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Effects of Diet Composition and Insulin Resistance Status on Plasma Lipid Levels in a Weight Loss Intervention in Women

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Background—Optimal macronutrient distribution of weight loss diets has not been established. The distribution of energy from carbohydrate and fat has been observed to promote differential plasma lipid responses in previous weight loss studies, and insulin resistance status may interact with diet composition and affect weight loss and lipid responses.

Methods and Results—Overweight and obese women (n=245) were enrolled in a 1-year behavioral weight loss intervention and randomly assigned to 1 of 3 study groups: a lower fat (20% energy), higher carbohydrate (65% energy) diet; a lower carbohydrate (45% energy), higher fat (35% energy) diet; or a walnut-rich, higher fat (35% energy), lower carbohydrate (45% energy) diet. Blood samples and data available from 213 women at baseline and at 6 months were the focus of this analysis. Triglycerides, total cholesterol, and high- and low-density lipoprotein cholesterol were quantified and compared between and within groups. Triglycerides decreased in all study arms at 6 months ($P<0.05$). The walnut-rich diet increased high-density lipoprotein cholesterol more than either the lower fat or lower carbohydrate diet ($P<0.05$). The walnut-rich diet also reduced low-density lipoprotein cholesterol in insulin-sensitive women, whereas the lower fat diet reduced both total cholesterol and high-density lipoprotein cholesterol in insulin-sensitive women ($P<0.05$). Insulin sensitivity and C-reactive protein levels also improved.

Conclusions—Weight loss was similar across the diet groups, although insulin-sensitive women lost more weight with a lower fat, higher carbohydrate diet versus a higher fat, lower carbohydrate diet. The walnut-rich, higher fat diet resulted in the most favorable changes in lipid levels.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT01424007. (*J Am Heart Assoc*. 2016;5: e002771 doi: 10.1161/JAHA.115.002771)

Key Words: insulin resistance • lipids • walnuts

Dyslipidemia is a major health concern in the United States as a risk factor for cardiovascular disease that also may increase risk for other comorbidities.[1–3] According to the Centers for Disease Control and Prevention, it was estimated as of 2012 that 28% of US adults aged >40 years were on some type of lipid-lowering medication, and of that population, 93% were using a statin.[4] Although these medications are very effective, they may put patients at risk for drug interactions and undesirable adverse effects. In addition,

overweight or obese persons are more likely to present with dyslipidemia than leaner persons.[5] In addition to cardiovascular disease, strong evidence shows that obesity and fat distribution are associated with risk for many types of cancer.[6] There also is compelling evidence that overweight status and obesity influences overall health outcomes and likelihood of comorbidities such as cardiovascular disease among those who have been diagnosed with cancer.[7] Weight loss and diet modification are strategies that may reduce risk of cardiovascular and other diseases linked to dyslipidemia and atherosclerosis.

Therapeutic lifestyle changes are the first-choice therapy recommended to lower low-density lipoprotein cholesterol (LDL-C) levels and to improve the lipid profile.[1,2] Historically, the most common dietary recommendation to reduce risk for cardiovascular disease has been to consume a low-fat, higher carbohydrate diet.[8] This dietary approach serves to lower the intake of saturated fat, which has had the strongest association with risk of cardiovascular disease; however, dietary patterns that replace saturated fat with unsaturated fat rather than carbohydrate, such as the Mediterranean diet,

can substantially reduce cardiovascular disease risk.[9] Furthermore, current evidence suggests that persons who are insulin resistant or who have type 2 diabetes may benefit more from a higher fat, lower carbohydrate diet if those fats are monounsaturated and polyunsaturated fat.[2,10] For patients with metabolic syndrome, a condition of insulin resistance, replacing saturated fat with unsaturated fat rather than carbohydrate and avoiding very low-fat diets has been recommended to avoid further worsening high triglyceride and low high-density lipoprotein cholesterol (HDL-C) levels.[8] Current dietary guidelines for lowering LDL-C recommend limiting the intake of saturated fat by adhering to either a higher fat (eg, Mediterranean) or lower fat (eg, Dietary Approaches to Stop Hypertension, or DASH) dietary pattern.[1]

A recent meta-analysis of 32 studies comparing low- and high-fat diets found significant reduction in total cholesterol and LDL-C in low-fat diets compared with high-fat diets and a significant increase in HDL-C and decrease in triglycerides in high-fat compared with low-fat diets.[11] Another meta-analysis of studies of the effects of dietary fat on HDL-C metabolism also found that replacing carbohydrate with fat (saturated or unsaturated) significantly increased HDL-C.[12]

Numerous clinical studies of nut consumption, focused primarily on the effects on cardiovascular disease risk factors and markers of inflammation, have observed a minimal (if any) effect on body weight, despite the potential additional energy intake contributed by the addition of nuts to the diet.[13,14] A recent meta-analysis of 13 short-term walnut feeding and walnut-rich diet intervention studies concluded that a walnut-enriched diet significantly decreased total cholesterol and LDL-C compared with a control diet.[15] Furthermore, a randomized crossover feeding trial compared the effects of a Mediterranean diet (high monounsaturated fat, lower carbohydrate) with those of a walnut-enriched diet (high polyunsaturated fat, lower carbohydrate) in 21 hypercholesterolemic men and women and found that substituting walnuts for monounsaturated fatty acids significantly reduced total cholesterol and LDL-C.[16]

A primary aim of this analysis was to examine the effects of diet composition on the plasma lipid profile at 6 months in overweight and obese women participating in a behavioral weight loss intervention. A secondary aim was to examine the lipid profile in consideration of insulin resistance status. The diets compared were lower fat (20% energy), higher carbohydrate (65% energy); lower carbohydrate (45% energy), higher fat (35% energy); and walnut-rich (18% energy), higher fat (35% energy), and lower carbohydrate (45% energy). Increased knowledge of how lipid responses may be influenced by diet composition and insulin resistance may allow more patient-specific recommendations to optimize the diet prescription for managing dyslipidemia and reducing disease risk.

Methods

Study Participants

The behavioral weight loss intervention trial randomized 245 overweight and obese women from a screened sample of 1559 women. To be included in the study, women had to meet the following criteria: age ≥21 years; body mass index (in kg/m²) between 27 and 40; willingness and ability to participate in clinic visits, group sessions, and telephone and Internet communications; ability to provide data through questionnaires and telephone; willingness to maintain contact with investigators for 12 months; willingness to allow blood collections; no known allergy to tree nuts; and ability to perform a simple test for assessing cardiopulmonary fitness.[17] Exclusion criteria were any of the following factors: inability to participate in physical activity due to severe disability, history or presence of a comorbid disease for which diet modification and increased physical activity may be contraindicated, self-reported pregnancy or breast-feeding or planning a pregnancy within the next year, current involvement in another diet intervention study or weight loss program, and history or presence of a significant psychiatric disorder or any condition that would interfere with participation in the trial.[17] The University of California San Diego institutional review board approved the study protocol, and all participants provided written informed consent.

Prior to enrollment, women were screened for diabetes and were considered ineligible with a fasting blood glucose level ≥125 mg/dL. Women were also asked to report all prescription medications and were asked if they had ever been told by a doctor that they had high blood cholesterol.

Once enrolled, participants were randomly assigned to 1 of the 3 study arms: lower fat (20% energy), higher carbohydrate (65% energy) diet; higher fat (35% energy), lower carbohydrate (45% energy) diet; or walnut-rich (18% energy), higher fat (35% energy), lower carbohydrate (45% energy) diet. All diet prescriptions limited saturated fat, so guidance for the higher fat diet emphasized lean meats and reduced-fat dairy foods and encouraged monounsaturated fat as a major (although not sole) source of fat in the diet. In all diets, prescribed protein intake exceeded recommended levels, although it was slightly lower for the lower fat diet compared with the others (15% versus 20% of energy). Randomization was stratified by menopausal status (aged ≥55 or <55 years as a proxy) and by insulin resistance status (insulin sensitive or insulin resistant), with a homeostasis model assessment value of >3.0 indicative of insulin resistance. Fasting glucose and insulin were measured at the screening clinic visit to calculate homeostasis model assessment for the baseline characterization of insulin resistance status. Data collection, anthropometric

measurements, and blood sample collection were conducted at clinic visits at baseline and at 6 and 12 months.

Intervention

Following randomization, participants were provided with a detailed diet prescription and sample meal plans in an individual counseling session with a dietitian, with follow-up at regular intervals by telephone or email to provide additional support and reinforce adherence. The overall goal of the dietary guidance was to promote a reduction in energy intake, aiming for a 500- to 1000-kcal/day deficit relative to expenditure, according to the individualized prescribed diet plan (1200, 1500, or 1800 kcal/day). Specific instructions for the lower fat diet were to choose lean protein sources and reduced-fat dairy foods and to emphasize vegetables, fruit, and whole grains as healthy high-carbohydrate choices. Participants assigned to the lower carbohydrate diet were educated about higher versus lower carbohydrate choices and lean protein sources and were instructed to achieve a high monounsaturated fat intake; examples and recipes were discussed.

Participants assigned to the walnut-rich study group were also educated about higher versus lower carbohydrate choices and lean protein sources. In addition, they were instructed to consume an average of 42 g (1.5 oz) of walnuts per day, within their energy-reduced diet plan, and were provided meal and snack suggestions and recipes to facilitate adherence. Walnuts were distributed to participants assigned to that group approximately every 2 weeks, and participants were instructed to record walnut consumption on a simple form. Diet prescriptions for participants assigned to the other 2 study groups excluded nuts.

All participants were encouraged to use Web-based tracking programs that guide dietary intake toward the prescribed macronutrient distribution. To facilitate self-monitoring, all participants were provided with a scale and a pedometer, to monitor weight and steps (aiming for >10 000 steps per day) on a daily basis, as well as measuring cups and exercise videos.

The group-based behavioral weight loss intervention involved an intensive 6-month intervention period during which participants met in closed group sessions weekly for the first 4 months, met biweekly for the following 2 months, and then met monthly for the remaining 6 months of the program. In addition to group meetings, participants had telephone and email counseling contacts with their group coleaders, who had backgrounds in dietetics, psychology, and/or exercise physiology, to individualize the experience by setting specific behavioral goals and monitoring progress.[17] In addition to reducing energy intake, the physical activity goal was an average of at least 60 min/day of purposeful exercise at a moderate level of intensity plus increased lifestyle activity. Strategies and approaches that were applied in this intervention included self-monitoring of food intake and exercise; setting realistic goals; using behavior-specific goals and a stepwise approach to progress to promote self-efficacy; addressing body image concerns; training and role playing in problem solving; preventing relapse; and modifying problematic thoughts and attitudes about weight, food, and physical activity.

Outcomes

At data collection clinic visits, weight and height (baseline only) were measured, body mass index (weight in kilograms/ height in Should be meters squared) was calculated, and questionnaires were collected by research staff. Fasting (≥6 hours) blood samples were collected at each clinic visit. Two-thirds of blood draws were in the early morning after an overnight fast, and 82% of participants had both fasting draws within ±3 hours of each other. Glucose, total cholesterol, triglycerides, and HDL-C were measured with the Kodak Ektachem Analyzer system (Johnson & Johnson Clinical Diagnostics). LDL-C values were calculated by the Friedewald equation.[18] The ADVIA Centaur assay (Siemens), a double-antibody immunoassay with chemiluminescent detection, was used for insulin quantification, and high-sensitivity C-reactive protein (CRP) was assayed using an electochemiluminiscence polystyrene-enhanced turbidimetric in vitro immunoassay (MesoScale Discovery) at the Laboratory for Clinical Biochemistry Research, University of Vermont. Accuracy and precision were monitored by the use of an in-house quality control pool and laboratory participation in the College of American Pathologists quality assurance program.

As a biomarker of adherence to the prescribed walnut intake, the fatty acid profile in red blood cells was determined using gas liquid chromatography methodology, conducted at the Wake Forest School of Medicine Lipid and Lipoprotein Analytic Laboratory. Briefly, the fatty acid moieties of the red blood cell lipids were converted to more volatile fatty acid methyl esters following saponification of an aliquot in ethanolic potassium hydroxide, removal of nonsaponifiables, and extraction of the acidified fatty acids into hexane. The fatty acid methyl esters were extracted into isooctane, then the isooctane was dried with sodium sulfate, and a sample was injected onto a gas liquid chromatography column. Each chromatogram was examined for correct identification of fatty acid peaks and quality control. Results were reported as the amount and percentage of each identified fatty acid.

Statistical Analysis

Baseline means and ranges for age and body mass index were summarized, along with baseline insulin resistance status and

self-reported race and ethnicity. Percentages of participants reporting any prescription medication for high cholesterol or any diagnosis of high cholesterol at study entry were tabulated.

Means and standard errors for levels of cholesterol, triglycerides, HDL-C, and LDL-C and proportions of participants with high cholesterol (≥200 mg/dL) at baseline and at 6 months for each study arm (and for all participants aggregated) were examined. In addition to the lipid analysis, CRP, insulin, and homeostasis model assessment were also examined. Analytes that did not conform to a Gaussian distribution (triglycerides, CRP) were log transformed in analysis. Mixed models were used to test differences between lipid levels by diet group assignment and time period and in a group–time interaction. Mixed models including baseline insulin resistance status (insulin sensitive versus insulin resistant) were then tested for differences between these fixed effects. Mixed models (our primary analysis method) included partitioned analysis of the least squares means for an interaction, which is equivalent to custom contrast statements comparing specific diets, study time, and insulin resistance status.

Multivariate regression models predicted 6-month levels of cholesterol, triglycerides, HDL-C, and LDL-C, and potentially associated factors were baseline level, baseline weight, percentage weight change at 6 months, age, diet group (reference was the lower carbohydrate, higher monounsaturated fat diet group), insulin resistance status, and self-reported prescription lipid-lowering medication.

Significance level was set at $\alpha=0.05$ for the primary mixed model analysis and at $\alpha=0.01$ for the regression models. No additional correction for multiple comparisons was used. All analyses were conducted in SAS version 9.4 (SAS Institute).

Results

The baseline characteristics of the participants are presented in Table 1. The study enrolled 245 overweight and obese women, and weight and lipid data were available at 6 months for 213 women. At enrollment, these women had a mean age of 50 years and a mean body mass index of 33.5. When categorized by insulin resistance status, 119 (48.6%) women were insulin sensitive and 126 (51.4%) were insulin resistant. Also at baseline, 56 (22.9%) of the 245 women self-reported that they had high cholesterol; however, only 29 (11.8%) of those women reported that they were taking a lipid-lowering medication.

At study entry, participants in each of the 3 diet arms had a mean percentage of red blood cell α-linolenic acid of 0.12% (SEM 0.01%). At 6 months, α-linolenic acid levels were unchanged in the lower fat and lower carbohydrate diet arms, but in women prescribed the walnut-rich diet, the percentage had increased to 0.17% (SEM 0.01%) α-linolenic acid ($P<0.01$). Baseline red blood cell linoleic acid averaged 13.3% (SEM 0.3%) in all participants, but at 6 months, the walnut-rich diet participants had a mean of 14.0% (SEM 0.3%) compared with lower fat diet participants (12.2% [SEM 0.3%], $P<0.01$) and lower carbohydrate diet participants (13.1% [0.3%], $P=0.03$). Group-by-time P value in a mixed model for the 2 biomarkers was $P<0.05$. Approximately 93% of participants assigned to the walnut-rich diet reported compliance with the regimen,

Table 1. Baseline Characteristics of Overweight and Obese Women Enrolled in the Weight Loss Study (N=245)

Variables	Mean (Range)	Lower Fat	Lower Carbohydrate	Walnut-Rich
Age, mean (range), y	50 (22–72)	50 (25–68)	50 (25–72)	51 (22–67)
Race/ethnicity, n (%)				
White non-Hispanic	181 (73.9)	60 (73.2)	63 (77.8)	58 (70.7)
Hispanic	42 (17.1)	16 (19.5)	11 (13.6)	15 (18.3)
Black	12 (4.9)	3 (3.7)	2 (2.5)	7 (8.5)
Asian American	4 (1.6)	0	3 (3.7)	1 (1.2)
Mixed or other	6 (2.5)	3 (3.7)	2 (2.5)	1 (1.2)
BMI, mean (range), kg/m²	33.5 (27–40)	33.2 (27–40)	33.6 (27–40)	33.6 (27–40)
Insulin resistance status, n (%)				
Insulin sensitive	119 (48.6)	39 (47.6)	39 (48.2)	41 (50.0)
Insulin resistant	126 (51.4)	43 (52.4)	42 (51.9)	41 (50.0)
Self-reporting cholesterol medication, n (%)	29 (11.8)	10 (12.2)	8 (9.9)	11 (13.4)
Self-reporting history of high cholesterol, n (%)	56 (22.9)	18 (22.0)	15 (18.5)	23 (28.1)

BMI indicates body mass index.

although there was some variability across the year of study participation because of illness, travel, and holidays.

At 6 months, average weight loss was 7.5% of initial weight in the total sample, and weight loss did not differ significantly across the diet groups. Percentage weight loss was 8.5% (SEM 0.7%), 6.3% (SEM 0.7%), and 7.5% (SEM 0.7%) for the lower fat, lower carbohydrate, and walnut-rich higher fat diets, respectively. Insulin-sensitive women assigned to the lower fat diet lost more weight than those assigned to the lower carbohydrate diet (8.3% [SEM 1.0%] versus 5.4% [1.0%], respectively) but not those assigned to the walnut-rich diet (7.9% [SEM 0.9%]; $P<0.05$). Differential degree of weight loss in the insulin-resistant women across the diet groups was not observed, averaging 8.7% (SEM 0.9%), 7.2% (SEM 1.0%), and 7.1% (SEM 1.1%) for the lower fat, lower carbohydrate, and walnut-rich diets, respectively. Overall, 37% of insulin-resistant women became insulin sensitive at 6 months (35% in lower fat, 35% in lower carbohydrate, and 40% in walnut-rich diet groups). CRP decreased in all diet groups (Table 2).

Table 2 shows the changes in lipid levels of the women from baseline to 6 months, grouped by diet composition assignment. At baseline, there were no differences in any of the lipid levels between the 3 study arms. At 6 months, there was a significant reduction in triglycerides compared with baseline in all diet groups ($P<0.05$), and a reduction in HDL-C level was observed in the lower fat diet group ($P<0.01$). A significant decrease in LDL-C was observed at 6 months in the lower fat ($P<0.05$) and walnut-rich ($P<0.01$) diet groups. There was also a significant reduction in total cholesterol in all 3 of the study groups at 6 months ($P<0.01$); however, only the walnut-rich diet group had a notable reduction in the percentage of participants with high cholesterol ($P<0.05$). The walnut-rich diet group increased HDL-C more than the lower fat diet group ($P<0.01$) and more than the lower carbohydrate diet group ($P<0.05$).

The lipid levels in response to diet composition categorized by insulin resistance status are presented in Table 3. At baseline, statistically significant differences were found in the level of triglycerides between insulin-resistant and insulin-sensitive participants within each study arm and all inclusively, regardless of study group assignment ($P<0.01$ for each). Women who were insulin resistant within the

Table 2. Lipid, Weight, and CRP Levels at Baseline and 6 Months

	Lower Fat	Lower Carbohydrate	Walnut-Rich	All
	Mean (SEM)	Mean (SEM)	Mean (SEM)	Mean (SEM)
Baseline, n	82	81	82	245
Triglycerides, mg/dL*	131 (8)	128 (7)	112 (5)	124 (4)
HDL-C, mg/dL	60 (2)	58 (2)	60 (2)	59 (1)
LDL-C, mg/dL	118 (4)	122 (3)	125 (4)	122 (2)
Total cholesterol, mg/dL	204 (4)	205 (4)	207 (4)	205 (2)
Percentage with high cholesterol	54 (6)	51 (6)	59 (5)	54 (3)
Weight, kg	89.7 (1.2)	90.0 (1.4)	90.0 (1.3)	89.9 (0.7)
BMI, kg/m^2	33.2 (0.3)	33.6 (0.4)	33.6 (0.4)	33.5 (0.2)
CRP, µg/mL*	5.04 (0.54)	5.21 (0.64)	4.36 (0.58)	4.86 (0.34)
6 months, n	76	66	71	213
Triglycerides, mg/dL*	118 (6)[†]	108 (5)[†]	97 (5)[†]	108 (3)[†]
HDL-C, mg/dL	57 (2)[‡]	57 (2)[‡]	63 (2)[‡]	59 (1)
LDL-C, mg/dL	111 (3)[†]	117 (4)	113 (4)[†]	114 (2)[†]
Total cholesterol, mg/dL	191 (4)[†]	196 (4)[†]	196 (4)[†]	194 (2)[†]
Percentage with high cholesterol	43 (6)	50 (6)	45 (6)[†]	46 (3)[†]
Weight, kg	82.0 (1.3)[†]	84.1 (1.8)[†]	83.0 (1.3)[†]	83.0 (0.9)[†]
BMI, kg/m^2	30.4 (0.4)[†]	31.4 (0.5)[†]	31.0 (0.4)[†]	30.9 (0.3)[†]
CRP, µg/mL*	3.95 (0.46)[†]	3.53 (0.44)[†]	3.42 (0.48)[†]	3.64 (0.27)[†]

BMI indicates body mass index, CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
*Untransformed means are shown, but values were log-transformed in analysis.
[†]$P<0.05$ within group compared with baseline, mixed model.
[‡]Significant group–time interaction in which the walnut-rich diet group increased HDL-C more than the lower fat diet group ($P<0.01$) and more than the lower carbohydrate diet group ($P<0.05$).

Table 3. Lipid, Weight, HOMA, and C-Reactive Protein Levels at Baseline and 6 Months by Insulin Resistance Status

	Lower Fat		Lower Carbohydrate		Walnut-Rich		All	
	IS	IR	IS	IR	IS	IR	IS	IR
Baseline								
n	39	43	39	42	41	41	119	126
Triglycerides, mg/dL*	107 (7)†	154 (14)†	109 (11)†	146 (9)†	89 (5)†	136 (7)†	101 (5)†	145 (6)†
HDL-C, mg/dL	64 (3)†	56 (2)†	61 (2)	55 (2)	64 (2)†	56 (2)†	63 (1)†	55 (1)†
LDL-C, mg/dL	115 (5)	121 (6)	120 (5)	125 (5)	123 (5)	126 (6)	119 (3)	124 (3)
Total cholesterol, mg/dL	201 (5)	206 (7)	202 (7)	208 (5)	205 (5)	208 (6)	203 (3)	207 (3)
Percentage with high cholesterol	51 (8)	56 (8)	46 (8)	55 (8)	63 (8)	54 (8)	54 (5)	55 (4)
Percentage using lipid medication	10 (5)	14 (5)	10 (5)	10 (5)	5 (3)†	22 (7)†	8 (3)	15 (3)
Insulin, μIU/mL	9 (0.4)†	18 (1)†	8 (1)†	22 (0.4)†	9 (0.3)†	19 (1)†	9 (0.2)†	20 (1)†
HOMA	2.2 (0.1)†	4.6 (0.3)†	2.0 (0.1)†	5.7 (0.4)†	2.2 (0.1)†	4.9 (0.3)†	2.1 (0.1)†	5.1 (0.2)†
Weight, kg	87 (2)†	92 (2)†	87 (2)†	92 (2)†	87 (2)	93 (2)	87 (1)†	93 (1)†
BMI, kg/m²	33 (0.4)	34 (1)	33 (1)†	34 (1)†	33 (1)†	35 (1)†	33 (0.3)†	34 (0.3)†
CRP, μg/mL*	4.3 (0.7)	5.7 (0.8)	3.0 (0.4)†	7.2 (1.1)†	3.6 (0.7)	5.1 (0.9)	3.7 (0.4)†	6.0 (0.5)†
6 Months								
n	36	40	35	31	36	35	107	106
Triglycerides, mg/dL*	100 (8)†	135 (9)†	95 (6)†	122 (8)†‡	82 (5)†	112 (9)†‡	92 (4)†	123 (5)†‡
HDL-C, mg/dL	60 (2)†‡	53 (2)†	60 (3)	54 (3)	69 (3)†	57 (2)†	63 (2)†	55 (1)†
LDL-C, mg/dL	108 (4)	114 (5)	113 (4)	123 (6)	108 (4)‡	119 (6)	110 (2)‡	118 (3)‡
Total cholesterol, mg/dL	189 (4)‡	194 (6)‡	192 (5)‡	200 (7)	193 (6)‡	198 (7)‡	191 (3)‡	197 (4)‡
Percentage with high cholesterol	36 (8)	50 (8)	46 (9)	55 (9)	36 (8)‡	54 (9)	39 (5)‡	53 (5)
Percentage using lipid medication	11 (5)	15 (6)	11 (5)	6 (4)	6 (4)†	23 (7)†	9 (3)	15 (3)
Insulin, μIU/mL	9 (1)†	14 (1)†‡	9 (1)†	16 (1)†‡	10 (1)†	17 (2)†	10 (0.4)†	16 (1)†‡
HOMA	2.1 (0.2)†	3.4 (0.2)†‡	2.1 (0.1)†	4.0 (0.4)†‡	2.3 (0.2)†	4.2 (0.5)†‡	2.2 (0.1)†	3.9 (0.2)†‡
Weight, kg	80 (2)‡	84 (2)‡	83 (3)‡	85 (2)‡	81 (2)‡	85 (2)‡	81 (1)‡	85 (1)‡
BMI, kg/m²	30 (1)‡	31 (1)‡	31 (1)‡	32 (1)‡	30 (1)‡	32 (1)‡	30 (0.4)‡	31 (0.4)‡
CRP, μg/mL*	3.7 (0.7)‡	4.2 (0.7)‡	2.9 (0.6)†	4.2 (0.6)†	2.7 (0.5)‡†	4.1 (0.8)†	3.1 (0.4)†	4.2 (0.4)†‡

Values shown are mean (SEM). BMI indicates body mass index, CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment; IR, insulin resistant; IS, insulin sensitive; LDL-C, low-density lipoprotein cholesterol.

*Untransformed means are shown, but values were log-transformed in analysis.

†IR different from IS within group and time ($P<0.05$), mixed model.

‡$P<0.05$ within group and insulin resistance status compared with baseline, mixed model.

lower fat and walnut-rich diet arms also differed in HDL-C, with significantly lower HDL-C ($P<0.01$). As expected, insulin differed by insulin resistance status ($P<0.05$) and declined significantly in insulin-resistant women. CRP generally declined in association with weight loss, also as expected.

At 6 months, there were significant decreases in triglycerides within all 3 diet arms in women who were insulin resistant ($P<0.05$). There was a significant decrease in HDL-C in insulin-sensitive women in the lower fat diet group ($P<0.05$). A significant decrease in LDL-C was observed only in the insulin-sensitive women of the walnut-rich diet arm ($P<0.01$). Total cholesterol decreased from baseline in all

groups except in the insulin-resistant women in the lower carbohydrate diet study arm ($P<0.05$).

The lower carbohydrate diet study arm was used as the reference group to examine different factors that were potentially associated with the lipid levels at 6 months (Table 4). The baseline levels were all significantly positively associated with the 6-month follow-up measurement ($P<0.01$), as expected. In addition, there was a significant association between percentage weight change and total cholesterol, triglycerides, and LDL-C ($P<0.01$ for each). Insulin resistance was also positively associated with triglyceride level at 6 months ($P<0.01$). Moreover, it should be noted that a positive relationship was seen between the lower fat diet

Table 4. Factors Associated With Lipid Levels at 6 Months

	Cholesterol R^2=0.53	HDL-C R^2=0.61	LDL-C R^2=0.50	Triglycerides R^2=0.41
Baseline level	0.629*	0.804*	0.581*	0.380*
Baseline weight	0.003	−0.019	0.011	−0.038
Percentage change in weight	1.113*	−0.075	0.751*	2.129*
Age	0.292	−0.011	0.233	0.357
Diet composition				
Lower fat	−1.642	−3.135	−2.446	13.377[†]
Walnut-rich	−0.598	2.277	−3.912	−3.498
Lipid-lowering medication use	0.590	2.739	−5.396	−4.277
Insulin resistance status	3.885	−0.943	5.827	14.947*

Values shown are β coefficients in a regression model for each lipid factor at 6-month follow-up. Each model included baseline level of the analyte, baseline weight, percentage change in weight, age, diet composition, use of lipid-lowering medications, and insulin resistance status, with no additional adjustment factors. HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
*$P \leq 0.01$.
[†]P=0.04.

assignment and triglyceride level (P=0.04); however, this did not meet the multiple comparisons threshold of $P<0.01$ for significance.

Discussion

Overall weight loss in the behavioral weight loss intervention was similar across the diet groups, although women who were insulin sensitive lost more weight with a lower fat diet than a lower carbohydrate diet. Weight loss was generally associated with an improvement in the lipid profile, but there were differences depending on insulin resistance status and diet composition. The walnut-rich diet resulted in the most favorable changes in lipid levels while promoting a degree of weight loss that was comparable to the lower fat diet.

Differential weight loss in response to diet composition in relation to insulin resistance status has been observed in 2 previous studies.[10,19] In those studies, insulin sensitivity was associated with more weight loss in response to a lower fat (versus lower carbohydrate) diet, and insulin resistance was associated with more weight loss in response to a lower carbohydrate (versus lower fat) diet. We observed differential weight loss in the insulin-sensitive but not the insulin-resistant women, although the lipid response to differential diet composition was more marked among those who were insulin resistant. Furthermore, insulin-resistant women in all diet groups demonstrated significant improvement in their homeostasis model assessment levels.

Obesity and overweight, associated with excess adipose tissue, result from energy intake that exceeds expenditure. Excessive accumulation of adipose tissue can lead to inflammatory responses that can upregulate the release of free fatty acids into the blood, resulting in increased triglyceride levels, especially in those with insulin resistance.[20,21] These results suggest that weight loss promoted by reduced energy intake can potentially have positive influences on triglyceride levels for women who are insulin resistant, given that the women lost weight and, presumably, reduced central fat deposits.

Women who were insulin resistant, but not those who were insulin sensitive, had a reduction in triglyceride levels by 6 months in all 3 diet groups. These findings are consistent with current understanding of insulin resistance, in which the impaired ability to manage glucose leads to a higher level of triglycerides in response to carbohydrate consumption. McLaughlin et al suggested that when laboratory data for insulin concentration are not readily available, quantifying plasma triglyceride levels would be a practical option for identifying insulin resistance in overweight persons.[22] The beneficial effects of the walnut-rich diet on lipids were especially pronounced for the insulin-resistant women.

Regular consumption of nuts has been consistently associated with reduced risk of cardiovascular disease.[23] Compared with most nuts, which contain monounsaturated fatty acids, walnuts are a rich source of polyunsaturated fatty acids, particularly the ω-3 fatty acid, α-linolenic acid, and ω-6 linoleic acid. Walnuts also provide several bioactive constituents that may exert anti-inflammatory effects, such as antioxidants (tocopherols, phenolic compounds, and ellagic acid).[24] Several studies have observed decreased inflammatory markers with the consumption of walnuts.[25–27] The additional anti-inflammatory properties of walnuts provide potential benefits in addition to effects on lipids.

A meta-analysis conducted by Yanai et al found that HDL-C increased when carbohydrates were substituted by fatty acids, and the reverse was also observed.[12] Our findings support those prior reports. Increased HDL-C in response to the walnut-rich diet in this study may result from the high polyunsaturated fat content of walnuts. HDL-C increased in the walnut-rich study arm, and the effect was most substantial in women who were insulin sensitive. An established inverse relationship between HDL-C and triglycerides[28] could suggest that the higher levels of triglycerides found in women who were insulin resistant may have attenuated the response of HDL-C to the walnut-rich diet.

Women in both the lower fat and walnut-rich diet groups demonstrated a significant reduction in LDL-C, particularly for insulin-sensitive women in the walnut-rich diet group. There have been consistent findings of decreased LDL-C in both low- and modified-fat diets in numerous studies.[16,29,30] All 3 diets examined in this study were low in saturated fat; therefore, the decrease in LDL-C was consistent with the relationship between saturated fat intake and LDL-C. Although it was observed that LDL-C was reduced with each of these diet compositions, a study conducted by Ros et al found greater reduction in LDL-C in response to a walnut-rich diet compared with a Mediterranean diet.[16] Antioxidants from walnuts also may help reduce the amount of oxidized LDL-C, suggesting additional potential benefits beyond effects on lipid levels.

Strengths of this study included its randomized design and its in-depth comparison of lipid levels in response to diet composition with further analysis based on insulin resistance status. This lipid study was an analysis within a weight loss intervention study, and the statistical power assessed for the project was determined for weight loss and not changes in lipid levels. In addition, although 245 women were enrolled, when participants were divided into individual diet groups and further stratified by insulin resistance status, statistical power was reduced because of small sample sizes in the subgroups that were compared. Further investigation with power assessed specifically for lipid analysis should be considered for future studies. Another limitation is the lack of assessment of lipoprotein particle size, known to be associated with cardiovascular risk and responsive to dietary intervention. The sample consisted of women, so these results may not be generalizable to men.

Finally, a limitation is the lack of detailed information about dietary intake and specific adherence to the prescribed diets. The target was a free-living population, so variability in adherence is likely. Self-reported dietary data have well-recognized limitations of accuracy, characterized as substantial underreporting and misreporting among overweight and obese persons. An implication of this limitation is that it is not known whether the differential responses among those assigned to the various diets may be due to differential adherence. Given the weight loss demonstrated by most study participants, we can assume that most were adhering to a reduced-energy diet. In addition, changes in the dietary biomarkers (red blood cell fatty acids) indicate good adherence in the walnut-rich diet group, as observed in previous walnut feeding and walnut-rich diet intervention studies.[9,16]

In conclusion, results from this study provide better understanding of lipid response to diet composition in consideration of insulin resistance status. Weight loss in the behavioral weight loss intervention was comparable across lower fat, higher carbohydrate and walnut-rich diets. Weight loss was generally associated with an improvement in the lipid profile but was influenced by insulin resistance status and diet composition. Decreased CRP was observed in all diet groups as well as improved insulin sensitivity in insulin-resistant women. The walnut-rich diet resulted in the most favorable changes in lipid levels while still associated with a degree of weight loss that was comparable to the lower fat diet.

Disclosures

None.

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