (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs), mean ± SD</td>
<td>29.7 ± 4.6</td>
<td>29.9 ± 4.6</td>
<td>29.0 ± 4.6</td>
</tr>
<tr>
<td>Infants’ age (wks), mean ± SD</td>
<td>17.5 ± 8.2</td>
<td>17.4 ± 8.1</td>
<td>18.1 ± 9.0</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²), mean ± SD</td>
<td>25.5 ± 4.7</td>
<td>25.2 ± 4.8</td>
<td>26.5 ± 4.3</td>
</tr>
<tr>
<td>BMI (kg/m²), mean ± SD</td>
<td>27.2 ± 4.9</td>
<td>26.9 ± 5.0</td>
<td>28.3 ± 4.1</td>
</tr>
<tr>
<td>Body fat (%), mean ± SD&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.8 ± 6.6</td>
<td>30.6 ± 6.9</td>
<td>31.6 ± 5.4</td>
</tr>
<tr>
<td>Waist (cm), mean ± SD</td>
<td>91.9 ± 12.8</td>
<td>91.6 ± 13.5</td>
<td>93.0 ± 9.6</td>
</tr>
<tr>
<td>Hip (cm), mean ± SD</td>
<td>108.7 ± 10.4</td>
<td>108.5 ± 10.9</td>
<td>109.2 ± 8.8</td>
</tr>
<tr>
<td>Waist:hip ratio (cm), mean ± SD</td>
<td>0.84 ± 0.07</td>
<td>0.84 ± 0.07</td>
<td>0.85 ± 0.06</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>97 (75.2)</td>
<td>76 (74.5)</td>
<td>21 (77.8)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>32 (24.8)</td>
<td>26 (25.5)</td>
<td>6 (22.2)</td>
</tr>
<tr>
<td>College degree, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>89 (69.0)</td>
<td>68 (66.7)</td>
<td>21 (77.8)</td>
</tr>
<tr>
<td>Formula supplementation, n (%)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34 (26.8)</td>
<td>19 (18.8)</td>
<td>15 (57.7)</td>
</tr>
<tr>
<td>Assigned to intervention diet, n (%)</td>
<td>65 (50.4)</td>
<td>53 (52.0)</td>
<td>12 (44.4)</td>
</tr>
</tbody>
</table>

<sup>P</sup> values calculated using t-tests for continuous variables and Chi-square tests for categorical variables
n=109; Significant between group difference (P<0.001); n=127.
CHAPTER 3

MEDITERRANEAN-STYLE AND MYPYRAMID DIETS ARE EFFECTIVE IN PROMOTING WEIGHT LOSS IN POSTPARTUM, BREASTFEEDING WOMEN

Nicole R. Stendell-Hollis, Patricia A. Thompson, Julie L. West, Betsy C. Wertheim, Cynthia A. Thomson

aNutritional Sciences Department, University of Arizona, 1177 E. 4th St., Tucson, AZ, 85721, USA; bArizona Cancer Center, University of Arizona, 1501 N. Campbell Ave., Tucson, AZ, 85719, USA; c College of Public Health, University of Arizona, 1295 N. Martin Ave., Tucson, AZ, 85724, USA

Submitted to JADA 3/2011
Abstract

**Background.** Increases in pre-pregnancy weight from one pregnancy to the next are associated with greater weight retention over time contributing to increased risk of long-term obesity; a factor which plays a role in numerous chronic diseases.

**Objective.** To determine if breastfeeding women randomized to a MED diet would demonstrate significantly greater reduction in body weight and body fat compared to women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet).

**Design.** A four-month randomized, controlled dietary intervention trial.

**Participants/setting.** At baseline, women (n=129) were 29.7±4.6 years, primarily non-Hispanic (75.2%), and overweight (BMI: 27.2±4.9 kg/m²). The majority of women were exclusively breastfeeding (73.6%) and a mean 17.5 weeks postpartum.

**Intervention.** Study participants received dietary education, incorporating common behavioral change techniques, specific to either a MED diet or the USDA’s MyPyramid diet for Pregnancy and Breastfeeding.

**Main Outcome Measures.** Adherence to the MED diet was evaluated via calculation of the MED diet score from validated FFQs administered pre- and post- the diet intervention. Anthropometric measurements (body weight, body fat and waist:hip ratio) were collected at baseline and four months (diet completion).
Statistical analyses performed. Paired sign-rank tests were used to assess changes in dietary and body size measurements within groups. Changes between diet groups were evaluated using linear regression models and Mann-Whitney U tests.

Results. The MED diet score was increased by 0.68±2.74 and 0.27±1.57 for the MED and control group, respectively. Increases in fish and dairy intake and a decrease in meat/poultry intake were significantly different between diet groups ($P<0.05$). Participants in both diet groups demonstrated significant ($P<0.001$) reductions in body weight (-2.31±3.42 kg and -3.11±3.35 kg for the MED diet and control diet, respectively), as well as significant ($P<0.002$) reductions in all other anthropometric measurements; no significant between group differences were shown.

Conclusions. Both the MED diet and the USDA's MyPyramid diet were effective in promoting postpartum weight loss in breastfeeding women.
Introduction

Approximately four million women in the U.S. deliver an infant annually [8]. Of these women, an estimated 34% are overweight/obese prior to pregnancy [9], and more than 30% gain weight that exceeds the IOM's recommendations [10]. Most women return to their pre-pregnancy weight within one year of parturition if they do not exceed the IOM's recommendations for GWG [11]; however, 15 – 20% will retain ≥ 5 kg [12]. Increases in pre-pregnancy weight from one pregnancy to the next are associated with greater weight retention over time [13, 14], an important predictor of long-term obesity [12, 15]. Epidemiological studies consistently demonstrate a positive association between overweight/obesity, percent body fat, and adult weight gain with the risk of numerous chronic diseases, including dyslipidemia, CVD, HTN, DM, and some cancers [48, 51, 86, 87].

Successful interventions aimed at preventing excess GWG are sparse and even fewer studies exist targeting a reduction in body weight during the postpartum period whether a woman is breastfeeding or not [55, 88-90]. This time period is of particular interest as mothers tend to be motivated for diet change in order to enhance the health of their newborns; and the postnatal period is a time when medical care is routinely provided supporting the potential to develop and disseminate new clinical guidelines for care. Identification of effective dietary interventions during the postpartum period that promote appropriate weight loss,
and maintenance of a healthy weight thereafter, is an important clinical goal for optimal long-term health.

The MED diet, rich in whole grains, fruits and vegetables, legumes and nuts, fish, olive oil, and low-fat dairy products, has been promoted as an effective diet for the promotion of weight control [38, 39]. In fact, a systematic review of several weight loss trials suggested that the MED diet may be effective in promoting weight loss [91, 92], but this has not been consistently demonstrated [93]. Given the lack of weight control interventions in breastfeeding women and the evolving evidence supporting the use of the MED diet to promote weight control, we sought to test this diet for weight loss/control in a sample of breastfeeding mothers. We hypothesized that breastfeeding women randomized to a MED diet for a four-month period would demonstrate a significantly greater reduction in body weight and body fat than women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet) [59].

METHODS

Research Design

This randomized, controlled dietary intervention trial was designed to test the hypothesis that breastfeeding women who adhered to a MED diet will show a significant reduction in body weight and body fat as compared to breastfeeding women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding.
(control diet). Participants completed a one-week run-in period during which they were advised to consume a diet that targeted habitual intake of the same food daily (i.e., 6 oz of orange juice and 1 oz of cheese consumed twice daily) along with their regular diet to evaluate likelihood for ongoing retention in a diet study.

Randomization was performed using a table of random numbers, independent of study personnel, at the Biometry Shared Service at the Arizona Cancer Center. Following randomization, a four-week washout period consisting of five or fewer servings of fruits and vegetables daily and elimination of the intake of nuts was completed prior to study initiation (Figure 3). All subjects completed the consent process before study enrollment. The University of Arizona Internal Review Board Human Subjects Committee approved the study protocol, and all participants provided written informed consent.

**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 diabetes, liver or kidney disease, or cancer (other than non-melanoma skin cancer). Women were eligible for study enrollment if their infants were between two weeks and six months of age and the mothers were willing to meet and maintain the following eligibility criteria: breastfeed ≥ three times per day for a minimum of six additional months; if supplementing with formula use a non-soy based formula; 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). Women were also asked to be available for clinic visits and telephone contact throughout the four-month study period and to complete study questionnaires. Women were required to limit infant supplementation to a non-soy based formula to avoid possible confounding as we plan to assess infant health outcomes in relation to study assignment, which could potentially be associated with estradiol levels in breast milk. Women were ineligible if they used tobacco products or had a personal/family history of food allergies.

**Recruitment**

Recruitment and retention strategies have been described in detail previously [94]. Briefly, recruitment strategies included the distribution of brochures and flyers at local hospitals, OB/GYN and pediatric offices, W.I.C. 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, OB/GYN 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 two-month visit; and a first-aid kit at the four-month visit.

**Study Diets**

After completion of the five-week run-in/washout period, study participants received dietary education specific to the MED diet emphasizing walnuts or normal diet recommended for lactation as described by the USDA’s MyPyramid for Pregnancy and Breastfeeding [59]. Participants in both groups received one-on-one diet counseling with a Registered Dietitian regarding the assigned diet and target dietary behaviors. Counseling methods incorporated common behavioral change techniques, including: self-efficacy promotion, goal-setting and goal-pursuit, and self-monitoring; in order to promote adoption and adherence to the assigned study diet. Counseling visits were repeated onsite at the study clinic at two weeks and two months and were complemented with written materials (diet education notebook specific to study diet randomization) as well as telephone consultations with a Registered Dietitian twice-monthly for the first two months on study and once during the third month.

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
(including walnuts), fish and poultry, olive oil, and low-fat dairy products; while limiting the intake of red meat and processed foods [95]. Specifically, participants in the intervention group were instructed to consume study-provided walnuts (28 g/day), 1 – 2 T/day olive oil, and seven or more servings/day fruits and vegetables for the duration of the diet intervention. The addition of walnuts to the diet was chosen for their proposed beneficial effects on weight control through increased satiation [41, 42]. Participants randomized to the control diet were provided with general nutrition education guidelines based on the USDA’s MyPyramid diet for Pregnancy and Breastfeeding emphasizing healthy eating choices. 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

All 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 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 AFFQ at baseline and four months [60, 61]. The AFFQ, a scannable 153 food/beverage item questionnaire, is a regionally appropriate modification of the food frequency component of the validated Block NCI Health Habits and History Daily Eating Pattern Assessment and includes responses on serving sizes and frequency of intake [96]. Questionnaires were reviewed for completeness by study personnel blinded to study arm assignment, and participants were contacted by telephone to ascertain missing data. Using this approach there were no AFFQs with missing items; however, seven women did not return either the baseline or four-month AFFQ, resulting in 95 AFFQs to be included in this analysis. Nutrient analyses of the AFFQs were completed by the Behavioral Measurement Shared Services at the Arizona Cancer Center using the proprietary Metabolize Software, developed by programming professional staff of the University of Arizona specifically for the quantification of nutrient intake derived from the AFFQ. Metabolize, the AFFQ analysis program, is a four-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 CSFII 1994–1996, 1998 and the NDS-R (versions 11–13) [97, 98].

The MED diet score is the sum of scores from nine different food groups from the AFFQ: vegetables, legumes, fruits and nuts, whole grain cereals, fish, meat/poultry, dairy, MUFA:SAT fat ratio, and ethanol [31]. A score of one is awarded for each food group in which the target grams (median from the total
population at baseline) are met or exceeded in the case of vegetables, legumes, fruits and nuts, whole grain cereals, fish, and the median monounsaturated:saturated fat ratio; in which the target grams are not exceeded in the case of meat/poultry and dairy; or the target grams are within a specified range in the case of ethanol (5 – 25 g/day). The MED diet score results in a range of scores of zero to nine, with nine indicating the best adherence.

**Body Weight and Body Composition Outcome Measures**

All anthropometric measurements were assessed at baseline and four months (diet completion). Body weight, height, and waist and hip circumference were measured following standardized protocols [99, 100]. Body composition measurements were assessed using the BIA (OMRON Body Fat Analyzer, OMRON Healthcare, Inc., Vernon Hills, IL).

**Statistical Analysis**

The power analysis for sample size was calculated using nQuery Advisor software comparing sample sizes of similar diet intervention studies from published literature. Two groups, two-sided t-tests of mean changes at 0.05 significance, 95% power, and varying effect sizes (ranging from 0.74 to 2.75) were calculated based on analysis of similar research designs. The calculated sample sizes ranged from six to 106, thus, a sample size of 100 subjects was identified as providing sufficient statistical power to test our study hypothesis [92, 101]. This estimated sample size
(n=100) included the consideration of a likely 10 - 15% attrition rate commonly seen with clinical diet intervention studies conducted in adults [102].

Differences between diet groups were tested using Fisher’s exact tests for categorical variables and Wilcoxon rank-sum tests for continuous variables. Changes in dietary (or body size) measures between baseline and four months (end of study) were tested using paired sign-rank tests. Changes in dietary measures over time between the two diet groups were tested using linear regression models, adjusted for the baseline dietary measure and baseline energy intake. Changes in body size measures over time between groups defined by formula supplement use (yes versus no) were tested using Mann-Whitney U 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). All tests were two-sided, and all analyses were performed using Stata 11.1 (StataCorp, College Station, Texas).

RESULTS

One hundred thirty-eight women were consented and enrolled into the study. Of these, nine discontinued study participation prior to the baseline visit: two reported cessation of breastfeeding; four were lost to follow-up; and three reported other reasons. Of the 129 women who initiated the study diets, 102 women completed the four-month dietary intervention. Among the 27 women who
discontinued study participation after initiation of the study diet, 11 reported cessation of breastfeeding, seven were lost to follow-up, and nine reported other reasons, resulting in a final attrition rate of 20.9% among women who initiated the study diet. These data have been reported in detail previously [94]. There were no significant associations between attrition rate and diet group assignment.

At baseline, women were an average age of 29.7 years and were 17.5 weeks postpartum (Table 3). Participants were overweight with a mean BMI of 27.2 kg/m² and a self-reported average pre-pregnancy BMI of 25.5 kg/m². The women were predominantly non-Hispanic white (75.2%) and had attained a college degree or higher level of education (69.0%), and 26.8% reported supplementation of the infant’s diet with formula at baseline. There were no significant differences in baseline characteristics between assigned diet groups. However, significant differences were noted among women who completed all study requirements (n=95) compared with the women who did not (n=34). Women who completed all study requirements had a significantly lower median pre-pregnancy BMI (23.5 vs. 27.5 kg/m²; \( P=0.006 \)), lower median baseline BMI (25.7 vs. 28.5 kg/m²; \( P=0.010 \)), and lower median percent body fat (30.5% vs. 34.0%; \( P=0.050 \)). They were also less likely to supplement infant breastfeeding with formula (18.1% vs. 51.5%; \( P<0.001 \)).

Participants in both diet groups demonstrated significant decreases in energy intake (-251.2±865.3 and -437.5±1331.0 kcal/day for the MED diet and control group, respectively) between baseline and four months (Table 4). The MED diet group demonstrated significant increases in intake of legumes (7.17±50.7
g/day), whole grains (30.8±75.9 g/day), and dairy (132.8±309.6 g/day). The control group demonstrated significant increases in vegetable intake (63.8±170.6 g/day). Linear regression models adjusted for baseline dietary measures and baseline energy intake indicated a statistically significant effect of diet group assignment on change in dietary measures for fish intake (MED, 4.61±22.2 vs. control, -1.33±12.0 g/day; \(P=0.001\)), meat/poultry intake (MED, -16.8±69.0 vs. control, 24.0±80.9 g/day; \(P=0.017\)), and dairy intake (MED, 132.8±309.6 vs. control, 4.82±284.9 g/day; \(P=0.019\)). The change in total MED diet score for the MED diet and control diet was 0.68±2.74 and 0.27±1.57, respectively; however, these changes did not reach statistical significance within or between groups.

Both diet groups demonstrated significant (\(P\leq 0.002\)) reductions from baseline in all anthropometric measurements (weight, BMI, waist, hip, waist:hip ratio, and percent body fat) at four months (Table 5). There were no statistically significant differences in the change in any of the anthropometric measures over time between groups even when controlling for baseline values.

Table 6 shows change in anthropometrics among all study participants stratified by breastfeeding status: exclusive breastfeeding versus breastfeeding and formula supplementation. Women who reported exclusive breastfeeding demonstrated significant (\(P<0.001\)) reductions in all anthropometric measurements (weight, BMI, waist, hip, waist:hip ratio, and percent body fat) between baseline and four months. Women who reported breastfeeding and formula supplementation demonstrated significant reductions in waist circumference (\(P=0.005\)) and percent
body fat ($P=0.004$) only. There were no significant between-group differences in the change in anthropometric measurements.

**DISCUSSION**

Results from this RCT in breastfeeding women suggest that both the MED diet and USDA’s MyPyramid diet, when adopted postpartum, can effectively promote weight loss/control. These findings do not support our *a priori* hypothesis that women randomized to a MED diet for 4-months would demonstrate greater reductions in body weight and body composition compared to women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding. In fact, the data suggest that the diets seem to be equally effective in the promotion of postpartum weight loss as well as a favorable change in body fat as assessed by the BIA method. These results further indicate that either dietary approach has potential to modify the risk of adult-onset obesity-associated disease including CVD, HTN, DM, and select cancers [48-51, 103].

Regardless of diet assignment, women demonstrated significant decreases in reported total energy intake between baseline and four-months and this reduction resulted in significant weight loss (-2.31±3.42 kg and -3.11±3.35 kg for the MED diet and control diet, respectively ($P<0.001$)). This finding was even demonstrated for women randomized to the MED diet group despite the addition of 28 g/day of walnuts as a targeted behavior to increase MED diet score. Our results support
previous research showing that daily walnut consumption, when added to the usual diet, does not result in weight gain but may actually contribute to weight loss through increased satiety [41, 42]. The self-report data also suggest that women randomized to the MED diet demonstrated a 52% increase in nut consumption (intake ≥ 5–6x/week) between baseline and four months \((P<0.001)\) compared with women in the control diet demonstrating a non-significant increase of 4.5% in nut consumption (intake ≥ 5–6x/week) during the same timeframe (data not shown); but may not have met other dietary behavioral targets of the MED diet consistently. The MED diet score increased by 0.68±2.74 points for a final score of 4.76±2.04 in the MED diet group, and it increased by 0.27±1.57 points for a final score of 4.27±1.68 in the control diet group and were not significantly different over time or between diet groups. The lack of a significant increase in mean MED diet score may reflect the fact that the study sample overall demonstrated a higher MED diet score at baseline (4.08±2.12 and 4.00±1.51 for the MED diet and control diet groups, respectively) than would have been anticipated from a population consuming a Western-style diet. The Western diet is characterized as being relatively high in fat and sugar, and low in fiber; and includes the consumption of eggs, high-fat dairy, refined grains, gravies and sauces, tomato sauces, fast foods, red and processed meats, potatoes, sugar and high-fat, high-sugar desserts [104]; all foods generally recommended to be avoided/limited for a MED diet pattern of eating [95] as well as the MyPyramid dietary guidelines [59].
To our knowledge, no MED diet interventions for the promotion of weight loss/control have been conducted previously in breastfeeding women. Yet, the MED diet has been promoted as an effective diet for weight loss/control in adults [38, 39, 105]. In our study, women randomized to the MED diet demonstrated significant reductions in all anthropometric measurements; but changes were not significantly different from the MyPyramid group, as hypothesized. The results demonstrated here are supported by a 24-month trial conducted among 322 moderately obese adult men and women who were randomized to either a low-fat, calorie-restricted diet; a MED, calorie-restricted diet; or a low-carbohydrate, non-calorie-restricted diet, that also showed a significant but modest weight loss with the MED and alternate diets, although women who were randomized to the MED diet tended to lose more weight than women randomized to one of the other diets [105]. It is possible that the lack of a statistically significant difference in weight loss between groups in this population may be due to the absence of a significant change in MED diet score within and between groups.

Breastfeeding is one factor that may further contribute to postpartum weight loss, as it has been estimated to increase metabolic expenditure by 480 kcals/day [17]; however, changes in body weight and body composition in response to the metabolic load imposed by lactation are highly variable among and within diverse populations [16]. When all study participants were stratified by breastfeeding status (exclusive breastfeeding or breastfeeding ≥ 3x/day + formula supplementation), statistically significant reductions were demonstrated in all
anthropometric measurements among the women who exclusively breastfed; findings not shown among women who were using formula supplementation. This may be explained by the fact that women who breastfed and formula fed their infants also initiated the study with a non-significantly higher baseline weight compared with women who exclusively breastfed (77.3±15.6 versus 73.0±14.9 kg; \( P = 0.260 \)), although controlling for baseline weight still resulted in non-significant differences in weight loss between the two diet groups. A study by Hatsu et al., observed that exclusive breastfeeding promotes greater weight loss than breastfeeding with formula supplementation among mothers in the early postpartum period, even though the exclusively breastfeeding mothers consumed more calories than mothers using a combined feeding approach [106]. Additional evidence also has suggested that exclusive breastfeeding promotes greater postpartum weight loss than breastfeeding with formula supplementation [107-109]; although not consistently [110, 111].

Few studies have examined what is considered to be the optimal amount of time required to return to pre-pregnancy weight. Therefore, it could be argued that the amount of weight loss attained in both diet groups in this research was related to continued, regular and gradual postpartum weight loss, given that study assignment did not result in significant between group reductions. Two studies that included repeat weight measurements during the postpartum period (0 – 12 months) reported that 75 – 80% of GWG is lost by two – six weeks postpartum [112, 113]. Women in this study demonstrated a mean postpartum time of 17.5±8.2
weeks, and thus it is unlikely, based on the time of the intervention, that the reduction in weight shown represented usual weight change independent of energy restriction.

One limitation to this research is that in order to reduce participant burden for study participation, we did not ask participants to maintain food diaries for self-monitoring or hold group counseling sessions, two methods which have been proven to be effective for increased adherence to dietary interventions [65, 114]. It is possible that participants randomized to the MED diet group would have demonstrated greater dietary change, significantly increasing the MED diet score and possibly achieving greater weight loss, had we made these study requirements. A second limitation of this research is the attrition rate of 20.9%, which may have introduced sampling bias. Yet, attrition rates in previous trials involving postpartum breastfeeding women range from 31 – 43% [8, 55-57], indicating that retention is particularly challenging in this study population and that our attrition rates were below expected rates. Further, when comparing the demographics of women who completed the trial versus not, we found no significant differences except for exclusivity of breastfeeding [94]. This study also had numerous strengths including the randomized, controlled study design, the use of Registered Dietitians to provide consistent nutritional counseling, and the use of a Lactation Consultant to provide breastfeeding support to the study participants to encourage maintenance of breastfeeding and continued study participation. This intervention tested the efficacy of a novel eating pattern, the MED diet, and compared it to current dietary
guidelines for healthy eating postpartum. Finally, the study was of ample sample size to test our *a priori* hypothesis and provides guidance regarding dietary support for weight control after pregnancy among breastfeeding mothers.

**CONCLUSIONS**

This research provides evidence that either a MED diet or the USDA’s MyPyramid diet is effective in the promotion of postpartum weight loss in breastfeeding women. Adult overweight/obesity remains a significant public health concern with 68% of Americans classified as overweight [115]. Pregnancy-associated weight retention is one factor contributing to long-term obesity. Overweight/obesity, percent body fat, and adult weight gain are associated with increased risk for several obesity-related, adult-onset diseases including CVD, HTN, DM, and select cancers [48-51, 103]. Interventions promoting weight loss/control during this vulnerable period of weight retention may help to reduce the risk for a pattern of continuing adult weight gain leading to obesity-associated disease risk. Future research should continue to examine intervention strategies to promote successful weight loss/control during the postpartum period. In addition, studies should be conducted to examine the effects of weight loss/control on biomarkers of inflammation, oxidative stress, and changes in metabolic health; additional risk factors associated with increased risk of several chronic diseases [116-118].
ACKNOWLEDGEMENTS

The authors would like to acknowledge Bayer HealthCare, LLC (Morristown, NJ) for donating One-A-Day Women’s Prenatal vitamins and Medela for donating the manual breast pumps.

FUNDING/SUPPORT DISCLOSURE

This research was funded by the California Walnut Commission, the Nutritional Sciences Department at the University of Arizona, and the USDA HATCH grant # 136833-H-23-145.

CONFLICT OF INTEREST DISCLOSURE

The authors have no conflict of interests to disclose.
Recruit postpartum women who intend to breastfeed for at least 6 additional months postpartum

Enroll and consent process

1 wk run-in:
6oz orange juice + 1 oz cheese (2x/day)
no citrus/walnut intake

Randomize into study diet groups

4 wk washout period:
No nut intake and ≤ 5 servings/day fruits/vegetables

Obtain baseline measurements from women:
- Blood – TNF-α, IFN-γ, IL-6, fatty acid profile
- Breast milk – TNF-α, IFN-γ, IL-6, fatty acid profile
- Anthropometric data (mother and infant)
- PBMCs – store for future research

Group 1: (4 months)
- General Mediterranean diet
- 28 g walnuts/day
- 1 – 2 T. olive oil/day
- ≥ 7 servings/day fruits/vegetables

Group 2: (4 months)
- USDA’s MyPyramid for Pregnancy and Breastfeeding

Obtain repeat measurements at 4 months and analyze data

Inclusion Criteria:
- age 18 – 40 y
- plan to breastfeed ≥ 6 mo
- no hormonal contraceptive use
- breastfeeding ≥ 3x/day
- no food allergy
Table 3. Baseline characteristics of study participants.\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Mediterranean</th>
<th>MyPyramid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 129)</td>
<td>(n = 65)</td>
<td>(n = 64)</td>
</tr>
<tr>
<td>Age (y), mean ± SD</td>
<td>29.7 ± 4.6</td>
<td>30.1 ± 4.5</td>
<td>29.4 ± 4.7</td>
</tr>
<tr>
<td>Hispanic ethnicity, n (%)</td>
<td>32 (24.8)</td>
<td>15 (23.1)</td>
<td>17 (26.6)</td>
</tr>
<tr>
<td>College degree, n (%)</td>
<td>89 (69.0)</td>
<td>42 (64.6)</td>
<td>47 (73.4)</td>
</tr>
<tr>
<td>Infant's age (weeks), mean ± SD</td>
<td>17.5 ± 8.2</td>
<td>17.5 ± 8.0</td>
<td>17.5 ± 8.5</td>
</tr>
<tr>
<td>Formula supplementation use\textsuperscript{b}, n (%)</td>
<td>34 (26.8)</td>
<td>18 (28.2)</td>
<td>16 (25.4)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m\textsuperscript{2}), mean ± SD</td>
<td>25.5 ± 4.7</td>
<td>25.4 ± 4.7</td>
<td>25.6 ± 4.6</td>
</tr>
<tr>
<td>Weight (kg), mean ± SD</td>
<td>74.1 ± 14.3</td>
<td>75.0 ± 16.1</td>
<td>73.2 ± 12.3</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2}), mean ± SD</td>
<td>27.2 ± 4.9</td>
<td>27.1 ± 5.2</td>
<td>27.2 ± 4.6</td>
</tr>
<tr>
<td>Body fat (%), mean ± SD</td>
<td>30.8 ± 6.6</td>
<td>30.8 ± 7.0</td>
<td>30.8 ± 6.2</td>
</tr>
<tr>
<td>Waist circumference (cm), mean ± SD</td>
<td>91.9 ± 12.8</td>
<td>91.4 ± 12.6</td>
<td>92.5 ± 13.0</td>
</tr>
<tr>
<td>Hip circumference (cm), mean ± SD</td>
<td>108.7 ± 10.4</td>
<td>109.1 ± 11.6</td>
<td>108.2 ± 9.2</td>
</tr>
<tr>
<td>Waist:hip ratio, mean ± SD</td>
<td>0.84 ± 0.07</td>
<td>0.84 ± 0.05</td>
<td>0.85 ± 0.08</td>
</tr>
</tbody>
</table>

\textsuperscript{a}There are no significant differences between diet groups.

\textsuperscript{b}n = 127

\textsuperscript{c}n = 109
### Table 4. Change in dietary measures between baseline and 4 months, by diet group. (mean ± SD)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Mediterranean diet (n = 50)</th>
<th></th>
<th>MyPyramid diet (n = 45)</th>
<th></th>
<th>P(^b)</th>
<th>P(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 months</td>
<td>Change</td>
<td>P(^b)</td>
<td>Baseline</td>
<td>4 months</td>
</tr>
<tr>
<td>Energy (kcal/day)(^d)</td>
<td>2711 ± 1305</td>
<td>2460 ± 1072</td>
<td>-251.2 ± 865.3</td>
<td>0.045</td>
<td>2950 ± 1363</td>
<td>2513 ± 1128</td>
</tr>
<tr>
<td>Vegetables (g/day)</td>
<td>281.1 ± 218.6</td>
<td>339.0 ± 251.1</td>
<td>57.9 ± 22.35</td>
<td>0.057</td>
<td>236.9 ± 123.8</td>
<td>300.7 ± 174.9</td>
</tr>
<tr>
<td>Legumes (g/day)</td>
<td>62.7 ± 75.4</td>
<td>69.8 ± 61.2</td>
<td>7.17 ± 50.7</td>
<td>0.044</td>
<td>77.1 ± 94.1</td>
<td>86.4 ± 96.0</td>
</tr>
<tr>
<td>Fruits and nuts (g/day)</td>
<td>291.1 ± 393.0</td>
<td>270.6 ± 127.7</td>
<td>-20.5 ± 349.3</td>
<td>0.106</td>
<td>236.9 ± 127.7</td>
<td>310.4 ± 227.1</td>
</tr>
<tr>
<td>Whole grains (g/day)</td>
<td>82.6 ± 50.3</td>
<td>113.3 ± 90.3</td>
<td>30.8 ± 75.9</td>
<td>0.011</td>
<td>91.5 ± 54.7</td>
<td>112.4 ± 62.2</td>
</tr>
<tr>
<td>Fish (g/day)</td>
<td>15.0 ± 14.9</td>
<td>19.6 ± 17.3</td>
<td>4.61 ± 22.2</td>
<td>0.120</td>
<td>9.74 ± 12.5</td>
<td>8.42 ± 7.98</td>
</tr>
<tr>
<td>Meat/poultry (g/day)</td>
<td>94.4 ± 66.0</td>
<td>77.6 ± 55.3</td>
<td>-16.8 ± 69.0</td>
<td>0.113</td>
<td>82.8 ± 53.8</td>
<td>106.8 ± 87.4</td>
</tr>
<tr>
<td>Dairy (g/day)</td>
<td>512.8 ± 347.0</td>
<td>645.6 ± 419.2</td>
<td>132.8 ± 309.6</td>
<td>0.009</td>
<td>505.0 ± 267.3</td>
<td>509.8 ± 310.7</td>
</tr>
<tr>
<td>Monounsaturated:saturated fat ratio</td>
<td>1.22 ± 0.35</td>
<td>1.27 ± 0.30</td>
<td>0.05 ± 0.44</td>
<td>0.128</td>
<td>1.18 ± 0.27</td>
<td>1.23 ± 0.31</td>
</tr>
<tr>
<td>Alcohol (g/day)</td>
<td>2.21 ± 1.98</td>
<td>1.45 ± 2.56</td>
<td>-0.76 ± 5.02</td>
<td>0.519</td>
<td>1.55 ± 4.45</td>
<td>1.09 ± 2.70</td>
</tr>
<tr>
<td>Total Mediterranean diet (score)</td>
<td>4.08 ± 2.12</td>
<td>4.76 ± 2.04</td>
<td>0.68 ± 2.74</td>
<td>0.091</td>
<td>4.00 ± 1.51</td>
<td>4.27 ± 1.68</td>
</tr>
</tbody>
</table>

\(^a\)There were no significant differences between diet groups at baseline; \(^b\)Wilcoxon signed-rank test for within group changes between baseline and 4 months; \(^c\)Wald statistic P value for effect of diet group on change in dietary measure in linear regression model, adjusted for baseline dietary measure and baseline energy intake; \(^d\)Missing energy data for 1 participant in MyPyramid group.
Table 5. Change in anthropometrics between baseline and 4 months, by diet group. (mean ± SD)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>Mediterranean diet (n = 53)</th>
<th></th>
<th>MyPyramid diet (n = 49)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (kg)</td>
<td>4 months (kg)</td>
<td>Change (kg)</td>
<td>(P^b)</td>
<td>Baseline (kg)</td>
<td>4 months (kg)</td>
</tr>
<tr>
<td>Weight</td>
<td>74.7 ± 16.8</td>
<td>72.4 ± 17.6</td>
<td>-2.31 ± 3.42</td>
<td>&lt; 0.001</td>
<td>72.7 ± 12.9</td>
<td>69.6 ± 13.8</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>27.1 ± 5.29</td>
<td>26.2 ± 5.58</td>
<td>-0.85 ± 1.24</td>
<td>&lt; 0.001</td>
<td>26.7 ± 4.82</td>
<td>25.6 ± 5.24</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>91.1 ± 13.3</td>
<td>87.6 ± 14.0</td>
<td>-3.47 ± 4.46</td>
<td>&lt; 0.001</td>
<td>92.2 ± 13.9</td>
<td>87.6 ± 14.1</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>109.2 ± 12.0</td>
<td>107.0 ± 11.7</td>
<td>-2.19 ± 3.97</td>
<td>&lt; 0.001</td>
<td>107.8 ± 9.52</td>
<td>104.9 ± 10.7</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.83 ± 0.06</td>
<td>0.82 ± 0.07</td>
<td>-0.02 ± 0.04</td>
<td>0.002</td>
<td>0.85 ± 0.08</td>
<td>0.83 ± 0.07</td>
</tr>
<tr>
<td>Body fat (%)(^d)</td>
<td>30.9 ± 7.20</td>
<td>29.7 ± 7.79</td>
<td>-1.19 ± 2.43</td>
<td>0.001</td>
<td>30.6 ± 6.47</td>
<td>28.4 ± 7.13</td>
</tr>
</tbody>
</table>

\(^a\) There were no significant differences between diet groups at baseline; \(^b\) Wilcoxon signed-rank test for within-group changes between baseline and 4 months; 
\(^c\) Mann-Whitney U test for difference between diet groups at 4 months; \(^d\) Missing body fat data for 13 participants (7 in Mediterranean group; 6 in MyPyramid group). There was no effect of diet group on change in anthropometrics in linear regression models adjusted for baseline anthropometric measurements and use of formula supplementation at baseline.
Table 6. Change in anthropometrics between baseline and 4 months, stratified by formula supplementation use. (mean ± SD)\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Exclusive breastfeeding (n = 82)</th>
<th></th>
<th>Breastsfeeding and formula supplementation (n = 19)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 4 months Change</td>
<td>Baseline 4 months Change</td>
<td></td>
<td>Baseline 4 months Change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.0 ± 14.9 70.1 ± 15.4 - 2.92 ± 3.34 &lt; 0.001</td>
<td>77.3 ± 15.6 75.6 ± 17.9 - 1.69 ± 3.65 0.064</td>
<td></td>
<td></td>
<td></td>
<td>0.260</td>
</tr>
<tr>
<td>BMI (kg/m\textsuperscript{2})</td>
<td>26.6 ± 5.02 25.6 ± 5.22 - 1.07 ± 1.21 &lt; 0.001</td>
<td>28.2 ± 5.24 27.6 ± 6.14 - 0.61 ± 1.32 0.080</td>
<td></td>
<td></td>
<td></td>
<td>0.234</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>90.9 ± 13.4 86.8 ± 13.6 - 4.13 ± 4.50 &lt; 0.001</td>
<td>95.0 ± 14.5 91.5 ± 15.9 - 3.44 ± 4.23 0.005</td>
<td></td>
<td></td>
<td></td>
<td>0.214</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>108.0 ± 10.8 105.1 ± 10.4 - 2.82 ± 4.31 &lt; 0.001</td>
<td>110.9 ± 11.3 109.6 ± 14.1 - 1.30 ± 6.40 0.061</td>
<td></td>
<td></td>
<td></td>
<td>0.305</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.84 ± 0.06 0.82 ± 0.07 - 0.02 ± 0.04 &lt; 0.001</td>
<td>0.86 ± 0.09 0.83 ± 0.07 - 0.02 ± 0.05 0.059</td>
<td></td>
<td></td>
<td></td>
<td>0.639</td>
</tr>
<tr>
<td>Body fat (%)\textsuperscript{d}</td>
<td>30.3 ± 6.83 28.6 ± 7.42 - 1.69 ± 2.89 &lt; 0.001</td>
<td>32.8 ± 6.87 31.3 ± 7.76 - 1.52 ± 1.72 0.004</td>
<td></td>
<td></td>
<td></td>
<td>0.098</td>
</tr>
</tbody>
</table>

\textsuperscript{a} There were no statistically significant differences in anthropometric measurements at baseline; \textsuperscript{b} Wilcoxon signed-rank test for within-group changes between baseline and 4 months; \textsuperscript{c} Mann-Whitney U test for difference between breastfeeding groups at 4 months; \textsuperscript{d} Missing body fat data for 13 participants (10 with exclusive breastfeeding; 3 with formula supplementation). There was no effect of formula supplementation on change in anthropometrics in linear regression models adjusted for anthropometric measurements at baseline.
CHAPTER 4

CHANGE IN BIOMARKERS OF INFLAMMATION IN RESPONSE TO A
MEDITERRANEAN-STYLE DIET INTERVENTION IN BREASTFEEDING WOMEN.

Nicole R. Stendell-Hollis\textsuperscript{a}, Patricia A. Thompson\textsuperscript{b,c}, Betsy C. Wertheim\textsuperscript{b}, Cynthia A. Thomson\textsuperscript{a,b,c}

\textsuperscript{a}Nutritional Sciences Department, University of Arizona, 1177 E. 4\textsuperscript{th} St., Tucson, AZ, 85721, USA; \textsuperscript{b}Arizona Cancer Center, University of Arizona, 1501 N. Campbell Ave., Tucson, AZ, 85719, USA; \textsuperscript{c}College of Public Health, University of Arizona, 1295 N. Martin Ave., Tucson, AZ, 85724, USA

Not yet submitted.
ABSTRACT:

Obesity-associated chronic diseases are a major public health concern; and persistent elevated inflammation is common in obesity. Excess postpartum weight retention increases the risk of adult overweight/obesity contributing to an increased risk of chronic disease. This RCT sought to determine the effect of a 4 month traditional MED diet intervention on inflammation in overweight postpartum breastfeeding women as compared to the USDA’s MyPyramid diet (control diet). At baseline, women (N=129) were 29.7±4.6 years, overweight (BMI: 27.2±4.9 kg/m2), and primarily non-Hispanic white (75.2%). The majority of women were exclusively breastfeeding (73.6%) and a mean 17.5 weeks postpartum. Anthropometric measurements and biosamples were collected at baseline and 4 months. Biomarkers of inflammation (IL-6 and TNF-α) were assessed via ELISA. Both diet groups demonstrated significant reductions in all anthropometric measurements (weight, body fat, BMI, and waist and hip circumference). A significant decrease in TNF-α, but not IL-6, was demonstrated in both diet groups. When all participants were stratified by clinically relevant weight loss (< 10% or ≥ 10%), a significant between group difference in IL-6 was noted in the model adjusted for baseline body weight and baseline biomarker level (< 10%: 0.04±0.88 compared to ≥ 10%: -3.45±5.85; P=0.045). Decreases in TNF-α were demonstrated in both weight loss with a significant between group difference in change in TNF-α in the unadjusted model (P<0.001); which was no longer significant in the adjusted
model \( (P=0.110) \). In conclusion, both the MED diet and USDA’s MyPyramid diet were effective in reducing body weight and biomarkers of inflammation in this target population who without weight loss intervention are at risk for persistent overweight status.
INTRODUCTION

Chronic diseases such as CVD, DM, stroke, and cancer, are the leading cause of mortality in the U.S. accounting for approximately 70% of all deaths annually [119]. Additionally, an estimated 1 out of every 2 adults is currently suffering from at least one type of chronic disease [1]. Numerous risk factors have been identified to increase risk of chronic disease, including excess adiposity, HTN, hyperlipidemia, and hyperinsulinemia [120-124]. All of these modifiable risk factors are also associated with a persistent elevated inflammatory state [3-6], suggesting the inflammatory pathway may be a primary target for chronic disease risk reduction. Dietary modifications have been identified as one therapeutic strategy for lowering chronic inflammation [125, 126].

The traditional MED diet is characterized as being rich in whole grains, fruits and vegetables, legumes and nuts, fish, olive oil, and low-fat dairy products; as well as a high MUFA:SAT ratio. Accumulating evidence from several large epidemiological studies suggest that greater adherence to the MED diet confers a significant protection against total mortality, as well as the occurrence of CVD and other major chronic diseases [31-35]. In a prospective investigation by Trichopoulou et al, a strong inverse association between adherence to a MED diet and total mortality (HR: 0.75, 95% CI: 0.64-0.87) was identified [31], and a study by Salas-Salvadó et al, demonstrated that regular consumption of foods common to the MED diet (cereals and fruits) was associated with a lower risk of inflammation as
measured by the biomarker, IL-6 ($P=0.005$) [127]. Several other studies also support the modification of inflammation by MED-associated diets [92, 125, 128-131].

Walnuts are a common component of the MED diet and are unique from other nuts in the greater concentration of ALA [46]; a nutrient with known anti-inflammatory properties [132, 133]. In a cross-sectional analysis lead by Salas-Salvadó in 772 asymptomatic men and women at high risk for cardiovascular disease, researchers found a significantly lower concentration of ICAM-1, a biomarker of inflammation, in those with the highest consumption of nuts ($P$ for trend=0.003); while not specific to walnuts, these data suggest that nut intake may be an effective adjuvant treatment for reducing chronic inflammation. In support of this, a study by Zhao et al also demonstrated that a diet high in ALA, obtained from walnuts, walnut oil and flaxseed oil, was effective in lowering inflammation as measured by the biomarkers of inflammation, hsCRP, ICAM-1, VCAM-1, and E-selectin [46]. Thus, it is feasible that a traditional MED diet in combination with regular walnut consumption would be effective in lowering inflammation and the risk of inflammatory-associated disease.

One time-period that has been associated with an up-regulation of inflammatory cytokines is the early postpartum period [19-21]; most likely as a well-controlled protective, biological response to the stress of parturition [22] and uterine involution [23]. While the exact magnitude and length of this up-regulated state is unclear, previous research examining lymphocytes from breastfeeding
mothers in *ex vivo* cell cultures demonstrated an increased production of Th1 cytokines (IL-2, INF-γ, and TNF-α) in breastfeeding women compared to formula feeding women and non-postpartum controls that persisted through postpartum months 1 – 12 [19, 24]. While this elevated inflammatory state is likely to be a normal, protective response to parturition; it is possible a *prolonged* inflammatory state as well as an *excessive* level of inflammation following pregnancy may increase the woman’s risk of developing chronic diseases later in life, particularly in women with chronic, low-grade, obesity-related inflammation.

The postpartum period is an opportune time for behavioral modification as new mothers tend to be motivated for diet change in order to increase the health of their newborns as well as promote postpartum weight control. The postpartum period is also a time of increased inflammation; thus, this research sought to test the hypothesis that breastfeeding women randomized to a MED diet emphasizing daily walnut consumption for 4 months would demonstrate a reduction in biomarkers of inflammation (IL-6 and TNF-α) and further, that the MED diet with walnuts would reduce biomarkers of inflammation more so than the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet) [134]). The biomarkers of inflammation, IL-6 and TNF-α, were chosen as they have consistently been shown to be elevated in postpartum women [19, 135], as well as to be favorably modulated by a MED diet [92, 132].

**METHODS**
Research Design

This RCT was designed to assess the efficacy of a MED diet versus the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet) for 4 months in breastfeeding women on change in biomarkers of postpartum inflammation. This study design has been described in detail previously [94]. Briefly, study participants completed a 1-week run-in period during which they were instructed to consume the same food each day (i.e., 6 oz of orange juice and 1 oz of cheese consumed twice daily) along with their regular diet to assess likelihood for ongoing retention in a diet study. Participants completed a 4-week wash-out period consisting of 5 or fewer servings of fruits and vegetables per day and elimination of the intake of nuts was completed prior to study initiation and were then randomized to the one of the 2 diet groups. All subjects completed the consent process and provided written informed consent prior to study enrollment. The University of Arizona Internal Review Board Human Subjects Committee approved the study protocol.

Study Population and Eligibility Criteria

Recruitment and retention strategies have previously been described [94]. In brief, 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 non-melanoma skin cancer) were recruited for study participation. Women were
eligible for study enrollment if their infants were between 2 weeks and 6 months of age and the mothers were willing to meet and maintain the following eligibility criteria: breastfeed ≥ 3 times per day for a minimum of 6 additional months; if supplementing with formula, use a non-soy based formula; 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). Study participants were also asked to complete study questionnaires and be available for clinic visits and telephone contact throughout the 4-month study period. Women were required to limit infant supplementation to a non-soy based formula to avoid potential confounding as we plan to assess infant health outcomes in relation to study assignment, which could potentially be associated with estradiol levels in breast milk. Women were ineligible if they used tobacco products or had a personal/family history of food allergies.

Study Diets

After completion of the 5-week run-in/washout period, study participants received dietary education specific to the assigned diet; either the MED diet emphasizing walnuts or normal diet recommended for lactation as described by the USDA’s MyPyramid diet for Pregnancy and Breastfeeding. Participants received one-on-one diet counseling with a Registered Dietitian specific to the assigned diet and target dietary behaviors. Counseling methods incorporated common behavioral
change techniques such as self-efficacy promotion, goal-setting and goal-pursuit, and self-monitoring; to promote adoption and adherence to the assigned study diet. Dietary counseling was repeated onsite at the study clinic at 2 weeks and 2 months and was supplemented with written materials (diet education notebook specific to study diet randomization) as well as telephone consultations with a Registered Dietitian twice-monthly for the first 2 months on study and once during the third month.

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 (including walnuts), fish and poultry, olive oil, and low-fat dairy products; while limiting the intake of red meat and processed foods [95]. Specifically, participants in the intervention group were instructed to consume study-provided walnuts (28 g/day), 1 – 2 T/day olive oil, and 7 or more servings/day fruits and vegetables for the duration of the diet intervention. Participants randomized to the control diet were provided with general nutrition education guidelines based on the USDA’s MyPyramid diet for Pregnancy and Breastfeeding emphasizing healthy eating choices. All study participants 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
All study participants completed questionnaires related to demographic and lifestyle characteristics upon study enrollment. Assessment of breastfeeding patterns was completed with each clinic visit or telephone 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 AFFQ at baseline and 4 months [136, 137]. The AFFQ, a scannable 153 food/beverage item questionnaire, is a regionally appropriate modification of the food frequency component of the validated Block NCI Health Habits and History Daily Eating Pattern Assessment and includes responses on serving sizes and frequency of intake [29]. Nutrient analyses of the AFFQs were completed by the Behavioral Measurement Shared Services at the Arizona Cancer Center using the proprietary Metabolize Software, developed by programming professional staff of the University of Arizona specifically for the quantification of nutrient intake derived from the AFFQ.

The MED diet score is the sum of scores from 9 different food groups from the AFFQ: vegetables, legumes, fruits and nuts, whole grain cereals, fish, meat/poultry, dairy, MUFA:SAT ratio, and ethanol [31]. A score of 1 is awarded for each food group in which the target grams (median from the total population at
baseline) are met or exceeded in the case of vegetables, legumes, fruits and nuts, whole grain cereals, fish, and the median MUFA:SAT fat ratio; in which the target grams are not exceeded in the case of meat/poultry and dairy; or the target grams are within a specified range in the case of ethanol (5 – 25 g/day). The MED diet score results in a range of scores of 0 to 9, with 9 indicating the best adherence.

**Body Weight and Body Composition Outcome Measures**

All anthropometric measurements were assessed at baseline and 4 months (diet completion). Body weight, height, and waist and hip circumference were measured following standardized protocols [99, 100]. Body composition measurements were assessed using the BIA method (OMRON Body Fat Analyzer, OMRON Healthcare, Inc., Vernon Hills, IL).

**Analysis of Inflammatory Biomarkers**

Eight-hour fasting venous blood samples were collected at baseline and 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 1500x g for 15-minutes, with serum aliquoted into 1.5 mL cryovials and stored at -80°C.

The Quantikine High Sensitivity Human IL-6 Immunoassay is a 5.5 hour solid-phase 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 has been pre-coated onto a microplate. Standards and
serum samples are pipetted into the wells and any IL-6 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-6 is added to the wells. Following 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 intra- and inter-assay CVs 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; Minneapolis, MN). Serum samples were analyzed in duplicate following the manufacturer's instructions. This assay also employs the quantitative sandwich enzyme immunoassay technique described above utilizing monoclonal and polyclonal antibodies specific to TNF-α. The intra- and inter-assay CVs were 10.9 and 13.9%, respectively.

**Statistical Analysis**

Differences between diet groups for continuous and categorical variables were tested using two-sample t-tests and chi-square tests, respectively. Changes in dietary intake between baseline and 4 months were tested using paired t-tests. Differences in dietary intake changes between diet groups were tested using the Wald statistic from linear regression models, adjusted for the baseline measure of the nutrient in question and energy intake. 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 follow-up. Linear regression models were also run to assess change in waist circumference and change in biomarkers, adjusted for baseline values. All two-sample t-tests were calculated assuming unequal variance using Welch’s formula. All statistical tests were two-sided with alpha of 5%. All statistical analyses were performed using Stata 11.2 (StataCorp, College Station, TX).

RESULTS

One hundred thirty-eight women completed the consent process and were enrolled into the study. Of these women, 9 discontinued study participation prior to the baseline visit: 2 reported cessation of breastfeeding; 4 were lost to follow-up; and 3 reported other reasons. One hundred twenty-nine women initiated the study diets and 102 women completed the 4-month dietary intervention. Of 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 the women who initiated the assigned study diet.
At baseline, study participants were an average 29.7±4.6 yr of age, overweight with a mean BMI of 27.2±4.9 kg/m², overweight pre-pregnancy with a self-reported BMI of 25.5±4.7, and 17.5±8.2 wk postpartum (Table 7). The majority of participants had attained a college degree or higher level of education (69.0%), was non-Hispanic white (75.2%), and was exclusively breastfeeding (73.2%). There were no significant differences in baseline characteristics between diet groups. However, the women who completed all study requirements (n=93) were half as likely to supplement infant breastfeeding with formula as compared to those who did not (n=36) (20.7% vs. 42.9%; P=0.012).

Significant decreases in energy intake were reported regardless of diet assignment (-251.2±865.3 and -437.5±1331.0 kcal/day for the MED diet and control group, respectively) between baseline and 4 months (Table 8). Significant increases in intake of whole grains (30.8±75.9 g/day) and dairy (132.8±309.6 g/day) were shown in the MED diet group; and significant increases in vegetable (63.8±170.6 g/day), and fruit and nut intake (73.5±204.2 g/day) were shown in the control diet group. Linear regression models adjusted for baseline dietary measures and energy intake indicated a statistically significant effect of diet group assignment on change in dietary measures for fish intake (MED, 4.61±22.2 vs. control, -1.33±12.0 g/day; P=0.001), meat/poultry intake (MED, -16.8±69.0 vs. control, 24.0±80.9 g/day; P=0.017), and dairy intake (MED, 132.8±309.6 vs. control, 4.82±284.9 g/day; P=0.019). The change in total MED diet score for the MED diet and control diet was 0.68±2.74 (P=0.085) and 0.27±1.57 (P=0.261), respectively.
Both diet groups demonstrated significant ($P \leq 0.002$) reductions from baseline in all anthropometric measurements (weight, BMI, waist, hip, waist:hip ratio, and percent body fat) at four months (Table 9). There were no statistically significant differences in the change in any of the anthropometric measures over time between groups.

No significant changes in IL-6 within or between diet groups were noted. Study participants in both diet groups demonstrated significant reductions in TNF-α (MED: -0.89±2.70 ($P=0.021$) vs. control: -0.53±0.98 ($P<0.001$)) (Table 10); however, these differences were not significantly different between groups. When all study participants were combined together to evaluate change in inflammatory biomarkers, results were similar (data not shown).

Given the demonstrated loss of body weight for both diets and the known association between weight loss and decreased inflammation [138, 139], we performed a post-hoc exploratory analysis wherein all participant data were stratified by weight loss (lost < 10% or lost ≥ 10%) (Table 11). Significant within group differences were observed in IL-6 for the participants who lost ≥ 10% of their body weight and these changes remained significant between groups when adjusted for baseline weight and biomarker level in question (< 10% weight loss: 0.04±0.88, ≥ 10% weight loss: -3.20±5.16; $P=0.045$). Study participants who demonstrated < 10% weight loss showed a significant decrease in TNF-α (-0.46±0.85, $P<0.001$); a finding not shown in those with ≥ 10% weight loss (-3.66±6.30, $P=0.144$). There
was a significant unadjusted between group difference ($P<0.001$), which was no longer significant when adjusted for baseline weight and biomarker level ($P=0.110$).

**DISCUSSION**

Results from this randomized, controlled, dietary weight loss trial in postpartum breastfeeding women demonstrated a significant decrease in the biomarker of inflammation, TNF-$\alpha$, but not IL-6. The change in TNF-$\alpha$ was not significantly different between groups as hypothesized, suggesting that both diets were effective in reducing TNF-$\alpha$. A post-hoc analysis was conducted combining all study participants and stratifying by amount of weight loss ($< 10\%$ or $\geq 10\%$) to assess change in biomarkers of inflammation. This percentage of weight loss was chosen based on results from a RCT in 93 obese subjects who maintained a VLED for 8 weeks followed by randomization to orlistat or placebo and lifestyle intervention for 3 years conducted by Madsen *et al.* Results from this study indicated that $\geq 10\%$ of weight loss was the minimum amount required in order to be effective in positively modulating biomarkers of inflammation as measured by hsCRP, adiponectin, and fibrinogen. In this research no significant within group difference was noted for IL-6.

Despite an emphasis on MED foods in the MED diet group, both diet groups increased total MED diet score to $4.76\pm2.04$ in the MED diet group and $4.27\pm1.68$ in the control diet group ($P=0.085$ and $0.156$, respectively); suggesting that both diets
promoted healthier food selections consistent with a traditional MED diet pattern. Our results suggest an inflammatory-modulating effect of both diets of similar MED diet score and this finding is supported by an increasing number of prospective epidemiological studies showing consumption of a traditional MED diet reduces the risk for inflammatory-associated chronic disease [95, 140, 141]. Specifically, the protective effect of the MED diet has been attributed to its ability to improve established chronic disease risk factors, such as adiposity, blood pressure, serum lipids, insulin resistance, and chronic inflammation [92, 131, 142-145]. This is likely due to the abundance of BAFCs found within the diet, including inflammation-modulating nutrients [146, 147]. Epidemiological and clinical research suggest that inflammation can be modified by MED-related dietary constituents such as MUFAs found in olive oil; PUFAs found in nuts; fiber found in whole grains, lentils and beans; and antioxidants found in vegetables, fruit, wine, nuts and seeds, [46, 148-154]. One cross-sectional study examining the association between specific components in the MED diet with circulating biomarkers of inflammation found that the consumption of fruits, cereals, virgin olive oil, and nuts were associated with lower inflammatory biomarkers (hsCRP, IL-6, ICAM-1, and VCAM-1) in subjects with increased risk for CVD [127]. Nuts, including walnuts, in particular are a good source of BAFCs as they are characterized as having a favorable fatty acid profile; and being a rich source of unsaturated fatty acids, fiber, phenolic compounds, L-arginine, and other anti-inflammatory and antioxidant molecules [155].
Change in the individual dietary components comprising the MED diet score did not show a significant increase in fruit and nut intake in the MED diet group, and in fact, showed a non-significant increase in fruit and nut intake in the control group. This may be attributed to the method by which the MED diet score is calculated which combines fruit and nut intake into one category; and therefore, may not have adequately represented change in nut consumption within or between the diet groups. When examining the self-report data obtained from the AFFQ specific to nut intake alone; a significant ($P<0.001$) 52% increase in nut consumption (intake $\geq 5 - 6x/week$) between baseline and 4 months was shown among women randomized to the MED diet as compared to women in the control diet who demonstrated a non-significant increase of 4.5% in nut consumption (intake $\geq 5 - 6x/week$) during the same timeframe (data not shown). However, when linear regression models stratified by diet group examining the association between nut consumption and change in inflammatory biomarkers adjusted for baseline nut consumption and biomarker level were run, no significant associations were observed; when combining all study participants together, results were similar (data not shown). Thus, while the women randomized to the MED diet group demonstrated a significant increase in nut intake compared to women randomized to the control diet, they may not have consistently met other dietary behavioral targets of the MED diet; which may explain the lack of a statistically significant difference in the biomarkers of inflammation between diet groups. Additionally,
walnut intake of 1 oz/day may not be sufficient exposure to ALA to modify inflammatory biomarkers.

Study participants in both diet groups demonstrated significant \( P \leq 0.002 \) decreases in all anthropometric measurements (weight, BMI, body fat, waist and hip circumference, and waist:hip ratio) between baseline and 4 months (unpublished data [156]) as planned for in the study protocol. Previous research has identified an association between weight loss and decreases in biomarkers of inflammation [138, 139]; particularly in relation to a reduction in waist:hip circumference indicating a decrease in abdominal obesity which is known for its pro-inflammatory effects [157, 158]. Thus, the significant reduction in TNF-\( \alpha \) demonstrated in this research was likely related to weight loss rather than diet group assignment, as both diet groups significantly reduced TNF-\( \alpha \). Yet, linear regression models examining the associations between change in body weight as well as change in waist circumference and change in inflammation demonstrated no significant associations (data not shown).

This research did not show a significant reduction in IL-6 within diet groups as expected. Previous studies have consistently demonstrated a down-regulation of circulating inflammatory biomarkers, including IL-6, in association with adherence to a MED-pattern of eating [127, 159, 160]. However, these studies have primarily been conducted in high-risk individuals who are more likely to demonstrate elevated concentrations of IL-6 at study entry. While our participants were considered, on average, to be overweight at study entry as well as through self-
reported pre-pregnancy BMI (a factor known to be associated with an elevated inflammatory state); participants nevertheless demonstrated relatively low levels of IL-6 at baseline (2.1±4.8 and 1.0±0.7 pg/mL for the MED and control diet groups, respectively) compared to studies in high-risk individuals who demonstrated higher baseline IL-6 levels ranging typically from 4.1 – 6.8 pg/mL [127, 159, 160]. Thus, it is likely that the relatively low baseline levels of IL-6 in this research made it more difficult to detect significant change. Yet, when the model was adjusted for baseline body weight and baseline biomarker value, there was a significant between group difference (< 10% weight loss: 0.04±0.88, ≥ 10% weight loss: -3.45±5.16; P=0.045). Study participants who demonstrated < 10% weight loss showed a significant decrease in TNF-α (-0.46±0.85, P<0.001); a finding not shown in those with ≥ 10% weight loss (-3.66±6.30, P=0.144). A significant between group difference (P<0.001) was noted in the unadjusted model; however, this was no longer significant in the adjusted model (P=0.110). It is possible that the lack of a significant difference in TNF-α, as well as in IL-6, in the greater weight loss category is related to the very small number of women who actually lost ≥ 10% of their total body weight (N=8). It is likely that with a larger sample size a significant reduction in the biomarkers would be detected.

There are some limitations to this research. First, using the traditional MED diet scoring system in a U.S. population typically consuming a Western-style diet may not have adequately represented conformity to a traditional MED diet as the median intake of some dietary components is likely to be lower than the median
intake in a Mediterranean population. It does emphasize key features of the diet, and may still confer a substantial benefit on total mortality in the U.S. as has been shown in previous research [34]. Second, it is possible that the duration of exposure to the diet intervention (4 months) was not long enough to significantly reduce IL-6. Yet, previous studies suggest that as few as 12 weeks is ample time to positively modulate this biomarker [160]; indicating our study duration was sufficient. Finally, this research also has numerous strengths, including: the RCT design; blinded inflammatory biomarker analyses; ample study sample size to test our a priori hypothesis; and the provision of guidance regarding dietary recommendations for the reduction of biomarkers of inflammation after pregnancy among breastfeeding mothers.

CONCLUSIONS

This research provides evidence that the traditional MED diet and the USDA’s MyPyramid diet for Pregnancy and Breastfeeding with resultant weight loss are effective in lowering chronic inflammation as measured by the biomarker, TNF-α, in postpartum breastfeeding women. This reduction in inflammation is influenced by percentage weight loss with the greatest reduction demonstrated in women reducing their weight by > 10% in 4 months. Persistent elevated inflammation is associated with an increased risk of numerous diseases, including CVD, stroke, DM, and select cancers [3-6]. Future research should include more targeted dietary
behaviors for the promotion of the anti-inflammatory response (e.g. curcumin, additional omega-3 fatty acids, etc) as well as assessment of the efficacy of diet in modifying additional biomarkers of inflammation such as ICAM-1, VCAM-1, and hsCRP; biomarkers also shown to be positively modulated by a MED diet [46, 159, 160].

ACKNOWLEDGEMENTS

The authors would like to acknowledge Bayer HealthCare, LLC (Morristown, NJ) for donating One-A-Day Women's Prenatal vitamins, Medela for donating the manual breast pumps, and Dr. Cynthia Thomson’s laboratory staff.

FUNDING/SUPPORT DISCLOSURE

This research was funded by the California Walnut Commission, the Nutritional Sciences Department at the University of Arizona, the USDA HATCH grant # 136833-H-23-145, and the Foundation of the American Dietetic Association.

CONFLICT OF INTEREST DISCLOSURE

The authors have no conflict of interests to disclose.
Table 7. Baseline characteristics of study participants.a

<table>
<thead>
<tr>
<th></th>
<th>Total (n = 129)</th>
<th>Mediterranean (n = 65)</th>
<th>MyPyramid (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y), mean ± SD</td>
<td>29.7 ± 4.6</td>
<td>30.1 ± 4.5</td>
<td>29.4 ± 4.7</td>
</tr>
<tr>
<td>Hispanic ethnicity, n (%)</td>
<td>32 (24.8)</td>
<td>15 (23.1)</td>
<td>17 (26.6)</td>
</tr>
<tr>
<td>College degree, n (%)</td>
<td>89 (69.0)</td>
<td>42 (64.6)</td>
<td>47 (73.4)</td>
</tr>
<tr>
<td>Infant’s age (weeks), mean ± SD</td>
<td>17.5 ± 8.2</td>
<td>17.5 ± 8.0</td>
<td>17.5 ± 8.5</td>
</tr>
<tr>
<td>Formula supplementation useb, n (%)</td>
<td>34 (26.8)</td>
<td>18 (28.2)</td>
<td>16 (25.4)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI (kg/m²), mean ± SD</td>
<td>25.5 ± 4.7</td>
<td>25.4 ± 4.7</td>
<td>25.6 ± 4.6</td>
</tr>
<tr>
<td>Weight (kg), mean ± SD</td>
<td>74.1 ± 14.3</td>
<td>75.0 ± 16.1</td>
<td>73.2 ± 12.3</td>
</tr>
<tr>
<td>BMI (kg/m²), mean ± SD</td>
<td>27.2 ± 4.9</td>
<td>27.1 ± 5.2</td>
<td>27.2 ± 4.6</td>
</tr>
<tr>
<td>Body fat (%), mean ± SD</td>
<td>30.8 ± 6.6</td>
<td>30.8 ± 7.0</td>
<td>30.8 ± 6.2</td>
</tr>
<tr>
<td>Waist circumference (cm), mean ± SD</td>
<td>91.9 ± 12.8</td>
<td>91.4 ± 12.6</td>
<td>92.5 ± 13.0</td>
</tr>
<tr>
<td>Hip circumference (cm), mean ± SD</td>
<td>108.7 ± 10.4</td>
<td>109.1 ± 11.6</td>
<td>108.2 ± 9.2</td>
</tr>
<tr>
<td>Waist:hip ratio, mean ± SD</td>
<td>0.84 ± 0.07</td>
<td>0.84 ± 0.05</td>
<td>0.85 ± 0.08</td>
</tr>
</tbody>
</table>

aThere are no significant differences between diet groups.

b n = 127

c n = 109
<table>
<thead>
<tr>
<th></th>
<th>Mediterranean diet (n = 50)</th>
<th></th>
<th>MyPyramid diet (n = 45)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline 4 months Change P&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Baseline 4 months Change P&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Pred P&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pred P&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Pred P&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Energy (kcal/day)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2711 ± 1305 2460 ± 1072 - 251.2 ± 865.3 0.046</td>
<td>2950 ± 1363 2513 ± 1128 - 437.5 ± 131 0.035</td>
<td>0.723</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetables (g/day)</td>
<td>281.1 ± 218.6 339.0 ± 251.1 57.9 ± 223.5 0.073</td>
<td>236.9 ± 123.8 300.7 ± 174.9 63.8 ± 170.6 0.016</td>
<td>0.536</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legumes (g/day)</td>
<td>62.7 ± 75.4 69.8 ± 61.2 7.17 ± 50.7 0.322</td>
<td>77.1 ± 94.1 86.4 ± 96.0 9.23 ± 65.2 0.348</td>
<td>0.580</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits and nuts (g/day)</td>
<td>291.1 ± 393.0 270.6 ± 127.7 - 20.5 ± 349.3 0.680</td>
<td>236.9 ± 127.7 310.4 ± 227.1 73.5 ± 204.2 0.020</td>
<td>0.241</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole grains (g/day)</td>
<td>82.6 ± 50.3 113.3 ± 90.3 30.8 ± 75.9 0.006</td>
<td>91.5 ± 54.7 112.4 ± 62.2 20.9 ± 78.8 0.082</td>
<td>0.692</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish (g/day)</td>
<td>15.0 ± 14.9 19.6 ± 17.3 4.61 ± 22.2 0.148</td>
<td>9.74 ± 12.5 8.42 ± 7.98 - 1.33 ± 12.0 0.461</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat/poultry (g/day)</td>
<td>94.4 ± 66.0 77.6 ± 55.3 - 16.8 ± 69.0 0.091</td>
<td>82.8 ± 53.8 106.8 ± 87.4 24.0 ± 80.9 0.053</td>
<td>0.017</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy (g/day)</td>
<td>512.8 ± 347.0 645.6 ± 419.2 132.8 ± 309.6 0.004</td>
<td>505.0 ± 267.3 509.8 ± 310.7 4.82 ± 284.9 0.910</td>
<td>0.019</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monounsaturated:saturated fat ratio</td>
<td>1.22 ± 0.35 1.27 ± 0.30 0.05 ± 0.44 0.433</td>
<td>1.18 ± 0.27 1.23 ± 0.31 0.06 ± 0.34 0.275</td>
<td>0.492</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol (g/day)</td>
<td>2.21 ± 1.98 1.45 ± 2.56 - 0.76 ± 5.02 0.290</td>
<td>1.55 ± 4.45 1.09 ± 2.70 - 0.44 ± 2.73 0.295</td>
<td>0.865</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Mediterranean diet score</td>
<td>4.08 ± 2.12 4.76 ± 2.04 0.68 ± 2.74 0.085</td>
<td>4.00 ± 1.51 4.27 ± 1.68 0.27 ± 1.57 0.261</td>
<td>0.156</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> There were no significant differences between diet groups at baseline; <sup>b</sup> Paired t-tests for within group changes between baseline and 4 months; <sup>c</sup> Wald statistic P value for effect of diet group on change in dietary measure in linear regression model, adjusted for baseline dietary measure and baseline energy intake; <sup>d</sup> Missing energy data for 1 participant in MyPyramid group.
Table 9. Change in anthropometrics between baseline and 4 months, by diet group. (mean ± SD)^a

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

^a There were no significant differences between diet groups at baseline; ^b Wilcoxon signed-rank test for within-group changes between baseline and 4 months; 
^c Mann-Whitney U test for difference between diet groups at 4 months; ^d Missing body fat data for 13 participants (7 in Mediterranean group; 6 in MyPyramid group). There was no effect of diet group on change in anthropometrics in linear regression models adjusted for baseline anthropometric measurements and use of formula supplementation at baseline.
Table 10. Change in biomarkers of inflammation among study participants, by diet group. (mean, SD)

<table>
<thead>
<tr>
<th></th>
<th>MED (n=52)</th>
<th>CNTRL (n=49)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 mos</td>
<td>Change</td>
<td>Baseline</td>
<td>4 mos</td>
<td>Change</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.98 (4.64)</td>
<td>1.59 (2.67)</td>
<td>-0.39 (2.47)</td>
<td>0.258</td>
<td>0.92 (0.73)</td>
<td>-0.03 (0.65)</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-a</td>
<td>4.19 (10.38)</td>
<td>3.30 (7.89)</td>
<td>-0.89 (2.70)</td>
<td>0.021</td>
<td>2.48 (3.46)</td>
<td>-0.53 (0.98)</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Paired t-tests for within group changes between baseline and 4 months; *unadjusted between group changes; *between group change adjusted for baseline weight and biomarker level.
Table 11. Change in biomarkers of inflammation among study participants, stratified by amount of weight loss. (mean, SD)

<table>
<thead>
<tr>
<th></th>
<th>lost &lt; 10% body weight (n=93)</th>
<th>lost ≥ 10% body weight (n=8)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>4 mos</td>
<td>Change</td>
<td>Pa</td>
<td>Baseline</td>
<td>4 mos</td>
<td>Change</td>
<td>Pb</td>
<td>Pc</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.02 (1.16)</td>
<td>1.06 (1.17)</td>
<td>0.04 (0.88)</td>
<td>0.650</td>
<td>6.64 (10.70)</td>
<td>3.45 (5.85)</td>
<td>-3.20 (5.16)</td>
<td>0.123</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>2.44 (3.50)</td>
<td>1.98 (2.84)</td>
<td>-0.46 (0.85)</td>
<td>&lt;0.001</td>
<td>14.00 (24.00)</td>
<td>10.30 (18.10)</td>
<td>-3.66 (6.30)</td>
<td>0.144</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Paired t-tests for within group changes between baseline and 4 months; *unadjusted between group difference; *between group difference adjusted for baseline weight and biomarker level.
CHAPTER 5

IMPLICATIONS AND FUTURE DIRECTIONS
Summary of Dissertation Work

Introduction

This randomized controlled diet intervention trial was designed to assess the efficacy of adherence to a traditional MED diet for 4-months in postpartum breastfeeding women on change in anthropometric measurements and biomarkers of inflammation as compared to postpartum breastfeeding women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet). The findings of this research suggest both diets were effective in reducing body weight and body fat, as well as modifying select biomarkers of inflammation in postpartum breastfeeding women.

Recruitment and Retention

One unanticipated challenge in this research was the recruitment and retention of postpartum breastfeeding women. We identified the use of advertisements specifically targeting new mothers to be the most effective approach to identify breastfeeding women for enrollment. Additionally, recruitment of women from local hospitals immediately postpartum was highly successful. Of the women randomized into the study, 65.9% were recruited from these sources.

The attrition rate for the study participants who initiated the intervention or control diet in this research was 20.9%. Attrition rates in weight loss trials involving non-postpartum women and men have been estimated to be
approximately 4 – 30% [65-67]; however, attrition rates for weight loss trials in postpartum women are estimated to be as high as 31 – 43% [8]. Poor retention rates in these studies suggest that lifestyle interventions for postpartum women are particularly challenging. Possible factors effecting attrition rates in weight loss studies have been identified and include but are not limited to: an increased likelihood of being overweight or obese at study entry; loss of motivation; and susceptibility to stress-related eating or eating out of boredom [65, 68]. One finding we observed as being an important predictive factor of retention when conducting clinical research in breastfeeding women is a commitment to exclusive breastfeeding. While this criterion is important in regards to study retention rates, researchers must keep in mind that it is also likely to introduce bias to the study as women who exclusively breastfeed are more likely to have higher levels of education and income, live with a partner, have had previous pregnancies, are at an older age at pregnancy and present with lower pre-pregnancy BMIs [81, 82]; making it difficult to extrapolate findings to a more general population of postpartum women.

**Change in Body Weight and Body Composition**

This research provides evidence that both a traditional MED diet and the USDA’s MyPyramid diet for Pregnancy and Breastfeeding are effective in the promotion of postpartum weight loss in breastfeeding women. These findings do not support our *a priori* hypothesis that women randomized to a MED diet for 4-
months would demonstrate greater reductions in body weight and body fat compared to women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding. In fact, the data suggest that the diets seem to be equally effective in the promotion of postpartum weight loss as well as in the promotion of a favorable change in body fat as assessed by the BIA method.

Adult overweight/obesity remains a significant public health concern with 68% of Americans classified as overweight [115]. Pregnancy-associated weight retention is one factor contributing to long-term obesity. Overweight/obesity, percent body fat, and adult weight gain are associated with increased risk for several obesity-related, adult-onset diseases including CVD, HTN, DM, and select cancers [48-51]. Interventions promoting weight loss/control during this vulnerable period of weight retention may help to reduce the risk for a pattern of continuing adult weight gain leading to obesity-associated disease risk.

**Change in Biomarkers of Inflammation**

Finally, this investigation provides evidence that both a traditional MED diet and the USDA’s MyPyramid diet for Pregnancy and Breastfeeding are effective in lowering chronic inflammation, as measured by the biomarker, TNF-α, in postpartum breastfeeding women. However, it is important to note that this reduction in inflammation may have primarily been influenced by weight loss; as suggested when we stratified all participants by percent of weight loss. When all study participants were combined together and stratified by amount of weight loss
(< 10% or ≥ 10%), no significant within group differences were noted for IL-6, however, there was an unadjusted between group difference (< 10% weight loss: 0.04±0.91, ≥ 10% weight loss: -3.66±5.39; P<0.001). Although this between group difference was no longer significantly different when the model was adjusted for baseline weight and biomarker level in question. Study participants who demonstrated < 10% weight loss showed a significant decrease in TNF-α (-0.46±0.85, P<0.001); a finding not shown in those with ≥ 10% weight loss (-3.66±6.30, P=0.144). However, similar to IL-6, there was a significant unadjusted between group difference (P<0.001), which was also no longer significant when adjusted for baseline weight and biomarker level. It is possible that the lack of a significant difference in TNF-α, as well as in IL-6, in the greater weight loss category is related to the very small number of women who actually lost ≥ 10% of their total body weight (TNF-α: N=8 , IL-6: N=7). It is likely that with a larger sample size a significant reduction in the biomarkers would be detected.

Persistent elevated inflammation is associated with an increased risk of numerous diseases, including CVD, stroke, DM, and select cancers [3-6]. Dietary interventions that are effective in reducing inflammation during a time-period that is associated with an up-regulated inflammatory state may present a low-risk method of reducing or delaying the onset of obesity-related chronic disease later in life.

**Future Directions**
Future researchers may consider commitment to exclusive breastfeeding a study criterion for clinical intervention trials involving breastfeeding women in order to promote greater retention rates. To promote breastfeeding, investigators could consider providing more intense and frequent breastfeeding support; the use of peer-counselors either in person, or via telephone or internet contact; and/or reaching mothers pre-parturition to provide antenatal breastfeeding education and counseling; all methods shown to be successful in the promotion of exclusive breastfeeding [83, 84]. Additionally, future research should continue to examine intervention strategies to promote successful weight loss/control during the postpartum period and may consider including formula feeding women and non-postpartum controls in order to better understand the effects of the diet intervention, separate from infant feeding status, on weight loss as well as on change in biomarkers of inflammation. Finally, future researchers may also consider including additional biomarkers of inflammation in order to detect significant change in relation to diet assignment.

**Conclusion**

This randomized, controlled trial examined the effects of adherence to a MED diet for 4 months in postpartum breastfeeding women compared to women randomized to the USDA’s MyPyramid diet for Pregnancy and Breastfeeding (control diet) on anthropometric measurements and biomarkers of inflammation. Our findings provide evidence that both diets are effective in the promotion of
postpartum weight loss as measured by body weight, body fat, and waist and hip circumference; and in the reduction of inflammation as measured by TNF-α. As both diet groups increased their MED diet score overall, it is feasible to conclude that dietary changes that reflect adherence to a more traditional MED pattern of eating will result in reductions of anthropometric measurements and chronic inflammation in a population of postpartum breastfeeding women.
APPENDIX A

STUDY TIMELINE FOR THE DIET STUDY IN LACTATING WOMEN
## Study Timeline for a Diet Study in Lactating Women

<table>
<thead>
<tr>
<th>STUDY ACTIVITY</th>
<th>IRB Approval</th>
<th>Recruitment</th>
<th>Consent &amp; Wash-out</th>
<th>Collect Baseline Data</th>
<th>Diet Intervention</th>
<th>Obtain Repeat Measures</th>
<th>Laboratory Assays</th>
<th>Statistics</th>
<th>Publication</th>
<th>Grant Writing</th>
</tr>
</thead>
<tbody>
<tr>
<td>7/08 – 1/09</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/09 – 3/09</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3/09 - 12/09</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/09 - 6/10</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/10 - 9/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9/10 – 12/10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12/10 – 6/11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
APPENDIX B

MEDITERRANEAN DIET SCORE
The Mediterranean-Diet Score System

<table>
<thead>
<tr>
<th>Beneficial Dietary Component</th>
<th>Consumption Below the Median</th>
<th>Consumption at or Above the Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetables</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Legumes</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fruits and nuts</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Cereal</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Fish</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Detrimental Dietary Component</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat /Poultry</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Dairy Products</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Monounsaturated to Saturated fat ratio</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alcohol (g/day)²</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 - 50</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5 - 25</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

¹Trichopoulou, 2003; A score of one is awarded for each food group in which the target grams (median from the total population at baseline) are met or exceeded in the case of vegetables, legumes, fruits and nuts, whole grain cereals, fish, and the median monounsaturated:saturated fat ratio; in which the target grams are not exceeded in the case of meat/poultry and dairy; or the target grams are within a specified range in the case of ethanol (5 – 25 g/day). The MED diet score results in a range of scores of zero to nine, with nine indicating the best adherence.
APPENDIX C

LIST OF ABBREVIATIONS
List of Abbreviations

AFFQ: Arizona Food Frequency Questionnaire
ALA: Alpha-Linolenic Acid
BAFC: Bioactive Food Component
BIA: Bioelectrical Impedance Analysis
BMI: Body Mass Index
CSFII: Continuing Survey of Food Intakes by Individuals
CVD: Cardiovascular Disease
DM: Diabetes
ELISA: Enzyme-Linked Immunosorbent Assay
FFQ: Food Frequency Questionnaire
GWG: Gestational Weight Gain
hsCRP: High Sensitivity C-Reactive Protein
HTN: Hypertension
ICAM-1: Inter-Cellular Adhesion Molecule-1
IFN-γ: Interferon-gamma
IL-2: Interleukin-2
IL-6: Interleukin-6
IOM: Institute of Medicine
MED: Mediterranean-style
MUFA: Monounsaturated Fatty Acid
NCI: National Cancer Institute
NDS-R: Nutrient Database for Standard Reference
OB/GYN: Obstetrics/Gynecology
PUFA: Polyunsaturated Fatty Acid
RCT: Randomized, Controlled Trial
RR: Relative Risk
SAT: Saturated Fatty Acid
TNF-α: Tumor Necrosis Factor-alpha
U.S.: United States
USDA: United States Department of Agriculture
VCAM-1: Vascular Cell Adhesion Molecule
VLED: Very Low Energy Diet
W.I.C.: The Special Supplemental Nutrition Program for Women, Infants, and Children
REFERENCES

2. Preventing and Managing Chronic Disease to Improve the Health of Women and Infants [http://www.cdc.gov/reproductivehealth/womensrh/ChronicDiseaseandRe productiveHealth.htm]


42. Brennan AM, Sweeney LL, Liu X, Mantzoros CS: Walnut consumption increases satiation but has no effect on insulin resistance or the metabolic profile over a 4-day period. *Obesity (Silver Spring)* 2010, 18(6):1176-1182.


65. Corbalan MD, Morales EM, Canteras M, Espallardo A, Hernandez T, Garaulet M: Effectiveness of cognitive-behavioral therapy based on the


75. State and County Quick Facts: Arizona [http://quickfacts.census.gov/qfd/states/04000.html]


87. Larsson B, Svardsudd K, Welin L, Wilhelmsen L, Björntorp P, Tibblin G: Abdominal adipose tissue distribution, obesity, and risk of


116. Probst-Hensch NM: *Chronic age-related diseases share risk factors: do they share pathophysiological mechanisms and why does that matter?* *Swiss Med Wkly* 2010, **140**:w13072.


149. Mori TA, Woodman RJ, Burke V, Puddey IB, Croft KD, Belin LJ: Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress


