Available at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

A Mediterranean-style, low–glycemic-load diet decreases atherogenic lipoproteins and reduces lipoprotein (a) and oxidized low-density lipoprotein in women with metabolic syndrome

Jennifer L. Jones[a], *Michael Comperatore*[a], *Jacqueline Barona*[a], *Mariana C. Calle*[a], *Catherine Andersen*[a], *Mark McIntosh*[b], *Wadie Najm*[c], *Robert H. Lerman*[d], *Maria Luz Fernandez*[a,*]

[a] *Department of Nutritional Sciences, University of Connecticut, Storrs, CT 06269, USA*
[b] *Department of Emergency Medicine, University of Florida, Jacksonville, FL, USA*
[c] *Department of Family Medicine, University of California Irvine, Irvine, CA, USA*
[d] *Metagenics Inc, Gig Harbor, WA, USA*

ARTICLE INFO

Article history:
Received 15 April 2011
Accepted 26 July 2011

ABSTRACT

The objective was to assess the impact of a Mediterranean-style, low–glycemic-load diet (control group, n = 41) and the same diet plus a medical food (MF) containing phytosterols, soy protein, and extracts from hops and *Acacia* (MF group, n = 42) on lipoprotein atherogenicity in women with metabolic syndrome. Plasma lipids, apolipoproteins (apos), lipoprotein subfractions and particle size, low-density lipoprotein (LDL) oxidation, and lipoprotein (a) were measured at baseline, week 8, and week 12 of the intervention. Three-day dietary records were collected at the same time points to assess compliance. Compared with baseline, women decreased energy intake from carbohydrate ($P < .001$) and fat ($P < .001$), whereas they increased energy intake from protein ($P < .001$). A significant increase in energy from monounsaturated fatty acids was also observed as well as increases in eicosapentaenoic acid and docosahexaenoic acid, whereas *trans*-fatty acid intake was reduced ($P < .00001$). The atherogenic lipoproteins, large very low-density lipoprotein ($P < .0001$) and small LDL ($P < .0001$), were reduced, whereas the ratio of large high-density lipoprotein to smaller high-density lipoprotein particles was increased ($P < .0001$). Apolipoprotein B was reduced for all women ($P < .0001$), with a greater reduction in the MF group ($P < .025$). Oxidized LDL ($P < .05$) and lipoprotein (a) ($P < .001$) were reduced in both groups at the end of the intervention. Consumption of a Mediterranean-style diet reduces the risk for cardiovascular disease by decreasing atherogenic lipoproteins, oxidized LDL, and apo B. Inclusion of an MF may have an additional effect in reducing apo B.

This trial was registered at clinicaltrials.gov as NCT01010841.

Author contributions: JLJ participated in data collection and interpretation and drafting of the manuscript. MLF evaluated the results, interpreted the data, and participated in manuscript preparation. MC, JB, MCC, CA, MM, and WN assisted in subject recruitment and data collection. RHL participated in experimental design and data interpretation. All authors read and approved the final manuscript.

* *Corresponding author*. Tel.: +1 860 486 5547; fax: +1 860 486 3674.
E-mail address: maria-luz.fernandez@uconn.edu (M.L. Fernandez).

0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.07.013

1. Introduction

The prevalence of overweight and obesity is increasing persistently. Currently, more than two thirds of adults in the United States are overweight or obese [1]. These characteristics are associated with multiple cardiometabolic risk factors that lead to the development of metabolic syndrome (MetS). *Metabolic syndrome* is defined as a clustering of abnormalities that include visceral adiposity, elevated blood pressure and fasting plasma glucose, and atherogenic dyslipidemia (reduced high-density lipoprotein cholesterol [HDL-C] and elevated plasma triglycerides [TG]) [2]. Metabolic syndrome exists as a constellation of metabolic disturbances with both genetic and environmental factors contributing to its pathogenesis [2,3].

A MetS classification is associated with a nearly 5-fold increase in the risk for development of type 2 diabetes mellitus and a 2-fold increase in the risk for development of cardiovascular disease (CVD) [3]. Modification of lifestyle is the first line of therapy to amend abnormalities related to MetS, with dietary intervention as a primary focus [4]. Controversy exists regarding the ideal dietary pattern to ameliorate MetS characteristics; but the Mediterranean-style dietary pattern has gained substantial consideration in this area, with epidemiological and experimental evidence to support its benefits [5-7]. The observed cardioprotective advantages can be attributed to the overall dietary pattern that encourages consumption of vegetables, legumes, fruits, fish, olive oil, and whole grains, providing monounsaturated fatty acids (MUFA), omega-3 fatty acids, dietary fiber, antioxidants, and phytosterols [8].

Many investigators have concentrated on the impact of a Mediterranean-style diet on specific MetS characteristics, but the potential dietary influence on emerging risk factors for CVD in the context of MetS has been less explored. To reduce risk for CVD, the primary lipid target is low-density lipoprotein cholesterol (LDL-C); but other viable targets have recently been used for risk evaluation and treatment. Apolipoprotein (apo) B in risk assessment indicates the total number of potentially atherogenic particles, including very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and lipoprotein (a) (Lp[a]), all of which contain one molecule of apo B-100 [9]. Elevated concentrations of other apos, namely, apo E and apo C-III, have been associated with the dyslipidemia observed in MetS [10,11].

In addition, analysis of LDL subpopulations may be valuable because the concentration of small, dense LDL particles serves as a better marker of risk than LDL-C [12,13]. A detailed examination of VLDL and HDL particle size distribution is also informative because higher numbers of both large VLDL and small HDL particles are suggestive of a more atherogenic lipoprotein profile [14,15]. High levels of oxidized LDL (oxLDL) and Lp(a) are other components of the abnormal lipoprotein profile associated with CVD, which merit additional study in individuals with MetS [16,17].

Previously, we evaluated the effect of a Mediterranean-style, low–glycemic-load diet (with or without a phytonutrient-rich medical food [MF]) in a 2-arm randomized trial and reported significant reductions of MetS variables in overweight and obese women with MetS [18]. The objective of the present study was to examine the impact of this diet on lipoprotein metabolism, specifically on atherogenic lipoprotein subfractions including oxLDL and Lp (a), lipoprotein particle size, and apo concentrations in this population. Furthermore, we aimed to determine if the diet supplemented with an MF containing soy protein, phytosterols, ρ iso-α acids, and *Acacia* proanthocyanidins provided additional cardiovascular benefits. We hypothesized that all women would present reductions in atherogenic lipoproteins and risk factors for CVD at the conclusion of the intervention and that the women consuming the MF would experience greater improvements.

2. Methods

2.1. Materials

Apolipoprotein kits were ordered from Millipore (Billerica, MA). The oxLDL kit was purchased from Alpco Diagnostics (Salem, NH), and the Lp(a) kit was from Cortex Diagnostics (Calabasas, CA). The MF, UltraMeal Plus 360°, in powdered beverage form, was provided by Metagenics (Gig Harbor, WA).

2.2. Study design and randomization

After approval from the Institutional Review Board at each site (University of Connecticut, Storrs; University of Florida, Jacksonville; and University of California, Irvine), 89 women, ages 20 to 75 years, were recruited. A total number of 83 subjects completed the dietary intervention. Participants were randomly assigned to the control group (n = 41) or the MF group (n = 42). The inclusion criteria were body mass index between 25 and 40 kg/m^2, LDL-C of at least 100 mg/dL, TG of at least 150 mg/dL, and 2 of the 4 remaining criteria for MetS according to the National Cholesterol Education Program–Adult Treatment Panel III definition [19]. Exclusion criteria included the use of hypoglycemic or cholesterol-lowering agents, and the existence of kidney, liver, or heart disease. Women in the control group were instructed to follow a modified Mediterranean-style, low–glycemic-load diet for the duration of the 12-week intervention, whereas those in the MF group were asked to consume the same diet plus the MF twice each day for 12 weeks. Study participants were brought on-site for visits at baseline and weeks 2, 4, 6, 8, 10, and 12. To measure compliance of MF intake, the unused portions were weighed at biweekly intervals. Details regarding diet and exercise have been reported elsewhere [18]. Dietary counseling was provided at each visit by a registered dietitian, following the dietary approach outlined by Schiltz et al [20].

2.3. Blood collection

At baseline, week 8, and week 12, 60 mL of blood was collected from each subject into EDTA tubes following a 12-hour overnight fast. Plasma was separated by centrifugation at 2000*g* for 20 minutes at 4°C, aliquoted to cryotubes, and stored at –80°C until analyses were performed.

2.4. Lipoprotein subfractions and particle size

Nuclear magnetic resonance was conducted by LipoScience (Raleigh, NC). A 400-MHz nuclear magnetic resonance analyzer was used to quantify lipoprotein subclasses based on particle diameters: large VLDL (35-60 nm), medium VLDL (27-35 nm), small VLDL (23-27 nm), large LDL (21.2-23 nm), medium LDL (19.8-21.2 nm), small LDL (18-19.8 nm), large HDL (8.8-13 nm), medium HDL (8.2-8.8 nm), and small HDL (7.3-8.2 nm). The weighted average particle size (diameter) was determined by multiplication of the diameter of each lipoprotein subclass by its relative concentration.

2.5. Plasma apos

Plasma concentrations of apo A-I and apo B were analyzed by Northwest Lipid Research Laboratories (Seattle, WA). Apolipoproteins A-II, C-II, C-III, and E were measured in duplicate using fasting plasma and xMAP technology on the Luminex IS 200 system (Austin, TX) with antibodies specific to the individual apos (Milliplex Human Apolipoprotein Panel) obtained from Millipore (Billerica, MA) [21].

2.6. Plasma oxLDL and plasma Lp(a)

Plasma oxLDL and plasma Lp(a) were measured using a solid-phase capture sandwich enzyme-linked immunosorbent assay. The microwells to which the samples and standards were added were coated either with anti-oxLDL antibodies or anti-Lp(a) antibodies. Reactions were carried out, and the absorbance for each well was read on a spectrophotometer (Spectramax Multimode Spectrophotometer, Sunnyvale, CA) at 450 nm.

2.7. Nutrient analysis

Dietary intake was analyzed at 4 time points (baseline, week 2, week 8, and week 12) using the Nutritional Data System for Research 8.0 (Minneapolis, MN). Participants kept 3-day dietary records, and the diets were analyzed for intakes (absolute and percentage of total energy) of the macronutrients and fatty acids.

2.8. Statistical analysis

Repeated-measures analysis of variance (ANOVA) was used to determine *diet* and *time* effects on dietary intake and the different biomarkers. Each individual's response to the intervention over time was the repeated measure (baseline, week 8, and week 12), and the diet interventions (control vs MF) were the between-subject factors. Post hoc tests were used to determine interactive effects. Statistical analyses were performed on SPSS (Chicago, IL) version 14.0 for Windows; and significance was placed on a P value < .05, with data values presented as mean ± SD. Initial body weight, waist circumference (WC), and plasma LDL were used as covariates to determine differences in apo B between control and MF groups.

3. Results

3.1. Energy intake

Significant changes in macronutrient intake were observed over the course of the intervention. The percentage of energy intake from carbohydrate (CHO) decreased over time, with no differences between groups. There was an interactive effect in the consumption of fat; participants in the control group consumed a higher amount of fat as a percentage of total energy (P < .05, Table 1). Conversely, energy intake from protein increased in both groups (P < .001). Percentage energy intake from saturated fat decreased in both groups, whereas that from MUFA increased (P < .001, Table 1). Intakes of *trans*-fatty acids (TFA) decreased in both groups (P < .001). In contrast, intake of the omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) increased over time (P < .0001, Table 2). In summary, no differences were found between groups in diet except for total fat that was higher for the control group.

3.2. Plasma lipids and atherogenic lipoproteins

Changes in plasma lipids have been previously reported [18]. Plasma LDL-C was reduced both in the control group (132.9 ±

Table 1 – Energy intake from CHO, protein, total fat, SFA, MUFA, and PUFA at baseline, week 8, and week 12 of women allocated to the MF group (n = 42) or the control group (n = 41)

Nutrient (% energy)	MF arm				Control arm				Time effect (P value)
	Baseline	Week 2	Week 8	Week 12	Baseline	Week 2	Week 8	Week 12	
CHO	44.8 ± 10.3	43.9 ± 9.7	40.4 ± 8.1[a]	41.1 ± 7.8[a]	43.2 ± 8.6	36.2 ± 9.0[a]	36.0 ± 9.6[a]	36.3 ± 8.9[a]	<.001
Protein	16.6 ± 4.7	26.3 ± 5.7[a]	25.4 ± 4.2[a]	25.3 ± 4.2[a]	16.3 ± 3.8	24.3 ± 5.6[a]	24.1 ± 5.6[a]	25.2 ± 5.4[a]	<.001
Fat[b]	36.1 ± 8.6	36.1 ± 8.6	33.7 ± 8.5[a]	32.5 ± 7.8[a]	38.4 ± 6.6	37.5 ± 8.2	37.3 ± 7.4	37.7 ± 8.2	<.001
SFA	12.3 ± 3.2	9.6 ± 3.4[a]	9.5 ± 2.5[a]	10.3 ± 1.9[a]	12.8 ± 2.8	10.1 ± 3.0[a]	10.6 ± 2.5[a]	10.3 ± 3.0[a]	<.001
MUFA	13.6 ± 4.1	14.4 ± 5.1[a]	16.4 ± 5.8[a]	15.5 ± 5.7[a]	14.2 ± 3.4	16.1 ± 5.1[a]	15.7 ± 4.2[a]	15.4 ± 4.1[a]	<.001
PUFA	7.3 ± 2.7	7.4 ± 3.3	8.7 ± 2.2	8.1 ± 3.2	8.2 ± 3.2	8.1 ± 2.2	7.6 ± 2.5	8.7 ± 3.5	NS

Numbers are expressed as mean ± SD for women in the MF and control groups. NS indicates nonsignificant.
[a] Indicates significantly different from baseline (P < .001) as determined by repeated-measures ANOVA.
[b] Subjects in the MF group consumed less fat as percentage energy at week 12. No diet or interactive effects were found for the other measured parameters.

Table 2 – Intake of TFA, EPA, and DHA at baseline, week 8, and week 12 of women allocated to the MF group (n = 42) or the control group (n = 41)

Nutrient (g/d)	MF arm				Control arm				Time effect (P value)
	Baseline	Week 2	Week 8	Week 12	Baseline	Week 2	Week 8	Week 12	<.0001
TFA	5.1 ± 3.2	1.4 ± 1.0[a]	1.6 ± 1.4[a]	1.4 ± 1.0[a]	5.3 ± 3.5[a]	1.7 ± 1.0[a]	1.8 ± 1.5[a]	1.5 ± 1.2[a]	<.0001
EPA	0.057 ± 0.093	0.090 ± 0.109[a]	0.096 ± 0.118[a]	0.109 ± 0.164[a]	0.034 ± 0.079	0.101 ± 0.122[a]	0.086 ± 0.100[a]	0.093 ± 0.091[a]	<.0001
DHA	0.094 ± 0.116	0.160 ± 0.168[a]	0.236 ± 0.393[a]	0.257 ± 0.340[a]	0.072 ± 0.115	0.198 ± 0.274[a]	0.183 ± 0.287[a]	0.232 ± 0.283[a]	<.0001

Numbers are expressed as mean ± SD for women in the MF and control groups. No diet or interactive effects were found for any of the measured parameters.
[a] Indicates significantly different from baseline ($P < .001$) as determined by repeated-measures ANOVA.

28.4 to 121.0 ± 27.9 mg/dL) and the MF group (135.6 ± 27.6 to 112.6 ± 28.4 mg/dL) ($P < .0001$), with a greater reduction in the MF group ($P < .05$). Similarly, plasma TG were reduced in the control group (193.8 ± 99.6 to 166.5 ± 86.3 mg/dL) and the MF group (179.5 ± 96.0 to 137.4 ± 60.8 mg/dL) ($P < .0001$). Plasma HDL-C concentrations were not different between groups, nor did they change between baseline and 12 weeks post-intervention (data not shown).

Decreases in large and small VLDL were observed for both groups ($P < .001$, Table 3), with no changes in medium VLDL (data not shown). Intermediate-density lipoprotein also decreased ($P < .001$, Table 3), with no difference found between the 2 groups. Finally, medium and small LDL decreased for both groups ($P < .001$, Table 3), whereas no changes were noted for large LDL (data not shown). No significant changes were found for large or small HDL in either group (data not shown), but there was a decrease in medium HDL in both groups ($P < .0001$, Table 3). Furthermore, the ratio of large HDL to medium + small HDL increased in both groups ($P < .05$); however, no differences were noted between groups.

3.3. *Plasma apos, oxLDL, Lp(a), and LDL size*

Plasma apos A-I, A-II, C-III, and E decreased in both groups from baseline to week 12 ($P < .001$, Table 4). Apolipoprotein B also decreased in both groups ($P < .0001$, Table 4); however, there was an interactive effect for this parameter because the degree of reduction was greater in the MF group. There was a 9% reduction in apo B from baseline to week 12, whereas there was a 17% reduction in apo B from baseline to week 12 ($P < .025$). The difference in changes in apo B persisted in the MF group after adjusting for initial body weight, LDL-C, and WC. No changes were observed for apo C-II (data not shown).

Compared with baseline, the mean concentration of oxLDL was decreased in week 8 for the control group and for both groups at week 12 ($P < .005$, Table 4). Lipoprotein (a) concentrations were lower at weeks 8 and 12 compared with baseline for both groups ($P < .001$, Table 4). Differences between groups were not significant. The mean size of LDL particles increased in both groups at weeks 8 and 12 when compared with baseline ($P < .005$, Table 4), with no differences between groups.

4. Discussion

A Mediterranean-style diet has been proposed as an option for dietary treatment of MetS components. Here we investigate whether this diet affects other risk factors for CVD, including atherogenic lipoproteins and oxLDL.

High glycemic load as a result of excess refined CHO consumption has been associated with increased CVD risk [22]. An association between high–glycemic-load dietary patterns and unfavorable lipoprotein and lipid profiles in

Table 3 – Atherogenic lipoproteins at baseline, week 8, and week 12 of women allocated to the MF group (n = 42) or the control group (n = 41)

Particle (mmol/L)	MF arm			Control arm			Time effect (P value)
	Baseline	Week 8	Week 12	Baseline	Week 8	Week 12	<.005
Large VLDL	7.3 ± 7.5	2.4 ± 2.9[a]	3.0 ± 4.4[a]	6.8 ± 6.2	3.4 ± 3.5[a]	3.8 ± 4.5[a]	<.005
Small VLDL	50.2 ± 18.5	44.9 ± 15.3[a]	46.1 ± 19.4[a]	58.5 ± 21.0	49.7 ± 19.4[a]	52.7 ± 16.8[a]	<.005
IDL	73.1 ± 52.2	54.3 ± 50.1[a]	51.6 ± 43.7[a]	86.7 ± 53.0	60.4 ± 59.9[a]	57.7 ± 52.1[a]	<.005
Medium LDL	251 ± 87	195 ± 76[a]	191 ± 80[a]	248 ± 87	205 ± 89[a]	210 ± 91[a]	<.005
Small LDL	949 ± 328	763 ± 314[a]	751 ± 331[a]	954 ± 382	796 ± 350[a]	811 ± 351[a]	<.005
Medium HDL (mmol/L)	5.68 ± 4.29	4.06 ± 3.62[a]	4.67 ± 4.51[a]	7.26 ± 5.51	4.10 ± 4.24[a]	4.49 ± 3.89[a]	<.005
Large HDL/medium + small HDL	0.193 ± 0.122	0.208 ± 0.110[a]	0.220 ± 0.130[a]	0.200 ± 0.127	0.215 ± 0.125[a]	0.220 ± 0.132[a]	<.005

Numbers are expressed as mean ± SD for women in the MF and control groups. No diet or interactive effects were found for any of the measured parameters.
[a] Indicates significantly different from baseline ($P < .005$) as determined by repeated-measures ANOVA.

Table 4 – Apolipoproteins, LDL size, Lp(a), and oxLDL at baseline, week 8, and week 12 of women allocated to the MF group (n = 42) or the control group (n = 41)

Apo (mg/L)	MF arm			Control arm			Time effect (P value)
	Baseline	Week 8	Week 12	Baseline	Week 8	Week 12	
Apo A-I	1487 ± 268	1349 ± 222 [a]	1391 ± 240 [a]	1562 ± 220	1473 ± 189 [a]	1484 ± 207 [a]	<.005
Apo A-II	184 ± 72	177 ± 80 [a]	161 ± 58 [a]	196 ± 70	175 ± 72 [a]	175 ± 63 [a]	<.005
Apo B [b]	1200 ± 213	985 ± 217 [a]	998 ± 203 [a]	1188 ± 221	1065 ± 257 [a]	1086 ± 239 [a]	<.005
Apo C-III	261 ± 123	223 ± 110 [a]	227 ± 92 [a]	271 ± 107	237 ± 110 [a]	231 ± 89 [a]	<.005
Apo E	68 ± 23	60 ± 18 [a]	59 ± 16 [a]	76 ± 35	68 ± 31 [a]	67 ± 26 [a]	<.005
LDL diameter (nm)	20.46 ± 0.68	20.59 ± 0.69 [a]	20.65 ± 0.77 [a]	20.52 ± 0.72	20.66 ± 0.74 [a]	20.67 ± 0.76 [a]	<.005
oxLDL (ng/mL)	105.7 ± 93.0	108.1 ± 99.1	98.3 ± 98.4 [a]	133.1 ± 100.6	122.3 ± 90.0 [a]	124.8 ± 97.7 [a]	<.005
Lp(a) (mg/dL)	24.0 ± 23.0	17.3 ± 15.1 [a]	10.4 ± 6.0 [a]	20.1 ± 19.7	14.5 ± 10.7 [a]	10.7 ± 5.9 [a]	<.005

Numbers are expressed as mean ± SD for women in the MF and control groups.
[a] Indicates significantly different from baseline ($P < .005$) as determined by repeated-measures ANOVA.
[b] The decrease in apo B was greater for the MF group before and after controlling for initial body weight, plasma apo B, and WC ($P < .025$). Data are not Bonferroni corrected. No other diet or interactive effects were found for any of the other measured parameters.

postmenopausal white women has been reported [23]. Other studies have observed that lower glycemic load reduces the inflammatory profile [24] and that glycemic load is more effective than a low-fat diet in subjects with MetS [25]. Moreover, a meta-analysis revealed that a Mediterranean-style dietary pattern is associated with improvements in MetS parameters and a reduction in MetS prevalence and progression [26]. In the present study, analysis of dietary records revealed compliance with the dietary prescription, as evidenced by the reduction in total energy intake from CHO and fat and in saturated fatty acids (SFA) and TFA.

Findings from controlled clinical trials and epidemiological studies have suggested that replacement of SFA with PUFA or MUFA may be a more efficient means of reducing CVD risk than reduction of total fat consumption [27]. In our participants, mean SFA intake decreased, whereas mean PUFA intake increased, after the 12-week intervention, in agreement with dietary recommendations. Dietary MUFA, a staple in the Mediterranean-style diet, have been shown to improve insulin sensitivity and glucose regulation, blood pressure, and plasma lipids [28,29]. Another important dietary change was the observed increases in EPA and DHA. It is possible that the increased intake of these fatty acids could have influenced the changes observed in VLDL size and number as a response to reductions in TG [30-32].

Significant reductions were also seen in small VLDL, IDL, and medium LDL of our study participants. Large VLDL particles are considered atherogenic because they can be taken up by macrophages in the arterial wall and they can be converted to small, dense LDL particles [33], which are also atherogenic because of their lower binding affinity for the LDL receptor, their prolonged plasma residency time, and their increased penetration into the arterial wall when compared with LDL particles of larger size [34-36]. Smaller LDL particles may also have a higher susceptibility to oxidation and a lower antioxidant carrying capacity [37]. Adherence to the dietary pattern proposed by this study resulted in a decrease in CHO intake, which could account for the observed reductions in oxLDL and the increase in mean LDL size [38].

High-density lipoprotein, generally considered an antiatherogenic particle, may lose some of its atheroprotective potential with a decrease in particle size [15]. In a nested case-control study, in which case subjects developed CVD during follow-up, a significant, inverse relationship between HDL size and CVD risk was reported [39]. Data from de Souza et al [40] suggested that, in the context of MetS, small HDL particles may be less protective. In this intervention, we did not observe a change in HDL-C [18]; however, we found a decrease in the medium HDL particles and an increase in the ratio of large HDL to medium + small HDL particles, suggestive of more atheroprotective HDL particles.

Following the 12-week intervention, substantial decreases were observed in both apo C-III and apo E. This paralleled a decrease in plasma TG [18], which may suggest that inhibition/displacement of apo C-II by these apos was reduced. Increased concentrations of apo E have also been associated with MetS [41]. Reductions in another atherogenic apo, apo B, a known marker for CVD risk [42], were also observed in response to Mediterranean diet. This decrease was greater in the MF group; but because no Bonferroni adjustments were performed, these data remain to be confirmed by future studies. These findings may be related to the high concentration of plant sterols in the MF (2000 mg per serving), which was previously shown to also have greater decreases in LDL-C [18]. Significant reductions were also noted for apo A-I and apo A-II that could be related to decreases in the medium-sized HDL. Apolipoprotein A-I is considered atheroprotective because of its role in reverse cholesterol transport via activation of lecithin-cholesterol acyltransferase. Whereas decreases in apo A-I are not considered desirable, decreases in apo A-II have been shown to exert atheroprotective effects [43].

Two emerging risk factors of CVD, oxLDL and Lp(a), were ameliorated by consumption of a Mediterranean-style, low–glycemic-load diet. A marker of oxidative stress associated with lipoproteins, oxLDL activates monocytes, facilitating their infiltration of the vascular wall [17]. In the Coronary Artery Risk Development in Young Adults (CARDIA) study, elevated oxLDL, but not higher LDL-C, was associated with increased incidence of MetS [44]. It is possible that, in our study population, the change in dietary habits provided sufficient antioxidants to interfere with lipid peroxidation of the LDL particles [45,46]. Elevated Lp(a) is a risk factor for atherosclerosis and thrombosis [47,48]. Lifestyle modifications

do not typically produce changes in Lp(a); however, reductions in TFA intake have been shown to reduce Lp(a) concentrations [49], which may explain the decreases observed in this study.

Although many researchers have reported benefits of a Mediterranean diet on lipid profiles in people with MetS, we aimed to provide a broad summary of the potential benefits that this dietary pattern can provide beyond basic lipid measurements. A strength of this study is the comprehensive evaluation of risk factors associated with increased risk of heart disease including apos, lipoprotein subfractions and size, oxLDL, and Lp(a). The results from our study suggest that a Mediterranean-style, low–glycemic-load diet alone has great potential in reducing not only traditional but also other emergent risk factors for CVD. Inclusion of the MF can have additional benefits on apo B, a well-known risk factor for heart disease, although this needs to be conclusively demonstrated in future studies. One limitation of the study is that the participants were women and mostly white. Additional studies using other populations need to be conducted to confirm our findings.

Funding

This study was funded by Metagenics, Gig Harbor, WA.

Conflict of Interest

MLF, MM, and WN received funds to conduct the study. RHL is employed by Metagenics. No other conflicts of interest exist for the remaining authors.

REFERENCES

[1] Flegal KM, Carroll MD, Ogden CL, et al. Prevalence and trends in obesity among us adults, 1999-2008. JAMA 2010;303:235-41.
[2] Grundy SM, Brewer Jr HB, Cleeman JI, et al. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.
[3] Lyssenko V, Sjögren M, Almgren P, et al. Genetic prediction of the metabolic syndrome. Diabetes Metab Syndr 2008;2:245-52.
[4] Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;112:2735-52.
[5] Djoussé L, Padilla H, Nelson TL, et al. Diet and metabolic syndrome. Endocr Metab Immune Disord Drug Targets 2010;10:124-37.
[6] Karampola M, Papandreou D, Makedou K. The role of Mediterranean diet in health and disease: an updated mini review. Nutr Food Sci 2011;41:63-72.
[7] Babio N, Bulló M, Basora J, et al. Adherence to the Mediterranean diet and risk of metabolic syndrome and its components. Nutr Metab Cardiovasc Dis 2009;19:563-70.
[8] Saura-Calixto F, Goni I. Definition of the Mediterranean diet based on bioactive compounds. Crit Rev Food Sci Nutr 2009;49:145-52.
[9] Vaverkova H. LDL-C or apo B as the best target for reducing coronary heart disease: should apo B be implemented into clinical practice? Clin Lipidol 2011;6:35-48.
[10] Tziomalos K, Athyros VG, Karagiannis A, et al. Apolipoproteins C-II and C-III and small dense low density lipoprotein: novel risk factors in metabolic syndrome? Arch Med Sci 2008;4:270-3.
[11] Kypreos KE, Karagiannides I, Fotiadou EH, et al. Mechanisms of obesity and related pathologies: role of apolipoprotein e in the development of obesity. FEBS J 2009;276:5720-8.
[12] Koba S, Hirano T, Ito Y, et al. Significance of small dense low-density lipoprotein-cholesterol concentrations in relation to the severity of coronary heart diseases. Atherosclerosis 2006;189:206-14.
[13] Rizzo M, Berneis K. Low-density lipoprotein size and cardiovascular risk assessment. QJM 2006;99:1-14.
[14] Freedman DS, Otvos JD, Jeyarajah EJ, et al. Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham Study. Clin Chem 2004;50:1189-200.
[15] Pascot A, Lemieux I, Prud'homme D, et al. Reduced HDL particle size as an additional feature of the atherogenic dyslipidemia of abdominal obesity. J Lipid Res 2001;42:2007-14.
[16] Onat A, Hergenç G, Özhan H, et al. Lipoprotein(a) is associated with coronary heart disease independent of metabolic syndrome. Coron Artery Dis 2008;19:125-31.
[17] Holvoet P, De Keyzer D, Jacobs DR. Oxidized LDL and the metabolic syndrome. Future Lipidol 2008;3:637-49.
[18] Jones JL, Fernandez ML, McIntosh ML, et al. A Mediterranean-style low–glycemic-load diet improves variables of metabolic syndrome in women, and addition of a phytochemical-rich medical food enhances benefits on lipoprotein metabolism. J Clin Lipidol 2011;5:188-96.
[19] Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421.
[20] Schiltz B, Minich DM, Lerman RH, et al. A science-based, clinically tested dietary approach for the metabolic syndrome. Metab Syndr Relat Disord 2009;7:187-92.
[21] Liu MY, Xydakis AM, Hoogeveen RC, et al. Multiplexed analysis of biomarkers related to obesity and the metabolic syndrome in human plasma, using the luminex-100 system. Clin Chem 2005;51:1102-9.
[22] Liu S, Willett WC, Stampfer MJ, et al. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in us women. Am J Clin Nutr 2000;71:1455-61.
[23] Shikany JM, Tinker LF, Neuhouser ML, et al. Association of glycemic load with cardiovascular disease risk factors: the Women's Health Initiative Observational Study. Nutrition 2010;26:641-7.
[24] Levitan EB, Cook NR, Stampfer MJ, et al. Dietary glycemic index, dietary glycemic load, blood lipids, and C-reactive protein. Metabolism 2008;57:437-43.
[25] Klemsdal TO, Holme I, Nerland H, et al. Effects of a low glycemic load diet versus a low-fat diet in subjects with and without the metabolic syndrome. Nutr Metab Cardiovasc Dis 2010;20:195-201.
[26] Kastorini CM, Milionis HJ, Esposito K, et al. The effect of Mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534,906 individuals. J Am Coll Cardiol 2011;57:1299-313.
[27] Hu FB, Manson JE, Willett WC. Types of dietary fat and risk of coronary heart disease: a critical review. J Am Coll Nutr 2001;20:5-19.

[28] Gillingham LG, Harris-Janz S, Jones PJH. Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors. Lipids 2011:1-20.
[29] Berglund L, Lefevre M, Ginsberg HN, et al. Comparison of monounsaturated fat with carbohydrates as a replacement for saturated fat in subjects with a high metabolic risk profile: studies in the fasting and postprandial states. Am J Clin Nutr 2007;86:1611-20.
[30] Tai CC, Ding ST. N-3 polyunsaturated fatty acids regulate lipid metabolism through several inflammation mediators: mechanisms and implications for obesity prevention. J Nutr Biochem 2010;21:357-63.
[31] Zuliani G, Galvani M, Leitersdorf E, et al. The role of polyunsaturated fatty acids (PUFA) in the treatment of dyslipidemias. Curr Pharm Des 2009;15:4087-93.
[32] Cicero AFG, Ertek S, Borghi C. Omega-3 polyunsaturated fatty acids: their potential role in blood pressure prevention and management. Curr Vasc Pharmacol 2009;7:330-7.
[33] Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 2002;43:1363-79.
[34] Palmer AM, Nova E, Anil E, et al. Differential uptake of subfractions of triglyceride-rich lipoproteins by THP-1 macrophages. Atherosclerosis 2005;180:233-44.
[35] Zambon A, Bertocco S, Vitturi N, et al. Relevance of hepatic lipase to the metabolism of triacylglycerol-rich lipoproteins. Biochem Soc Trans 2003;31:1070-4.
[36] Julius U, Dittrich M, Pietzsch J. Factors influencing the formation of small dense low-density lipoprotein particles in dependence on the presence of the metabolic syndrome and on the degree of glucose intolerance. Int J Clin Pract 2007;61: 1798-804.
[37] Rizzo M, Berneis K. Small, dense low-density-lipoproteins and the metabolic syndorme. Diabetes Metab Res Rev 2007;23: 14-20.
[38] Volek JS, Fernandez ML, Feinman RD, et al. Dietary carbohydrate restriction induces a unique metabolic state positively affecting atherogenic dyslipidemia, fatty acid partitioning, and metabolic syndrome. Prog Lipid Res 2008;47: 307-18.
[39] El Harchaoui K, Arsenault BJ, Franssen R, et al. High-density lipoprotein particle size and concentration and coronary risk. Ann Intern Med 2009;150:84-93.
[40] de Souza JA, Vindis C, Hansel B, et al. Metabolic syndrome features small, apolipoprotein A-I–poor, triglyceride-rich HDL3 particles with defective anti-apoptotic activity. Atherosclerosis 2008;197:84-94.
[41] Sima A, Iordan A, Stancu C. Apolipoprotein E polymorphism—a risk factor for metabolic syndrome. Clin Chem Lab Med 2007;45:1149-53.
[42] Contois JH, McConnell JP, Sethi AA, et al. Apolipoprotein B and cardiovascular disease risk: position statement from the AACC Lipoproteins and Vascular Diseases Division Working Group on Best Practices. Clin Chem 2009;55: 407-19.
[43] Tailleux A, Duriez P, Fruchart JC, et al. Apolipoprotein A-II, HDL metabolism and atherosclerosis. Atherosclerosis 2002;164:1-13.
[44] Holvoet P, Lee DH, Steffes M, et al. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. JAMA 2008;299:2287-93.
[45] Fitó M, Guxens M, Corella D, et al. Effect of a traditional Mediterranean diet on lipoprotein oxidation: a randomized controlled trial. Arch Intern Med 2007;167:1195-203.
[46] Raederstorff D. Antioxidant activity of olive polyphenols in humans: a review. Int J Vitam Nutr Res 2009;79:152-65.
[47] Galvano F, Malaguarnera M, Vacante M, et al. The physiopathology of lipoprotein (a). Front Biosci (Scholar edition) 2010;2:866-75.
[48] Bermúdez V, Arráiz N, Aparicio D, et al. Lipoprotein(a): from molecules to therapeutics. Am J Ther 2010;17:263-73.
[49] Ramsden CE, Faurot KR, Carrera-Bastos P, et al. Dietary fat quality and coronary heart disease prevention: a unified theory based on evolutionary, historical, global, and modern perspectives. Curr Treat Options Cardiovasc Med 2009;11: 289-301.