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Jacobs Journal of Food and Nutrition

Research Article

Weight Loss and Breast Cancer Biomarkers in a Randomized Dietary Trial Among Overweight/Obese Premenopausal Women

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Received: 08-29-2014
Accepted: 10-02-2014
Published: 10-06-2014

Abstract

Background: This was a pilot study that was conducted to: 1) determine feasibility ofrecruitment, adherence, and follow-up of calorie-restricted low-fat or low-carbohydrate diet plus exercise intervention among overweight and obese premenopausal women; 2) determine if these interventions promoted weight loss; and 3) investigate whether the interventions altered breast cancer biomarkers.

Methods: 79 overweight and obese premenopausal women were enrolled in a 52-weekrandomized intervention trial of calorie-restricted low-fat versus calorie-restricted low-carbohydrate diet along with physical activity. Changes in body mass index (BMI) and breast cancer biomarkers such as insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3) were measured at baseline, 12, 34, and 52 weeks.

Results: Among the 550 women screened, 81 were eligible and 79 agreed to participate. Boththe calorie-restricted low-fat and low-carbohydrate diets plus exercise resulted in significant weight loss (average of 3.2 kg across arms, p<0.01). There were no significant changes in IGF-1 or IGFBP-3 in either group; however, IGF-1 had a significant positive relationship with weight loss (p=0.03), with a similar trend observed in each group (p=0.14). Insulin levels decreased an average of 23% between baseline and 52 weeks (p=0.052), with the same trend observed in each group (p=0.88).

Conclusions: The feasibility of adherence to both calorie-restricted diets plus exercise over a52-week intervention was low; however, both resulted in weight loss. Weight loss may lead to an increase in IGF-1 and a decrease in insulin. Although this was a relatively small pilot study, a larger study is needed to follow-up on these results.

Keywords: Breast Cancer; Low-Fat; Low-Carbohydrate; Physical Activity; Insulin-LikeGrowth Factor-1

Introduction

Breast cancer is the most common type of cancer diagnosed among U.S. women, with approximately 232,670 newly diagnosed cancers estimated in 2014 [1]. It has been suggested that 30-50% of cancers may be attributed to potentially modifiable lifestyle factors, such as diet and exercise [2]. Additionally, the World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR) concluded that there is convincing evidence that being overweight or obese increases the risk of postmenopausal breast cancer [3]; however, obesity has been associated with a decreased risk of premenopausal breast cancer [4]. Furthermore, weight gain during adulthood, especially after menopause, has been consistently associated with an increased risk of breast cancer in several large cohort and case-control studies [5-8]. Therefore, due to its modifiable nature, weight loss prior to menopause is an attractive breast cancer prevention strategy because it would lead to lower rates of postmenopausal obesity.

There is considerable controversy regarding the association of specific dietary patterns with breast cancer risk. Several studies, along with the AICR/WCRF panel, have suggested that a diet high in fruits and vegetables and low in fat and alcohol, as well as regular physical activity, reduces the risk of breast cancer [3, 9, 10]. However, the Nurses' Health Study demonstrated that a low-carbohydrate diet was associated with decreased risk of estrogen receptor- (ER) negative breast cancer [11]. Unlike specific dietary patterns, the association between physical activity and breast cancer appears to be clearer. Several studies have found a dose-dependent, significant inverse association with breast cancer risk among both pre- and post-menopausal women and the average risk may be between 30-40% [2, 12, 13].

The biological mechanisms linking diet, physical activity, and breast cancer are unclear; however, they may involve alteration of insulin-like growth factor-1 (IGF-1). The bioavailabilityof IGF-1 depends in part on the presence of insulin-like growth factor binding proteins (IGFBPs) and, in particular, IGFBP-3 which binds approximately 75% of IGF-1 [14]. Many epidemiologic studies have shown that elevated levels of IGF-1, or the ratio of IGF-1 to IGFBP-3, are associated with an increased risk for breast cancer among premenopausal women, [15-19] as well as postmenopausal women [20]. Physical activity has been shown to affect IGF-1 and IGFBP-3 levels [21-24]. Finally, IGFBP activity has been noted to increase with physical activity leading to an overall decrease in IGF-1 bioavailability [25].

To the best of our knowledge, no randomized trials have been conducted among premenopausal women comparing a calorie-restricted low-fat diet plus exercise versus a calorie-restricted low-carbohydrate diet plus exercise on biomarkers of breast cancer risk. Therefore, the current study was conducted to determine the feasibility of recruiting premenopausal women to a calorie-restricted low-fat versus a calorie-restricted low-carbohydrate diet plus exercise intervention to determine adherence. Furthermore, we investigated whether these interventions would result in weight loss, or an alteration in biomarkers of breast cancer risk, including IGF-1 and IGFBP-3 levels.

Materials and Methods

Study Design

The Lifetime Eating and Fitness (LEAF) study was a 52-week randomized intervention trial of two calorie-restricted dietary patterns plus exercise. The diets consisted of a low-carbohydrate diet (40% of total calories from carbohydrates, 30% of calories from protein and 30% of calories from fat) or a low-fat diet (20% of total calories from fat, 20% of calories from protein and 60% of calories from carbohydrates). The low carbohydrate diet mimickedpreviously used low carbohydrate diets [26] while the low fat diet met the lower end of the US Dietary Guidelines for Americans which recommended 20-35% of total calories from fat [27]. The women were educated on calorie-restriction regardless of the type of diet and given an individualized physical activity prescription to facilitate weight loss. Women were randomized using stratified randomization. The stratification was based on BMI (<30kg/m2 vs. >30kg/m2) to ensure an equitable distribution of overweight and obese subjects.

Participants

Eligible participants consisted of premenopausal (as determined by a follicle-stimulating hormone (FSH) test) women aged 30 or older with no prior diagnosis of cancer (except non-melanoma skin cancer); BMI of 25-34 kg/m2; planned to live in the Columbus, OH, area during the 18 month follow-up; and presented a letter of medical clearance from their physician to participate in this study. Exclusion criteria included: being pregnant or planning to become pregnant during the study period (women who became pregnant while in the study were removed from the study); current participation in a formal weight loss program (such as Weight Watchers); medical history that precluded adherence to the dietary patterns (history of renal insufficiency, gluten enteropathy, Crohn's disease or other medical conditions that significantly impacted nutritional status or metabolism, except controlled Type 2 diabetes); and lack of controlled management of currently existing medical problems.

Women were recruited from physician offices and through media advertisements. Women called a designated phone number if they were interested in participating and a staff member from The Ohio State University (OSU) Comprehensive Cancer Center provided general information about the study and screened women for eligibility. Potential subjects were scheduled for an enrollment visit where the study was described in more detail; if they agreed toparticipate, an informed consent form was signed. Women were not compensated financially, but parking vouchers were provided when necessary. This study was approved by the OSU Institutional Review Board.

Among the 550 women screened, 82 women were eligible and consented to participation in this study; however, 3 women withdrew prior to the baseline visit. Therefore, 79 women were available to participate, with 41 women randomized to the low-fat arm and 38 randomized to the low-carbohydrate arm.

Intervention

Diet: The diet education was based on the Exchange System for Weight Management, aspublished by the American Dietetic Association and the American Diabetes Association [28]. A registered dietitian (RD) determined the level of calorie restriction to provide based upon resting metabolic rate determined from indirect calorimetry at the baseline visit. The RD used each woman's resting metabolic rate, as determined from indirect calorimetry and multiplied an activity factor determined after assessing each woman's current physical activity. After calculating caloric requirements, 250-500 calories per day were subtracted to arrive at a new goal calorie intake which could facilitate weight loss. Because it is difficult to meet nutrient needs with a diet that is less than 1200 calories per day, goal intakes were not recommended under 1200 calories. Once the reasonable caloric restriction was determined, the RD then developed a plan of servings (or "exchanges") from each food group which would match the macronutrient distribution assigned at randomization. After baseline measures were assessed, each woman met with the RD at the Clinical Research Center (CRC) at OSU once per week for the first month of the study, every three weeks during the second, third and fourth month of the study, and then every 6 weeks for the remainder of the study, except for weeks 34 and 52, which were clinicvisits. On occasion, the counseling sessions were conducted by phone if the participant had rescheduled her visit more than once and could not come to the CRC. The average attendance rate at the clinic was 80%.

Adherence to the diet was monitored through 7-day diet records. All women were asked to complete a detailed diet record for the first seven days of each month of the study and bring it to their next appointment for analysis. Therefore, diet records were collected a total of 12 times (once per month) during the intervention component and analyzed to check for adherence to the intervention diet and to direct individual nutrition counseling. Food Processor software (ESHA Research, Salem, Oregon) was used to analyze diet records and provide individualized nutrition feedback.

Physical Activity: Women were asked to monitor their physical activity by wearing aDigiwalkerTM pedometer and recording their total steps walked per day. Each woman was individually counseled to increase the number of steps taken each day with an ultimate goal of 10,000 steps per day, which has been shown to result in weight loss and other positive health outcomes in previous studies [29, 30]. Women were also encouraged to increase their lifestyle activity by using stairs, parking further away, etc. If women wished to participate in other activities in addition to walking, they were asked to record these activities in their physical activity logs.

Scheduled Clinic Visits: After an initial screening visit to determine eligibility andensure medical clearance, approximately 5 visits (screening, baseline, week 12, week 34 and end of study-week 52) to the CRC were required for anthropometric measurements and fasting blood draw. A baseline questionnaire was administered to assess demographic data, smoking and alcohol use, physical activity [31], and quality of life [32].

Anthropometric measures: Participants were weighed at the CRC using a standardizedscale with a precision of 0.1 pound. Waist and hip measurements were completed by the CRC staff using a tape measure. Waist circumference was measured between the costal margin and the iliac crest. Hip circumference was measured from the widest part of the hip, generally at the level of the greater trochanters.

IGF-1: Serum IGF-1 was analyzed at baseline, 12, 34, and 52 weeks using a free IGF-1enzyme immunoassay (EIA) (Diagnostic Systems Laboratory, Topsfield, MA) by the CRC clinical laboratory. The intra-assay variation was 4.0% and the inter-assay variation was 9.1%. The theoretical sensitivity was 0.15 ng/ml.

IGFBP-3: IGFBP-3 was analyzed at baseline, 12, 34, and 52 weeks with the ActiveIGFBP-3 EIA (Diagnostics Systems Laboratory) by the CRC clinical laboratory. The intra-assay variation was 8.8% and the inter-assay variation was 10%. The theoretical sensitivity was 0.04 ng/ml.

Insulin: Insulin was analyzed at baseline, 12, 34, and 52 weeks with achemilluminescence methodology using the Immulite 1000 (Siemens Medical Solutions Diagnostics, Tarrytown, NY) by the CRC clinical laboratory. The sensitivity for the assay was 2μIU/ml. The intra-assay coefficient of variation was 5.7% and the inter-assay coefficient variation was 6.7%.

Statistical analysis: Descriptive statistics (mean and standard deviation) were used to examinethe characteristics of women in each assigned group for the various parameters at randomization. Change in weight loss over weeks 1 to

52, waist circumference, waist-to-hip ratio, IGF-1, IGFBP-3, IGF-1:IGFBP-3 ratio, and insulin were examined using linear mixed models containing fixed effects for treatment arm, time, and a treatment-by-time interaction. The weightmodel included a random subject-specific intercept and time slope, but due to the fewer longitudinal measurements of waist, waist-to-hip, IGF-1, IGFBP-3, IGF-1:IGFBP-3 ratio, and insulin we could not estimate a random intercept and slope in these models; we instead used an autoregressive correlation structure for the residual errors (REPEATED statement in SAS PROC MIXED, covariance type = SP(POW)). The trend in time in the fixed effect portion of each model was determined by comparing linear, quadratic, and cubic trends and selecting the model with the smallest Bayesian Information Criterion. The Kenward-Roger method [33] was used to calculate the degrees of freedom in all hypothesis tests. To allow flexibility in timing of the final follow-up, measurements obtained by week 56 were included in the analysis.

All participants with at least one measurement obtained over weeks 1-56 of the study were included in the analysis. Therefore, all 79 participants were used in the weight and biomarker longitudinal analyses because they had at least a baseline or follow-up measurement. Women with a baseline weight and biomarker level and at least one follow-up measure of each were included in the analysis relating weight to change in biomarker levels (n=60).

Sensitivity analyses were conducted examining only those who adhered either to diet or physical activity. Diet adherence was monitored through diet records for the first seven days of each month of the study. Women were considered adherent if their fat or carbohydrate intake was within 80% of the intervention goal (for low-carbohydrate: 40% of total calories from carbohydrates and for low-fat diet: 20% of total calories from fat) according to their final diet record. Physical activity was self-monitored by the participants using daily physical activity logs. Women were considered adherent if their daily average steps were within 80% of the physical activity intervention goal of 10,000 steps per day. Dropout rates were also comparedacross arms. A dropout was defined as a woman who did not complete at least 80% of all study visits (clinic/counseling visits) or did not attend their last study visit.

The study was originally powered to have 80% power to detect a difference in mean IGF-1 levels (a primary endpoint) of 30.00 assuming a standard deviation of differences of 50.00 using a paired t-test with a 0.05 two-sided significance level. Therefore, the required sample size was 24 women; however 20% non-evaluable participants were added in, which increased the sample size to 30 participants per arm. All analyses were performed using SAS Version 9.2 (SAS Inc., Cary, NC).

Results

There were 550 women screened for the study with the most common reasons for ineligibility being postmenopausal or having a BMI below 25 (Figure 1). Seventy-nine women were eligible and agreed to participate in the study with 41 randomized to the calorie-restricted low-fat diet and 38 randomized to the calorie-restricted low-carbohydrate diet. Among these women, 48% had a BMI that categorized them as overweight, while 49% of them had a BMI that categorized them as obese. Demographic data illustrated the effectiveness of the randomization as the two arms were comparable with respect to race, education, marital status, smoking status, insurance, work status, occupation, and income (Table 1). Although there were no statistically significant differences in adherence rates between the different diets, adherence was low in both, with 29% of the women adherent to the low-carbohydrate intervention and 22% adherent to the low-fat intervention (data not shown). Women were more adherent to their physical activity goal with 61% of the women in the low-carbohydrate intervention and 66% of the women in the low-fat intervention adherent (data not shown). Finally, dropout rates were similar in both arms with 17 (41%) women dropping out of the low-fat arm and 21 (55%) women dropping out of the low-carbohydrate arm (p=0.21).

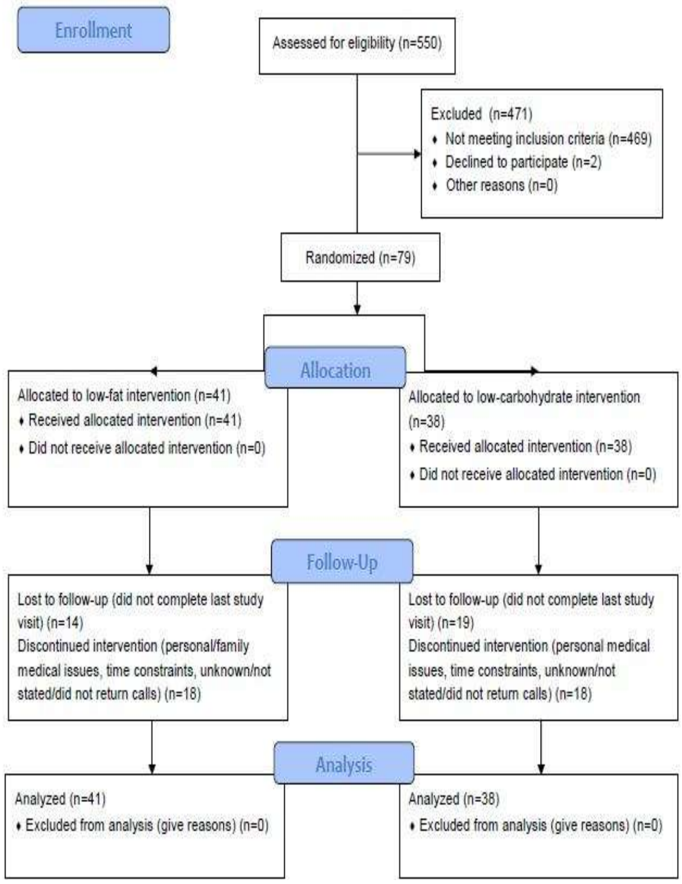

Figure 1. CONSORT Flow Diagram for the LEAF study.

Table 1. Demographic Characteristics of Study Participants	Total		Low-fat		Low-carbohydate	
	(n=79)		(n=41)		(n=38)	
	n (%)		n (%)		n (%)	
Age (yrs)						
30-34	12	(15)	7 (17)		5 (13)	
35-39	18	(23)	10	(24)	8 (21)	
40-44	25	(32)	14	(34)	11	(29)
45-49	19	(24)	9 (22)		10	(26)
50-54	5	(6)	1	(2)	4 (11)	
Race						
White	54	(68)	29	(69)	25	(68)
African American	21	(27)	11	(26)	10	(27)
Asian	1	(1)	1	(2)	0	(0)
American Indian	1	(1)	0	(0)	1	(3)
Other	2	(3)	1	(2)	1	(3)
Education						
High School/GED	3	(4)	1	(2)	2	(5)
Vocational/Training School	2	(3)	2	(5)	0	(0)
Some College	14	(18)	11	(26)	3	(8)
Associate Degree	7	(9)	3	(7)	4 (11)	
Bachelor's Degree	20	(25)	9 (21)		11	(30)
Master's Degree	9 (11)		4 (10)		5 (14)	
Doctorate/Advanced Degree	4	(5)	2	(5)	2	(5)
Unknown	20	(25)	10	(24)	10	(27)
Marital Status						
Married	65	(82)	35	(83)	30	(81)
Divorced/Separated	4	(5)	3	(7)	1	(3)
Single, never married	7	(9)	2	(5)	5 (14)	
Single, living as married	0	(0)	0	(0)	0	(0)
Widowed	2	(3)	2 (5)		0	(0)
Unknown	1	(1)	0	(0)	1	(3)
Smoking Status						
Never/former smoker	56	(71)	29	(69)	27	(73)
Current smoker	1	(1)	1	(2)	0	(0)
Insurance						
Private Insurance	54	(68)	28	(67)	26	(70)
Medicare	1	(1)	1	(2)	0	(0)
Military or VA Program	1	(1)	1	(2)	0	(0)
No insurance/self-pay	3	(4)	1	(2)	2	(5)
No insurance/no means to pay	1 (1)		1	(2)	0	(0)
Unknown	19	(24)	10	(24)	9 (24)	
Current Work Status						
Employed full time	38	(48)	24	(57)	14	(38)
Employed part time	9 (11)		3	(7)	6 (16)	
Employed/on medical leave	1	(1)	1	(2)	0	(0)
Self employed	6	(8)	4 (10)		2	(5)
Homemakeer	5	(6)	0	(0)	5 (14)	
Unknown	20	(25)	10	(24)	10	(27)
Occupation						
Professional, technical-managerial or administrative	43	(54)	20	(48)	23	(62)
Sales/clerical service	7	(9)	5 (12)		2	(5)
Craftsman, machine operator or laborer	1	(1)	1	(2)	0	(0)
Other	8 (10)		6 (14)		2	(5)
Unknown	20	(25)	10	(24)	10	(27)
Income						
<$10,000	1	(1)	1	(2)	0	(0)
$10,000-$19,999	2	(3)	0	(0)	2	(5)
$20,000-$34,999	4	(5)	3	(7)	1	(3)
$35,000-$49,999	7	(9)	3	(7)	4 (11)	
$50,000-$74,999	12	(15)	6 (14)		6 (16)	
$75,000-$99,999	15	(19)	5 (12)		10	(27)
$100,000-$149,999	10	(13)	6 (14)		4 (11)	
>$150,000	5	(6)	3	(7)	2	(5)
Don't know	1	(1)	1	(2)	0	(0)
Refused	3	(4)	2	(5)	1	(3)
Unknown	22	(28)	12	(29)	10	(27)

	mean (sd)	mean (sd)	mean(sd)
BMI	30.3 (2.8)	30.5 (2.9)	30.1 (2.6)
Physical Activity Expenditure (kcal/day)*	515.0 (176.	526.8 (204.6)	500.9 (137.1)
Diet**			
Total Calories	729.3 (409.1760.9 (299.0		1729.3 (409.2)
Carbohydrates (%)	50.2 (6.7)	53.5 (6.0)	46.9 (5.6)
Fat (%)	31.4 (4.9)	30.3 (5.1)	32.4 (4.4)
Protein (%)	18.8 (3.7)	17.2 (2.7)	20.3 (3.9)
*n=57			
**n=73			

The mean body weight by diet was examined from week 1 through 56. Women in both the low-fat and the low-carbohydrate groups lost a significant amount of weight during the study (p<0.01) (Table 2).

Table 2. Mixed model means of outcome measures. Overall estimates are averages across the twodiets. Estimates for weight and waist assume a cubic trend in time. All other estimates assume a linear trend in time.

		Baseline		Week 34		Week 52	
Outcome	**Diet**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Weight (kg)*	Low Fat	84.1	1.3	80.6	1.4	80.6	1.6
	Low Carb	83.6	1.4	80.0	1.5	80.7	1.6
	Overall	83.9	1.0	80.3	1.0	80.7	1.1
Waist (cm)**	Low Fat	92.3	1.2	89.7	1.3	89.6	1.4
	Low Carb	91.0	1.3	89.2	1.4	90.3	1.5
	Overall	91.5	0.9	89.5	0.9	89.9	1.0
Waist/Hip	Low Fat	0.82	0.01	0.82	0.01	0.82	0.01
	Low Carb	0.81	0.01	0.82	0.01	0.82	0.01
	Overall	0.81	0.01	0.82	0.01	0.82	0.01
IGF-1 (ng/ml)	Low Fat	285.1	17.4	270.1	17.7	261.9	22.5
	Low Carb	274.9	18.4	287.3	18.6	294.1	23.7
	Overall	280.0	12.7	278.7	12.8	278.0	16.4
IGFBP-3 (ng/ml)	Low Fat	45.6	1.5	43.1	1.5	41.8	2.0
	Low Carb	42.2	1.5	41.8	1.6	41.6	2.1
	Overall	43.9	1.1	42.5	1.1	41.7	1.4
IGF-1:IGFBP-3	Low Fat	6.36	0.37	6.48	0.38	6.54	0.50
	Low Carb	6.46	0.39	6.93	0.40	7.18	0.52
	Overall	6.41	0.27	6.70	0.27	6.86	0.36
Insulin (ln(uIU/ml))***	Low Fat	1.89	0.10	1.73	0.10	1.65	0.14
	Low Carb	1.72	0.11	1.54	0.10	1.44	0.16
	Overall	1.81	0.07	1.64	0.07	1.55	0.11

*Significant difference in trend by diet (p=0.050). No difference in net weight loss between diets (p=0.63).
**Significant trend in time across diets (p<0.01).
***Average trend in time across diets marginally significant (p=0.052).

There was a significant difference in the weight loss trend between the two arms (p=0.050) with women on the low-carbohydrate diet experiencing a steeper decline in weight initially, but at around week 22 began to rebound toward the weight loss level of the women on the low-fat diet. At the conclusion of the study (week 52), the net weight loss was similar across arms (3.5 kg for low-fat and 2.9 kg for low-carbohydrate, p=0.63) (Figure 2).

A sensitivity analysis examining weight loss among just those women who were adherent to the interventions demonstrated similar results to when all women were included. There was a significant overall decline in weight (p<0.01), but there was no difference between the two groups (p=0.42) (Table 3). Similar results were observed when looking at women who were adherent to physical activity. Among the 50 women who were adherent to physical activity, there was significant weight loss (p<0.01), but no difference between groups (p=0.59).

Waist circumference and waist-to-hip ratio were also examined in both interventions over the 52-week study

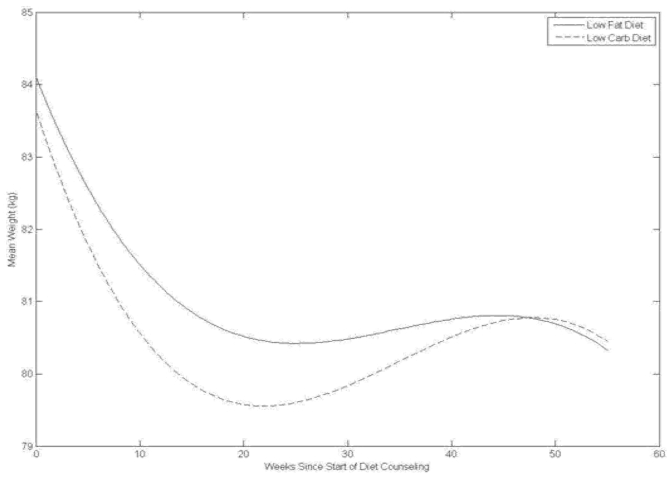

Figure 2. Mean body weight by dietary intervention. Estimates are from a linear mixed model with a cubic trend in time. A significant amount of weight was lost in both the low-fat and the low-carbo-

hydrate arms (p<0.01). The trend in weight loss differed between groups, though the net weight loss was the same at study conclusion (p=0.63).

(Table 2). There was a significant decrease in waist circumference means across groups (p=0.03), with a similar decline observed in each group (p=0.19). Results for waist circumference means were similar when analyses were restricted to physical activity adherent subjects (significant overall decline in waist circumference (p<0.01), no difference across groups). The overall decline in waist circumference among diet adherent subjects (2.7 cm) was similar to the decline among physical activity adherent subjects (2.6 cm), but was not significant (p=0.12) due to the small sample size (n=20 adherent subjects) (Table 3). Waist-to-hip ratio did not change in either group (p=0.88); similar results were observed among diet adherent and physical activity adherent subjects.

adherent to physical activity, changes in insulin were more pronounced when we focused on diet-adherent subjects: there was a 50% decrease in mean insulin between baseline and 52 weeks for diet adherers compared to only a 23% decrease overall (Table 3).

Lastly, levels of IGF-1 and IGFBP-3 were measured in association with changes in weight. There was no difference across the interventions in changes in IGF-1 associated with changes in weight (p=0.14) (Figure 3A). However, the average trend across groups was significant (p=0.04) and suggests that regardless of diet arm, an increase in weight loss was associated with an increase in IGF-1. IGFBP-3 did not significantly change with weight (p=0.81), with the results being consistent across arms (p=0.40) (data not shown). There was a significant positive association between IGF-1:IGFBP-3 ratio and weight loss (p<0.01), which did not differ by diet (p=0.12) (Figure 3B).

Table 3. Mixed model means of outcome measures among participants adherent to diet.
Overall estimates are averages across the two diets. Estimates for weight assume a cubic trend in time. All other estimates assume a linear trend in time.

		Baseline		Week 34		Week 52	
Outcome	**Diet**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
Weight (kg)*	Low Fat	88.4	3.1	84.1	3.4	83.3	3.7
	Low Carb	85.3	2.8	80.1	3.1	82.1	3.3
	Overall	86.8	2.1	82.1	2.3	82.7	2.5
Waist/Hip	Low Fat	0.82	0.02	0.83	0.02	0.84	0.03
	Low Carb	0.83	0.02	0.82	0.02	0.82	0.02
	Overall	0.82	0.01	0.83	0.02	0.83	0.02
IGF-1 (ng/ml)	Low Fat	239.3	37.9	237.4	39.5	236.3	47.0
	Low Carb	256.9	34.3	263.3	34.9	266.7	41.0
	Overall	248.1	25.6	250.3	26.4	251.5	31.2
IGFBP-3 (ng/ml)	Low Fat	41.3	3.0	38.4	3.1	36.8	3.9
	Low Carb	38.7	2.7	40.7	2.7	41.8	3.4
	Overall	40.0	2.0	39.5	2.1	39.3	2.6
IGF-1:IGFBP-3	Low Fat	5.80	0.68	6.32	0.72	6.60	0.94
	Low Carb	6.36	0.61	6.43	0.63	6.48	0.81
	Overall	6.08	0.46	6.38	0.48	6.54	0.62
Insulin (ln(uIU/ml))**	Low Fat	1.74	0.21	1.29	0.22	1.05	0.33
	Low Carb	1.94	0.19	1.50	0.19	1.25	0.29
	Overall	1.84	0.14	1.40	0.15	1.15	0.22

*Significant difference in trend by diet (p<0.01). No difference in net weight loss between diets (p=0.42).
**Average trend in time across diets significant (p=0.02).

A number of breast cancer biomarkers were measured at baseline, and weeks 12, 34, and 52, including IGF-1, IGFBP-3, and their ratio, and insulin (Table 2). No significant changes were observed in levels of IGF-1, IGFBP-3, or their ratio over the course of the intervention. However, there was a marginally significant decrease in insulin (overall decrease of 1.4 uIU/ml in the geometric mean across arms, p=0.052). There were no differences in the trend of any of the biomarker levels across the two interventions. Furthermore, the results for IGF-1, IGFBP-3, and IGF-1:IGFBP-3 ratio were consistent when examined only among the women who adhered either to the diet or physical activity. While the results for insulin were also consistent when the analysis was restricted to subjects

Discussion

Modifiable risk factors for breast cancer have been identified and are attractive targets for postmenopausal breast cancer prevention. Two of these risk factors, BMI and weight gain, have been associated with an increased risk of postmenopausal breast cancer, thus reduction and prevention of obesity prior to menopause is an appealing breast cancer prevention strategy. While calorie restriction and physical activity are two of the most effective methods to reduce obesity, the most effective diet modification for reducing obesity and impacting breast cancer prevention is currently unknown. The goal of this pilot intervention study was to determine the feasibility

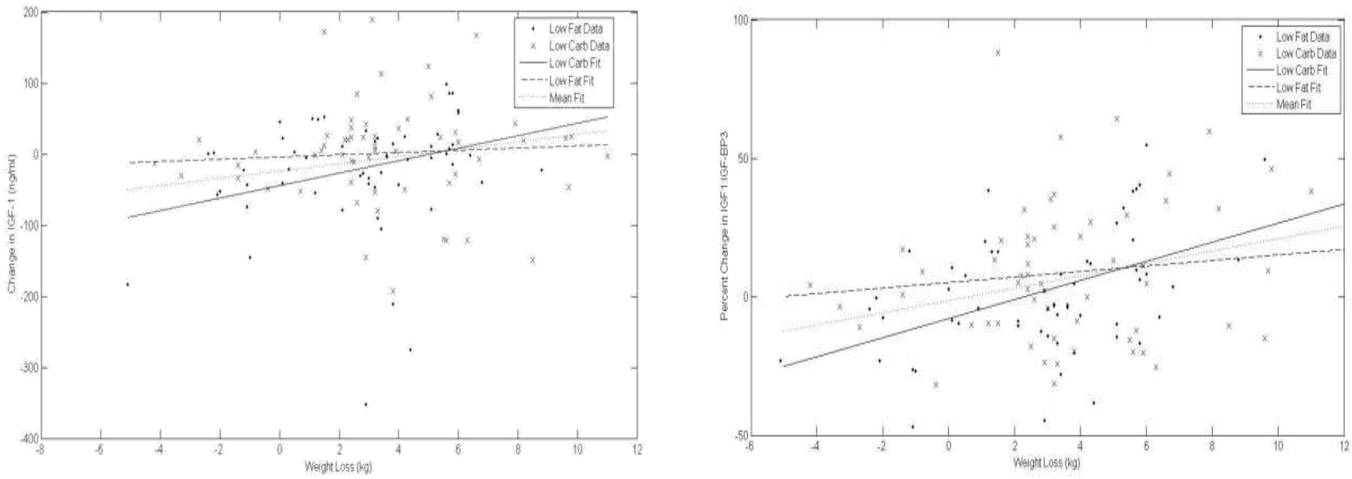

Figure 3. Percent change in IGF-1 and IGF-1:IGF-BP3 ratio with weight loss in the LEAF study. A) There was no difference between the low-fat and low-carbohydrate arms in change in IGF-1 with weight loss (p=0.14). The average trend across groups was significant (p=0.04). B) There was no difference between the low-fat and low-carbohydrate There was no difference between the low- fat and low-carbohydrate arms in change in the ratio with weight loss (p=0.12). The average trend across groups was significant (p<0.01).

of recruiting premenopausal women to a dietary plus exercise intervention and determining adherence and dropout rates over a 52-week period. Secondary goals included whether the interventions were effective on weight loss and altering biomarkers of cancer risk.

In this feasibility study, recruitment was a challenge. Although 550 women were screened, only 81 were eligible. However, of those 81, 79 agreed to participate. Adherence to the dietary intervention over the 52-week period was very low, similar to what others have observed [34, 35]. Only 29% adhered to the low-carbohydrate diet and 22% adhered to the low-fat diet. Adherence to physical activity, however, was higher at 61% and 66% for women in the low-carbohydrate and the low-fat interventions, respectively. In addition, a significant number of women in each arm were lost to follow-up, with 41% women dropping out of the low-fat arm and 55% women dropping out of the low-carbohydrate arm, similar to what has been observed in other studies [34, 35].

Both the calorie-restricted low-fat and low-carbohydrate interventions resulted in significant weight loss; however, no significant difference in weight loss between the two arms was found. Many studies have observed that a low-carbohydrate diet is more successful in creating short-term weight loss compared to a low-fat diet [36-40]. However, other studiesrandomizing men and women to a low-fat versus low-carbohydrate diet for one or two years observed similar results to ours, i.e., that there was weight loss, but no difference between arms [36, 41-44]. These studies also observed that most of the weight loss occurred in the first 6 months and weight loss occurred faster among those assigned to a low-carbohydrate diet [43, 44]. In addition, it is possible that the distribution of macronutrients in our diets may not have been different enough to detect differences in weight, however; our goal was to use nutritionally replete diets for free-living participants. Although the participants in these studies had a mean BMI similar to our study, most consisted of both men and women and many of the women in these studies were postmenopausal.

We did not observe any change in waist-to-hip ratio among women in either the low-fat or low-carbohydrate interventions. Similarly, another randomized study of low-fat or low-carbohydrate diets conducted among obese men and women did not observe a change in waist-to-hip ratio [42]. However, their follow-up was limited to three months, and in weight loss interventions, the biggest changes are usually observed within the first six months [40, 44, 45]. Few studies have examined the effect of these two diets on waist circumference but, of those studies, most have observed a significant decrease in waist circumference in both diets with no significant differences between diets [40, 41, 45, 46]. Furthermore, many studies have not observed a significant association between waist circumference and increased breast cancer risk after adjusting for BMI [47].

Levels of IGF-1 did not change significantly in either the low-fat or low-carbohydrate diet groups. The same was observed with levels of IGFBP-3. Mouse studies have demonstrated the ability of a low-carbohydrate diet to reduce IGF-1 levels [48-50] and increase IGFBP-3 levels [50]; however, some of these studies compared low-carbohydrate diets to calorierestriction and observed that the animals on a low-carbohydrate diet had higher levels of IGF-1 [51]. There are few studies in the current literature examining the effects of a low-fat or low-carbohydrate diet on IGF-1 and IGFBP-3 levels in humans. One study done only in men demonstrated an increase in IGF-1 levels among men on a low-fat diet; however, this was in comparison to a high fat diet and was conducted as a crossover feeding study [52]. This same study reported no differences in IGFBP-3 levels. The authors suggested this effect could have been due to a carryover effect [52]. Similar to our study, others have also detected no changes in IGF-1 levels [53, 54] or IGFBP-3 [53-56] in participants following a low-fat diet. There are a few studies, however, that have observed a decrease in IGF-1 [56-59] and an increase in IGFBP-3 [58] when a low-fat diet was consumed. There is a suggestion that simple overall calorie restriction may alter IGF-1 and IGFBP-3 levels in humans [60, 61]. One study conducted in premenopausal women demonstrated that calorie restriction significantly decreased IGF-1 and increased IGFBP-3 levels [60]. However, other studies have shown an increase in IGF-1 with a low calorie diet [62]. There also appears to be a link between changes in IGF-1 with physical activity. Among women, physical activity has been associated with a reduction in IGF-1 [63-66] and increase in IGFBP-3 levels [25, 63, 64]. However, some studies observed increases in IGF-1 with physical activity [25], while others observed no difference in IGF-1 or IGFBP-3 [67-69]. Therefore, additional investigations are required to elucidate the association between IGF-1, IGFBP-3, diet, and physical activity.

Although both the low-fat and low-carbohydrate diets resulted in significant weight loss, there was no significant change in IGFBP-3 with change in weight. However, IGF-1 was significantly positively associated with weight loss. Other studies have observed a significant increase in IGF-1 following weight loss [70-72]. The peptide hormone ghrelin is reduced inobese individuals and has been demonstrated to increase after weight loss [73-75]. It has been hypothesized that ghrelin may be one factor involved in the increase in IGF-1 after weight loss [71]. Insulin has also been hypothesized to stimulate production of IGF-1 in obese women [71, 76], although our results contradict this theory.

This study has some limitations. First, the number of participants was relatively small with 41 women randomized to a low-fat diet and 38 women randomized to a low-carbohydrate diet. Therefore, because this was a pilot study it is possible that we were underpowered to observe a difference in weight loss and biomarkers between dietary interventions. This study limited participants to premenopausal women with a BMI between 25 and 34, therefore, future studies are necessary to determine if similar results are observed in postmenopausal women and women with a BMI above 34. Second, adherence to the dietary interventions was low. Therefore, it is possible that this low adherence resulted in smaller changes to the biomarkers measured. Third, physical activity was self-monitored by the participants using a daily physical activity log. Although daily logs are useful because activity is recorded each day, they can sometimes be completed inaccurately and can influence a subject's behavior [77]. Diet records are also a limitation as they are also self-reported, they may not contain complete data, and subjects may alter their eating patterns due to the burden of recording their food consumption or to please the investigators [78]. Finally, there was a high drop-out rate, which is common in diet intervention studies.

Despite the limitations, the study had several strengths. This was a randomized trial comparing low-fat versus low-carbohydrate diets. There are relatively few studies comparing low-fat versus low-carbohydrate diets for greater than six months, and the current study followed women for a year. Furthermore, not only was weight loss measured, but the current study alsomeasured specific biomarkers that have been associated with breast cancer risk. These biomarkers were assessed at several time points during the study to examine changes over the course of the interventions. BMI was also assessed along with waist and hip measurements. Finally, the use of mixed models in our analysis allows the use of all available measurements and provides unbiased estimates of treatment and time effects under the assumption that missing follow-up measurements were missing at random [79].

Conclusion

Although this is a small randomized study, several important results can inform future studies, which are as follows: both diets may produce equivalent weight loss; adherence to diet changes is difficult to achieve while adherence to exercise regimens may be easier to attain; and biomarkers of breast cancer risk do not appear to be widely impacted by diet and physical activity changes. These findings, suggest the need for larger randomized studies examining diet, physical activity, and biomarkers of breast cancer risk to determine whether these modifiable factors can result in breast cancer prevention.

Acknowledgements:

The project described was supported by grants from the Breast CancerResearch Foundation, the National Center for Advancing Translational Sciences (8UL1TR000090-05), and the National Cancer Institute (K225K22CA140860-02). The content is solely the responsibility of the authors and does not necessarily represent the official views of the Breast Cancer Research Foundation, the National Center for Advancing Translational Sciences or the National Institutes of Health. There are no conflicts of interest to disclose.

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