Published in final edited form as:
Am J Cardiol. 2011 December 1; 108(11): . doi:10.1016/j.amjcard.2011.07.008.

Effects of High Adherence to Mediterranean or Low-Fat Diets in Medicated Secondary Prevention Patients

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Abstract

Although the Mediterranean diet (MD) and the low-fat Therapeutic Lifestyle Changes Diet (TLCD) promote equivalent increases in event-free survival in secondary coronary prevention, possible mechanisms of such complete dietary patterns in these patients, usually medicated, are unclear. The aim of this study was to investigate the effects of the MD versus the TLCD in markers of endothelial function, oxidative stress, and inflammation after acute coronary syndromes. Comparison was made between 3 months of the MD (n = 21; rich in whole grains, vegetables, fruits, nuts, and olive oil, plus red wine) and the TLCD (n = 19; plus phytosterols 2 g/day) in a highly homogenous population of stable patients who experienced coronary events in the previous 2 years (aged 45 to 65 years, all men) allocated to each diet under a strategy designed to optimize adherence, documented as >90%. Baseline demographics, body mass index and clinical data, and use of statins and other drugs were similar between groups. The MD and TLCD promoted similar decreases in body mass index and blood pressure ($p \leq 0.001$) and particularly in plasma asymmetric dimethylarginine levels ($p = 0.02$) and L-arginine/asymmetric dimethylarginine ratios ($p = 0.01$). The 2 diets did not further enhance flow-mediated brachial artery dilation compared to baseline (4.4 ± 4.0%). Compared to the TLCD, the MD promoted decreases in blood leukocyte count ($p = 0.025$) and increases in high-density lipoprotein levels ($p = 0.053$) and baseline brachial artery diameter. Compared to the MD, the TLCD decreased low-density lipoprotein and oxidized low-density lipoprotein plasma levels, although the ratio of oxidized to total low-density lipoprotein remained unaltered. Glucose, high-sensitivity C-reactive protein, triglycerides, myeloperoxidase, intercellular adhesion molecular, vascular cell adhesion molecule, and glutathione serum and plasma levels remained unchanged with either diet. In conclusion, medicated secondary prevention patients show evident although small responses to the MD and the TLCD, with improved markers of redox homeostasis and metabolic effects potentially related to atheroprotection.

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The role of prudent diets for secondary coronary artery disease management remains to be explored. Recent data have shown mortality and morbidity reductions in patients with coronary artery disease managed with adequate diets,[1] exercise, and smoking cessation.[2] However, the mechanisms of diet effects in these patients, who are generally exposed to several drugs, have been only superficially investigated. This includes a lack of information on markers of oxidative stress and inflammation, crucial determinants of endothelial function that are likely relevant also for secondary prevention.[3] Mechanistic information cannot be readily obtained from large-population randomized trials, because diet adherence is not optimized in these cases. The aim of our study was to compare, in medicated patients with coronary artery disease, the effects of aggressive treatment with the Mediterranean diet (MD) to those with the Therapeutic Lifestyle Changes Diet (TLCD), with a focus on endothelial function, inflammation, and oxidative stress.

Methods

We designed a prospective controlled clinical trial to assess 3-month effects of high compliance with the full-pattern MD compared to the TLCD on end point variables, assessed at the beginning and end of interventions. To maximize adherence, diet allocation was not randomized, while selection criteria were stringent to provide a high degree of homogeneity.

Forty-two men aged 45 to 65 years were selected from among consecutive outpatient appointments at our hospital. Eligibility criteria included ≥1 coronary event (myocardial infarction or unstable angina) occurring <24 and >4 months before enrollment, clinical stability and absence of secondary events, body mass index (BMI) 18.5 to 30.0 kg/m^2, nonsmoker or ex-smoker for >1 year, and fasting blood glucose <110 mg/dl. Exclusion criteria included a history of diabetes, chronic illnesses, or food allergy; serum low-density lipoprotein (LDL) >190 mg/dl; serum triglycerides >310 mg/dl; suspected or confirmed drug or alcohol addiction; and any condition that might impair participation in the study. Special care was taken to continue all medications, with dosages unchanged, during the study; nutritional supplements were not allowed. Exercise levels were kept unchanged. All patients gave written informed consent, and the study was approved by institutional ethics committee.

Without knowledge about specific dietary patterns under investigation, selected patients underwent baseline evaluations including clinical history, nutritional assessment, endothelial function testing, and laboratory measurements. Then, on the basis of previous cultural and dietary habits and 4-day food records, each patient was allocated to either the MD or the TLCD and given personalized dietary advice by a dietitian, together with the patient's partner. After 3-month interventions, each patient was reevaluated similarly to baseline. Laboratory analyses were conducted at baseline and after the 3-month dietary period.

All patients received cholesterol-lowering dietary advice before the study. During the study, patients were given printed copies of the MD or the TLCD and personalized advice about daily food plans, including portion size models, desired food intake frequency, and specific recipes. Individual food plans were tailored to nutritional assessments, including BMI, energy needs by the Harris-Benedict equation, and daily and cultural habits. Total energy was adjusted only for patients with BMIs >25 kg/m^2 at baseline. The 2 diets were adapted to Brazilian food habits (nutritional patterns are listed in Table 1). The advised MD pattern included (1) daily consumption of unrefined cereals and products (e.g., whole-grain bread, pasta, brown rice); fresh fruits (4 to 6 servings/day); varied raw or cooked vegetables and legumes (2 to 3 servings/day); extra-virgin olive oil (30 ml/day) as the main added fat; nonfat or low-fat dairy products (1–2 servings/day) and nuts (10 g/day); (2) weekly

consumption of fish (3 to 4 times/week), poultry (3 to 4 times/week), and eggs (0 to 4 per week) and low red meat consumption (once a week). Sweets were allowed only a few times per month; red wine consumption (250 ml/day) was recommended for all MD patients.[4] TLCD patients were advised to follow recommendations according to the National Cholesterol Education Program Third Adult Treatment Panel: decreased fat intake, particularly saturated and trans-fatty acids; increased intake of fruits, vegetables, legumes, whole grains, fat-free and low-fat dairy products; moderate amount of lean meat, fish, or poultry; and vegetable oil for cooking. TLCD patients received a list of soluble fiber-rich foods with daily consumption amounts; all were asked to avoid alcohol during the study.[5] All patients were provided with specific foods that could favor adherence, as follows: for the MD group, mixed plain nuts (Brazil nuts, almonds, and walnuts, 10 g/day), cabernet sauvignon wine (250 ml/day), and extra-virgin olive oil (15 ml, amber flasks); for the TLCD group: cholesterol-lowering spread (phytosterol rich) with a measuring cup (20 g/day). All MD and TLCD patients had similar continuous, individually scheduled and assisted dietitian access throughout the study. Diet composition was analyzed with Food Processor version 10.5 software (Esha Research, Salem, Oregon) adapted to Brazilian food databases.

Compliance with diets and specific foods was enforced through monthly interviews plus 24-hour recall analysis at follow-up visits, unscheduled quarterly 24-hour recalls by telephone and e-mail, patient and partner collaboration, and comparison between baseline and final (3-month) 4-day food-record analyses. At 3 months, adherence scores were also calculated from specific dietary intake questionnaires for the MD[6] and the TLCD.[7] Fatty acid composition analysis by gas chromatography[8] of the extra-virgin olive oil supplied to MD patients showed equivalence with the United States Department of Agriculture nutritional data-base, and the phytosterol-rich spread had undetectable trans-fatty acids. Anthropometric parameters were obtained by trained technicians using standard methods,[9] and BMI was calculated as weight in kilograms divided by the square of height in meters. Subscapular, triceps, biceps, abdominal, and suprailiac skin-fold thicknesses were measured in alternate triplicates with calipers (Lange, Ann Arbor, Michigan) and expressed as medians.

Plasma and sera from fasting venous blood samples were kept on ice for ≤1 hour and stored at −80°C. Methods were as follows: for oxidized LDL, monoclonal antibody–based immunoassay (Mercodia, Uppsala, Sweden); for soluble vascular cell adhesion molecule–1 and soluble intercellular adhesion molecule–1, enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minnesota); for myeloperoxidase, enzyme-linked immunosorbent assay (CardioMPO; PrognostiX, Cleveland, Ohio); and for glutathione, fluorescence assay (Arbor Assays LLC, Ann Arbor, Michigan). Glucose, plasma cholesterol, liver enzymes, triglyceride, high-density lipoprotein cholesterol and calculated LDL cholesterol, lipoprotein(a), apolipoprotein A-I and apolipoprotein B, high-sensitivity C-reactive protein, and total leukocyte count were measured at the hospital laboratory using standard methods. For asymmetric dimethylarginine (ADMA) analysis, plasma samples, shipped to the University of Florida, were loaded onto an Oasis SPX cation exchange column (Millipore, Billerica, Massachusetts) and basic amino acids eluted with 1 ml sodium hydroxide/methanol/water in a 10:40:50 ratio. Samples were dried, resuspended in mobile phase, derivatized with o-phtalaldehyde and assessed by high-performance liquid chromatography and electrochemical detection.[10] Mitochondrial deoxyribonucleic acid (DNA) copy number was assessed in peripheral blood mononuclear cells[11] by SYBR Green quantitative polymerase chain reaction (Applied Biosystems, Carlsbad, California), with single-copy gene hemoglobin-β as reference.[12] Primer (400 to 600 nmol/L) sequences were (5′ -3′): mitochondrial DNA–F: CCTAGCCGTTTACTCAATCCT; R: TGATGGCTAGGGTGACTTCAT; hemoglobin-β-F: GTGAAGGCTCATGGCAAGA; and

R: AGCTCACTCAGGTGTGGCAAAG. Conditions were: 40 cycles at 95°C for 15 seconds and 60°C for 1 minute.

Endothelium-dependent FMD and responses to sublingual isosorbide dinitrate 5 mg were assessed as described[13] under current guidelines.[14] Brachial artery diameters were assessed in the left arm in recumbent position, after 10-minute rest in a room kept at 20°C to 25°C, using a 7.5-MHz linear-array vascular ultrasound transducer and an Apogee 800 Plus ultrasound system (ATL Ultrasound, Bothell, Washington). Blood pressure and heart rate were monitored with an automated sphygmomanometer. Vessel diameter was measured with locally developed software.[13] Reactive hyperemia was induced by the inflation of a tourniquet around the forearm to 250 mm Hg, deflated after 5 minutes. Endothelium-dependent and independent dilation were calculated as the percentage change in brachial artery diameter ratio after reactive hyperemia or nitrate to baseline diameter. All tests were blindly performed and analyzed by a single dedicated ultrasonographer.

A sample size of 40 patients was calculated to detect a 10% difference in total cholesterol[5] (because this is a well-established variable), with a 24 mg/dl SD (determined from preliminary data), 5% type I error, and 10% type II error. Such a sample size is consistent with the recommendations of the International Brachial Artery Reactivity Task Force for endothelial function studies.[14] Results reflect data from 40 patients who completed the study; results including the 2 dropouts were similar. Continuous variables are presented as mean ± SD. Baseline characteristics were compared between groups using Student's *t* or chi-square tests. Two-factor repeated-measures analysis of variance was used to compare changes between groups (MD vs TLCD) and between time periods (baseline vs 3 months). Analysis of mitochondrial DNA copy number, including a normal control group, was performed using Kruskal-Wallis and Dunnet's post hoc tests. Statistical analysis was carried out using SPSS version 15.0 (SPSS, Inc., Chicago, Illinois). All tests were 2 tailed, and the significance level was $p < 0.05$.

Results

From 176,000 consecutive records screened over 31 months, 159 patients were interviewed, and 42 were eligible and assigned to the MD (n = 21) or the TLCD (n = 21); 2 TLCD patients dropped out because of family problems, so that 40 (95%) completed the study. Table 2 lists the results of all analyzed variables. At baseline, MD patients had higher weights and heights but similar BMIs compared to TLCD patients. Age, body composition, blood pressure, glucose levels, and oxidative, inflammatory, and endothelial function variables were similar between MD and TLCD patients, with the exception of higher oxidized LDL levels in TLCD patients. Histories of dyslipidemia and/or hypertension were present in >90% patients, myocardial infarction in 52%, percutaneous intervention in >68%, and surgical revascularization in 33%. None of these variables differed between groups. Eighty-six percent to 100% of patients were taking lipid-lowering, antihypertensive, and antiplatelet agents. Sedentary lifestyles were reported by 35% and weekly aerobic physical activity ≥90 minutes by 53% of patients, with exercise status maintained during the 3-month intervention in 97.5% of patients (data not shown). Baseline nutritional characteristics were similar between the groups, except for monounsaturated fats, which were higher in MD than in TLCD patients (Table 3). Food-record analysis before and after the intervention showed total energy reduction ($p < 0.001$) with the MD (464 kcal) and the TLCD (478 kcal). The TLCD significantly decreased total fat (38%) compared to the MD and to baseline, while the MD significantly decreased carbohydrates compared to the TLCD and to baseline. The MD increased monounsaturated fat from 9% to 15% kcal, while total fat increased by only 1%. Saturated fats decreased and omega-3 fatty acids reached 0.9% in the 2 groups. There were 199 attendances to 200 scheduled appointments (5 per patient). Additional quarterly

interviews by telephone (80%) or e-mail (20%) were well accepted.[15] Validated[6] adherence scores (highest MD adherence = 9) showed values of 7, 8, and 9, respectively, in 19%, 33%, and 48% of MD patients. Reasons for scores of 7 and 8 were fish intake <3 times/week and/or lower compliance with whole-grain cereals. The MED-FICTS questionnaire indicated high TLCD adherence, with scores <40 for all patients.[7] Questionnaires and 24-hour records showed high adherence to whole diet patterns as well as specific foods: extra-virgin olive oil, wine, and nuts with the MD and phytosterol-rich spread with the TLCD.

The MD and the TLCD promoted similar significant decreases in weight, BMI, waist circumference, and skin-fold thickness. Blood pressure also decreased with the MD and the TLCD, irrespective of antihypertensive use. The MD promoted a significant decrease (−533 ± 785) in leukocyte count, compared to an increase (+137 ± 1,028) with the TLCD. High-density lipoprotein cholesterol levels were nonsignificantly increased with the MD and unaltered with the TLCD. High-sensitivity C-reactive protein levels were not significantly changed. Total and LDL cholesterol significantly decreased with the TLCD compared to the MD. Plasma oxidized LDL decreased significantly with the TLCD compared to the MD and to baseline, although final levels for the 2 groups were similar. This was due to lower baseline values in MD patients. Ratios of oxidized to total LDL did not differ between the groups. Plasma and serum levels of apolipoprotein A-I, lipoprotein(a), glucose, myeloperoxidase, soluble vascular cell adhesion molecule, and soluble intercellular adhesion molecule and plasma and erythrocyte glutathione levels were unchanged in the 2 groups, while triglyceride levels nonsignificantly decreased with the 2 diets. Importantly, plasma ADMA levels decreased and L-arginine/ADMA ratios increased with the 2 diets. Flow-dependent brachial artery reactivity, within normal limits at baseline, remained unchanged in the 2 groups, similarly to postnitroglycerin dilation, while baseline flow velocity mildly increased with the 2 diets. Peripheral blood mononuclear cell mitochondrial DNA copy number was increased in all patients at baseline (Table 2) compared to age-matched healthy controls, which showed values for 2^{-DDCT} of 1.7 ± 1.4 ($p < 0.001$).

Discussion

Our results identified some inflammation and redox markers related to mechanisms underlying the effects of aggressive dietary intervention in secondary prevention patients. In contrast, despite optimal adherence, diet-induced absolute changes in such markers were small. This should be analyzed bearing in mind that secondary prevention patients were, as expected, medicated and had already received previous general diet advice. Importantly, in the long term, even the small improvements achieved in our study have the potential to affect event-free survival, as indeed observed in The Heart Institute of Spokane Diet Intervention and Evaluation Trial (THIS-DIET)[1] and in other secondary prevention studies addressing distinct interventions.[16] An important result of our study was the decrease in plasma ADMA levels and increase in L-arginine/ADMA ratios achieved with either diet. This may point to the peculiar sensitivity of this index to redox and nitric oxide homeostasis, even in medicated patients, and is in line with data showing that ADMA levels correlate with fatal and nonfatal cardiovascular events after myocardial infarction and coronary interventions.[17] Known correlations of BMI, cholesterol levels, sodium intake, and arterial pressure to ADMA levels might be involved in effects of our diets.[18] The observed lack of a correlation between decreased ADMA levels and vasomotor endothelial function is notable but not surprising, considering that higher baseline ADMA concentrations predict lower endothelial function response to statins.[17,19] Of note, we observed increase in mitochondrial DNA copy number in peripheral blood mononuclear cells from patients with coronary artery disease compared to normal controls. Although unaltered by dieting, this variable warrants further study as a risk marker, because mitochondrial DNA copy number reflects mitochondrial biogenesis, an adaptive response to several cell stresses.[20]

Some observed effects were specific to the MD compared to the TLCD, namely, increased high-density lipoprotein levels and a consistent decrease in circulating leukocyte count. A correlation of the latter to inflammation is possible but unclear, and cut-off values are nonexistent.[21] Remarkably, however, average decreases of 500 leukocytes/mm^3, equivalent to the effects of our MD, were reported to discriminate late occurrence of secondary postinfarction events.[21,22] Compared to the MD, the TLCD specifically decreased plasma total and LDL cholesterol levels. Because the amount of cholesterol and saturated fat was equivalent for the 2 diets, this effect may be due to phytosterol supplementation, as reported previously.[23] TLCD was also able to decrease oxidized LDL plasma levels; because the ratio of oxidized to total LDL remained unaltered, this likely reflects total lipid lowering rather than a direct antioxidant effect. Interestingly, baseline oxidized LDL was already low in our MD patients, possibly because of a marginally higher intake of monounsaturated fats.

Because we aimed to evaluate mechanisms related to the full potential of complete MD or TLCD patterns, we prioritized some characteristics. First, adherence was optimized through an approach in which cultural preferences were taken into account, increasing compliance with diet-specific foods. Near ideal adherence was enforced through meticulous individual follow-up and documented with validated scores, and the 2 groups were oriented under identical nutritional strategies. This is important, because diet adherence is often less than optimal in large randomized trials, limiting to some extent mechanistic inferences. Second, strict inclusion criteria allowed the selection of highly homogenous groups for the 2 diets, clearly validating their comparison in the absence of randomization. Together, this design likely promoted changes close to an achievable optimum for each diet. BMI and fat mass–related anthropometric variables were similarly decreased with the 2 diets. This accords with the previously reported negative correlation between BMI and MD adherence scores[24] and is relevant, considering the intake of 328 kcal/day from olive oil and nuts. Such decreases in BMI and arterial pressure likely reflect high diet adherence in our study, because this effect was not observed in other secondary prevention studies with MD and low-fat diets,[1,25] despite higher baseline BMIs.[1] Importantly, neither of our diets was low in calories. Decreases in BMI and arterial pressure have peculiar relevance in secondary prevention considering their reported roles in atherosclerosis regression.[26] Intrinsic limitations of our study include the short follow-up time and the nonrandomized design, which preclude extrapolations to sustained diet effects in the overall population. Also, the redox and inflammation variables addressed in our study present intrinsic limitations.[27]

Overall, the observed improvements with either the MD or the TLCD on some variables related to redox homeostasis and inflammation in secondary prevention patients deserve further investigation regarding their possible impact on cardiovascular risk.

Acknowledgments

We are indebted to Jacqueline Issa and Célia Strunz for support.

Supported by: Grants from: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo, Brazil), CNPq (Instituto Nacional de Ciência e Tecnologia de Processos Redox em Biomedicina - Redoxoma, Brazil), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil).

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Table 1 Energy and nutrient recommendations for Mediterranean diet and Therapeutic Lifestyle Changes Diet

Nutrient Goal	MD	TLCD
Energy	To maintain desirable weight	To maintain desirable weight
Protein	12%-17% of total calories	Approximately 15% of total calories
Carbohydrate	45%-50% of total calories	55%-60% of total calories
Total fat	33%-38% of total calories	25%-30% of total calories
Monounsaturated fat	20%-25% of total calories	Up to 20% of total calories
Polyunsaturated fat	Up to 10% of total calories	Up to 10% of total calories
Saturated fat	≤8% of total calories	≤7% of total calories
Omega-3 fats	>0.75% of total calories	*
Cholesterol	<200 mg/day	< 200 mg/day
Dietary fiber	20-30 g/day	20-30 g/day
Therapeutic lifestyle components of TLCD		
Plant stanols/sterols	*	2 g/day
Increased viscous fiber	†	10-15 g/day

*Value not established by National Cholesterol Education Program Third Adult Treatment Panel (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;106:3143-3421).

†Value not established by Mediterranean dietary patterns.

Table 2 Demographic, clinical, and laboratory variables at baseline and after 3-month intervention period

Variable	MD (n = 21)		TLCD (n = 19)		p Value Baseline*	p Value Between Groups¶		
	Baseline	3 Months	Baseline	3 Months		Time	Group Time X Group	
Age (years)	55.0 ± 4.6	NA	54.6 ± 5.0	NA	0.807	NA	NA	NA
Height (cm)	173 ± 5	NA	167 ± 5	NA	**0.001**	NA	NA	NA
Smoking status								
Ex-smoker for ≤ 1 year	17 (81%)	NA	10 (53%)	NA	0.091	NA	NA	NA
Educational level								
High school or beyond	21 (100%)	NA	16 (84%)	NA	0.058	NA	NA	NA
Medication								
Aspirin	20 (95%)	NA	17 (90%)	NA	0.489	NA	NA	NA
Other antiplatelet drugs	8 (38%)	NA	9 (47%)	NA	0.554	NA	NA	NA
Statins	17 (81%)	NA	16 (84%)	NA	0.787	NA	NA	NA
Statins plus ezetimibe	4 (19%)	NA	3 (16%)	NA	0.787	NA	NA	NA
Nitrates	4 (19%)	NA	3 (16%)	NA	0.787	NA	NA	NA
ACE inhibitors	11 (52%)	NA	13 (68%)	NA	0.301	NA	NA	NA
βblockers	18 (86%)	NA	19 (100%)	NA	0.087	NA	NA	NA
Body weight (kg)	79.3 ± 7.5	77.7 ± 7.5	73.6 ± 7.6	71.9 ± 6.7	**0.023**	**< 0.001**	**0.017**	0.879
BMI (kg/m^2)	26.5 ± 1.9	25.9 ± 1.8	26.3 ± 2.5	25.7 ± 2.4	0.827	**< 0.001**	0.783	0.819
Waist circumference (cm)	96 ± 6	94± 6	94± 8	92± 8	0.400	**< 0.001**	0.352	0.885
Waist/hip ratio	0.94 ± 0.05	0.94 ± 0.04	0.95 ± 0.07	0.94 ± 0.06	0.801	0.211	0.939	0.372
Skin-fold thickness sum (mm)	102 ± 18	99 ± 14	94 ± 25	88 ± 24	0.205	**0.004**	0.117	0.357
Systolic blood pressure (mm Hg)	138 ± 13	127 ± 11	134± 19	128 ± 13	0.479	**0.001**	0.791	0.246
Diastolic blood pressure (mm Hg)	87 ± 7	80± 7	87± 9	81± 8	0.971	**< 0.001**	0.731	0.637
Total cholesterol (mg/dl)	153 ± 33	155 ± 35	161 ± 24	147 ± 32	0.396	0.114	0.998	**0.029**
LDL cholesterol (mg/dl)	87 ± 26	90 ± 30	98 ± 23	86 ± 27	0.140	0.236	0.598	**0.034**
HDL cholesterol (mg/dl)	40 ± 7	43± 9	38± 7	38± 7	0.400	0.076	0.133	0.053
Oxidized LDL (U/L)	60 ± 21	59 ± 20	78 ± 24	62 ± 21	**0.019**	**0.002**	0.114	**0.009**
Oxidized LDL/total LDL	0.73 ± 0.25	0.67 ± 0.16	0.79 ± 0.23	0.73 ± 0.26	0.384	0.120	0.297	0.976
Triglycerides (mg/dl)	130 ± 74	110 ± 62	121 ± 59	112 ± 70	0.694	0.086	0.870	0.524
Apolipoprotein B (g/L)†	0.79 ± 0.16	0.78 ± 0.19	0.91 ± 0.24	0.80 ± 0.24	0.066	**0.027**	0.268	0.067

Variable	MD (n = 21)		TLCD (n = 19)		p Value Baseline*	p Value Between Groups¶		
	Baseline	3 Months	Baseline	3 Months		Time	Group Time X Group	
Apolipoprotein A-I (g/L)†	1.49 ± 0.22	1.48 ± 0.31	1.37 ± 0.21	1.28 ± 0.21	0.063	0.206	**0.019**	0.357
Lipoprotein(a) (mg/dl)	37 ± 29	40 ± 32	36 ± 28	38 ± 29	0.844	0.093	0.859	0.934
Glucose (mg/dl)	91 ± 8	93± 8	89± 8	88± 9	0.419	0.348	0.138	0.251
High-sensitivity C-reactive protein (mg/L)	1.65 ± 1.50	1.07 ± 0.93	1.38 ± 1.07	2.07 ± 2.99	0.510	0.874	0.437	0.058
Total leukocyte count ($\times 10^3/mm^3$)	6.3 ± 1.2	5.8 ± 1.2	6.1 ± 1.3	6.2 ± 1.5	0.495	0.176	0.888	**0.025**
Myeloperoxidase (pmol/l)‡	433 ± 86	376 ± 136	389 ± 79	384 ± 69	0.243	0.150	0.637	0.215
Soluble intercellular adhesion molecule-1 (ng/ml)	124 ± 29	122 ± 35	138 ± 35	127 ± 38	0.177	0.068	0.372	0.201
Soluble vascular cell adhesion molecule-1 (ng/ml)	314 ± 74	320 ± 84	317 ± 68	317 ± 75	0.891	0.645	0.995	0.637
L-arginine (μmol/L)	94.9 ± 11.4	91.5 ± 16.1	86.1 ± 20.2	85.5 ± 18.8	0.110	0.378	0.143	0.549
ADMA (/nmol/L)§	0.88 ± 0.22	0.77 ± 0.22	0.90 ± 0.22	0.82 ± 0.24	0.794	**0.021**	0.575	0.685
L-arginine/ADMA§	114.7 ± 32.2	125.1 ± 27.4	99.3 ± 25.8	111.8 ± 35.9	0.108	**0.012**	0.110	0.805
Plasma reduced (GSH)/oxidized (GSSG) glutathione	1.3 ± 0.7	1.3 ± 0.7	1.5 ± 0.9	1.4 ± 0.6	0.487	0.301	0.503	0.678
Erythrocyte GSH/GSSG§	5.5 ± 6.4	5.8 ± 5.0	5.8 ± 6.5	5.8 ± 3.5	0.881	0.921	0.916	0.885
Mitochondrial DNA copy number// 2^{-DDCT} (controls)	10.4 ± 10.1	8.6 ± 5.0	6.7 ± 3.3	10.3 ± 11.5	0.161	0.635	0.640	0.154
DCT (MctHBb-MctDNAmit)	7.5 ± 1.0	7.4 ± 0.7	7.1 ± 0.6	7.4 ± 1.0	0.185	0.528	0.447	0.270
Endothelial function†								
Endothelium-dependent flow-mediated dilation (%)	4.4 ± 3.6	4.9 ± 4.3	4.4 ± 5.5	4.9 ± 3.7	0.999	0.397	0.790	0.760
Endothelium-independent postnitroglycerin dilation (%)	23.1 ± 6.6	18.7 ± 8.7	19.2 ± 8.1	20.8 ± 8.8	0.099	0.238	0.785	0.082
Baseline brachial artery diameter (mm)	4.38 ± 0.39	4.44 ± 0.52	4.44 ± 0.51	4.34 ± 0.49	0.690	0.413	0.983	**0.035**
Baseline flow velocity (cm/s)	51 ± 10	54 ± 11	52 ± 14	56 ± 14	0.759	**0.046**	0.636	0.755
Hyperemic flow velocity (cm/s)	96 ± 20	99 ± 19	95 ± 21	88 ± 14	0.793	0.648	0.174	0.220

Data are expressed as mean ± SD or as number (percentage). Significant p values are shown in boldface type. NA = not applicable.

*Baseline characteristics for the 2 groups were compared using Student's or chi-square tests as appropriate.

Am J Cardiol. Author manuscript; available in PMC 2013 November 19.

† MD: n = 21; TLCD: n = 18.

‡ MD: n = 10; TLCD: n = 10.

§ MD: n = 20; TLCD: n = 19.

‖ MD: n = 18; TLCD: n = 17.

¶ Obtained using 2-factor repeated-measures analysis of variance.

Table 3

Nutritional characteristics at baseline and after 3-month intervention period

Nutritional Characteristic	MD (n = 21)		TLCD (n = 19)		p Value Baseline*	p Value Between Diets†		
	Baseline	3 Months	Baseline	3 Months		Time	Group	Time × Group
Total calorie intake	2,447 ± 648	1,983 ± 390	2,160 ± 513	1,682 ± 341	0.132	**<0.001**	**0.037**	0.921
Protein (g)	119 ± 33	87 ± 20	105 ± 25	86 ± 21	0.157	**<0.001**	0.289	0.159
Protein (% total calorie intake)	19	18	20	20				
Carbohydrate (g)	295 ± 103	224 ± 57	248 ± 56	229 ± 55	0.078	**<0.001**	0.281	0.03
Carbohydrate (% total calorie intake)	48	45	46	54				
Total fat (g)	82 ± 26	69 ± 13	79 ± 29	49 ± 13	0.762	**<0.001**	**0.046**	**0.028**
Total fat (% total calorie intake)	30	31	33	26				
Saturated fat (g)	27 ± 10	16 ± 5	27 ± 13	14 ± 5	0.984	**<0.001**	0.705	0.58
Saturated fat (% total calorie intake)	10	7	11	7				
Monounsaturated fat (g)	24 ± 7	32 ± 7	19 ± 6	13 ± 4	**0.024**	0.467	**< 0.001**	**< 0.001**
Monounsaturated fat (% total calorie intake)	9	15	8	7				
Polyunsaturated fat (g)	14 ± 5	13 ± 3	17 ± 6	15 ± 3	0.077	0.134	**0.022**	0.574
Polyunsaturated fat (% total calorie intake)	5	6	7	8				
Omega-3 fat (g)	1.3 ± 1.1	2.0 ± 0.7	1.8 ± 0.6	1.8 ± 0.6	0.164	0.105	0.57	0.099
Omega-3 fat (% total calorie intake)	0.5	0.9	0.7	0.9				
Omega-6 fat (g)	11.2 ± 5.2	10.1 ± 2.3	13.7 ± 4.3	12.9 ± 2.7	0.102	0.17	0.011	0.843
Omega-6 fat (% total calorie intake)	4.1	4.6	5.7	6.9				
Trans fat (g)	1.5 ± 0.9	0.9 ± 1.2	1.8 ± 1.2	0.6 ± 0.4	0.416	**<0.001**	0.979	0.162
Trans fat (% total calorie intake)	0.6	0.4	0.8	0.3				
Cholesterol (mg)	261 ± 84	179 ± 76	260 ± 99	166 ± 50	0.974	**<0.001**	0.721	0.675
Dietary fiber (g)	27 ± 12	31 ± 12	25 ± 11	26 ± 9	0.549	0.201	0.222	0.402
Sodium (mg)	2,128 ± 997	1,457 ± 621	1,706 ± 827	1,398 ± 609	0.155	**0.001**	0.252	0.191
Potassium (mg)	4,125 ± 4,935	3,257 ± 1,319	2,680 ± 806	2,764 ± 916	0.216	0.522	0.113	0.437
Calcium (mg)	928 ± 360	837 ± 246	874 ± 499	811 ± 381	0.697	0.26	0.687	0.845
Phosphorus (mg)	1,264 ± 345	1,146 ± 384	1,162 ± 347	1,107 ± 364	0.358	0.207	0.45	0.643
Vitamin (mg)	206 ± 193	243 ± 109	146 ± 137	146 ± 127	0.266	0.383	0.063	0.39

Nutritional Characteristic	MD (n = 21)		TLCD (n = 19)		p Value Baseline*	p Value Between Diets†		
	Baseline	3 Months	Baseline	3 Months		Time	Group	Time × Group
Selenium (μg)	12 ± 9	27 ± 37	14 ± 17	14 ± 12	0.763	0.083	0.271	0.153

Data are expressed as mean ± SD. Significant p values are shown in boldface type.

*Baseline characteristics for the 2 groups were compared using Student's *t* tests.

†Obtained using 2-factor repeated-measures analysis of variance.