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A Mediterranean Dietary Intervention in Healthy American Women Changes Plasma Carotenoids and Fatty Acids in Distinct Clusters

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Abstract

This study examined patterns of changes in plasma fatty acids and carotenoids when women were asked to follow a novel, Greek-Mediterranean exchange list diet. A total of 69 healthy, non-obese, women ages 25–59, were randomized to either continue their own usual diet or to follow a modified Mediterranean diet for six months. There were no significant changes in blood lipids, triacylglycerol, insulin, glucose or C-reactive protein. Mean plasma carotenoids increased by 55%, which is consistent with a large increase in fruit and vegetable consumption. Likewise, changes in fat intakes were reflected in blood fatty acids, with a 25% increase in mean plasma monounsaturated fatty acids (MUFA). Principal component analysis was conducted to examine the sources of inter-individual variation for changes in carotenoid and fatty acid levels. Changes in the Mediterranean diet were clustered together in four components that accounted for 78% of the variance in plasma levels. Increases in plasma lutein, α - and β -carotene clustered together in a “vegetable” pattern, and increases in carotenoids found in fruit, β -cryptoxanthin and zeaxanthin, also clustered together but accounted for less of the variance. Increases in plasma MUFA were clustered with a decrease in plasma polyunsaturated fatty acids, consistent with substitution in the type of oils consumed. The only association of fatty acid levels with carotenoids was that of lycopene, which clustered together with an increase in saturated fatty acids. The changes in blood levels indicate the exchange list diet was effective for targeting Mediterranean nutrient intakes using foods available in the United States.

Keywords

cancer prevention; monounsaturated fat; fruit; vegetables; olive oil; human; clinical trial; carotenoids

1. INTRODUCTION

A Greek-Mediterranean type of eating pattern has been suggested to be protective of diabetes, cardiovascular disease and cancer. In recent European studies, Mediterranean eating patterns have been shown to decrease all cause mortality and increase longevity [1,2]. Epidemiological evidence also points to the protective effects of a Greek-Mediterranean diet on risk of breast and other cancers [3–5]. A prominent feature of the traditional Greek diet was a high monounsaturated fatty acid (MUFA) intake, stemming largely from the use of olive oil [6,7]. Another prominent feature of the traditional Greek diet was a high consumption of fruit and

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vegetables. The 1960s diet of Crete included about 650 g/day of fruit and vegetables in a wide variety [6].

Both the type of fat intake and increased fruit and vegetable intakes could be protective of breast cancer risk by a variety of mechanisms. In animal models, polyunsaturated fats (PUFA) that contain high levels of linoleic acid have been shown to have strong promoting effects on mammary gland tumors, unlike other dietary fats [8,9]. This may be related to the ease with which PUFA are peroxidized [10]. Mediterranean diets also have been shown to improve insulin resistance and decrease estrogen levels [11,12]. In a meta analysis of 8 prospective cohort studies, the relative risks of breast cancer for each 5% increase in energy from fat (for pre- and postmenopausal women, respectively) were 1.10–1.07 for saturated fat, 0.87–0.81 for MUFA and 1.12–1.28 for PUFA [13]. There are also many protective compounds in fruits and vegetables, but the benefits of increased fruit and vegetable consumption on breast cancer risk have not been readily evident in epidemiological studies [14,15]. This may be due to problems inherent in dietary assessments. Three large prospective studies have measured blood levels of carotenoids, which are markers of FRUITS AND VEGETABLES intake, and the relative risk of breast cancer was 57–24% lower in women who had relatively higher levels of blood carotenoids [16–18]. Like fruits and vegetables, olive oil also contains many phytochemicals with antioxidant and anticancer properties [15,19].

The maximum protective effects of diet may be evident when all foods are considered as part of an eating pattern. Prospective studies have more recently indicated that overall quality of diet may be more important for cancer risk than any one dietary component. In a U.S. study, increased consumption of plant foods, whole grains and lean animal foods decreased risk of cancer mortality in women (multivariate RR=0.60) [20]. In a large Greek study, every 2-point increment in a Mediterranean Diet Score was associated with 24% decreased cancer mortality, but intakes of specific foods largely were not associated with mortality (except intakes of MUFA and fruits, and these were less protective than overall dietary pattern) [3]. Similar protective effects of a Mediterranean dietary pattern on breast cancer risk were found in a recent U.S. study [21]. Interventions in which eating patterns rather than specific nutrients are being modified, therefore, may hold great promise for cancer prevention [20,22].

We conducted a randomized trial to test implementation of a self-selected, exchange-list diet that had both high MUFA and high fruit and vegetable goals. The long-term goal for developing this modified Mediterranean exchange list was to test the diet in studies of breast cancer prevention. Self-reported compliance to the dietary goals was excellent [23]. Here we report on the extent of changes in blood fatty acids and carotenoids as markers of fat, fruit and vegetable intakes, and we evaluated how changes in these measures clustered together in this multi-faceted intervention. Our hypothesis was that the changes in blood measures would be correlated with each other, stemming from concurrent dietary changes in each food category. Observational studies have indicated that persons following a more traditional Mediterranean diet in Europe have relatively higher levels of blood carotenoids [24,25]. With regard to Mediterranean dietary interventions, most have been conducted in Europe, and they focused on increasing n-3 fatty acid intakes and/or MUFA intakes and less so on substitution of PUFA for MUFA with concurrent increases in fruits and vegetables [26]. Some Mediterranean interventions have even resulted in decreased MUFA levels [27–29]. The present intervention used an exchange list diet to target substitution of foods in an isocaloric manner. Blood levels of fatty acids and carotenoids both were evaluated to confirm compliance to the multi-faceted dietary goals and to examine patterns of changes within the Mediterranean arm.

2. MATERIALS AND METHODS

2.1 Subjects and intervention

The Mediterranean Eating Study was approved by the Institutional Review Boards of Wayne State University and University of Michigan, and the clinical trial registration number was NCT00120016. Women ages 25–65 were eligible and gave written, informed consent to participate. Details of study recruitment and retention have been published [23]. Recruitment spanned April 2004 - August 2005. Diets considered as eligible had a fat intake of at least 23% of calories from 7-day food records, with no more than 48% of fat intake being from MUFA. Fruit and vegetable intakes considered as eligible were below 5.5 servings/day, not including white potatoes and iceberg lettuce.

Other eligibility criteria were being in good general health, currently a non-smoker and in the normal to overweight range (body mass index 18.5 to 30 kg/m²). Exclusions included persons with chronic diseases such as diabetes mellitus, auto-immune disease or high blood pressure, on medically prescribed diets, taking dietary supplements above 150% of the RDAs, pregnant or lactating, or being treated with therapies or supplements that could obscure our ability to detect diet effects.

All study participants were asked to complete an assessment visit three times: at baseline, three months and six months. Subjects were paid \$20 for each study visit. At these visits, subjects were weighed, provided a fasting blood sample, filled out demographic questionnaires and returned 7-day food records. The food records were analyzed using the Nutrition Data System Research software from University of Minnesota (database version 35, software version 5.0).

Women assigned to the non-intervention group did not receive any dietary counseling with the exception of written materials to correct any nutritional deficiencies (below 67% of the RDA), and they received the National Cancer Institute's Action Guide to Healthy Eating. Women in the Mediterranean intervention received exchange goals that were designed to keep total fat and energy intake at baseline levels. The goal for fat intake was to achieve a PUFA:SFA:MUFA ratio of 1:2:5. The goal to increase in fruits and vegetables was accomplished by substituting fruits and vegetables for other carbohydrates. The fruits and vegetable goal was 7–9 servings per day, depending on energy intake, in specified variety: they were asked to consume at least once serving daily from each of the following groups: vitamin C fruit, other fruit, red vegetable, dark orange vegetable, dark green vegetable, other vegetable, allium vegetable and dark green herb [23].

2.2 Fatty Acid Analysis

Plasma lipids (from 300 µl plasma) were extracted with 10 volumes of Folch reagent (chloroform:methanol 2:1). Half of this extract was used for analysis of total fatty acids and half was used to prepare phospholipids. Phospholipid fatty acids have been measured in most epidemiological studies, but there is some evidence that total plasma fatty acids are better correlated with dietary intakes [30]. Phospholipid fatty acids were isolated by placing the chloroform extract on a silica cartridge, washing with chloroform and methanol before eluting with chloroform:methanol:water (3:5:2) based on published methods [31]. The internal standard, 17:0, was added to the phospholipid eluate and the total lipid chloroform extract, and each fraction was dried. Fatty acids were redissolved in 300 µl hexane: chloroform (1:1) and methyl esters prepared by adding 1/10 volume 2M KOH in dry methanol and vortexing for 2 minutes. Methanolic KOH, using dry conditions, esterifies all classes of fatty acids well [32].

Fatty acid methyl esters in the top layer were determined by gas chromatography with mass spectral detection (GC-MS) using a SP2330 capillary column and heating from 70°C to 220°C over 20 minutes. The system consisted of a HP5971 MSD, HP5890 GC and HP7673

autosampler (Agilent Technologies, Santa Clara, CA). The optimal ion for detection of each fatty acid was selected based on abundance and specificity, and these were (in m/z): 183.1 for 12:0, 199.1 for 14:0, 185.1 for 16:0, 194.1 for 16:1, 199.1 for 17:0, 298.1 for 18:0, 296.1 for 18:1, 294.1 for 18:2, 295.1 for 18:3, 292.1 for 20:0, 292.1 for 20:1, 203.1 for 20:4, 201.1 for 20:5, and 199.1 for 22:6. The system was calibrated using standard curves constructed with fatty acids from NuChek Prep (Elysian, MN).

2.3 Clinical Chemistry

Assays for cholesterol, HDL, and triacylglycerol (triglycerides) were done using a Cobas Mira Chemistry analyzer from Roche Diagnostics Corporation (Indianapolis, IN). LDL was calculated from the Friedewald equation [33]. High sensitivity C-reactive protein was measured using a latex immunoturbidimetric assay. Values above 10 mg/ml for C-reactive protein were excluded as a possible indication of infection or other acute inflammatory condition: this criterion excluded 6 of 191 samples. C-peptide (insulin), insulin-like growth factor 1 (IGF-1) and its binding protein 3 (IGF-bp3) were assayed using the Immulite chemiluminescent assay system from Diagnostic Products Corporation (Los Angeles, CA). Glucose was measured with a hexokinase colorimetric assay. These assays were done by the Michigan Diabetes Research and Training Center Core Chemistry Laboratory. The facility calibrates all assays with standards and analyzes quality control samples daily.

2.4 Carotenoids and Tocopherols

Plasma, 200 μ l, was mixed with 200 μ l ethanol containing 0.05% butylated hydroxytoluene (an antioxidant) and the internal standard, Tocol. This was extracted twice with 2 ml hexane, combined hexane extraction, dried and re-dissolved in 200 μ l of ethanol: hexane (7:3). The HPLC system was a Waters 600E pump and 715 autosampler (Milford, MA). Detection used both visible (450 nm) and electrochemical detection with an ESA 4-channel detector set at 310, 390 and 470 mV (ESA Biosciences, Chelmsford, MA). The separation of 8 major plasma carotenoids, 2,6-cyclolycopene-1,5-diol and tocopherols was achieved with a C30, YMC 3 μ m 4.6X250 mm column using a 20 mm guard column. The mobile phase A was methanol with 2% 1 M ammonium acetate. Mobile phase B was 75% methyl-tert-butyl-ether, 5% hexane, 18% methanol, with 2% 1 M ammonium acetate. The flow rate used was 1 ml/min with a gradient of 10% B to 100% B over 35 min. Serum samples from the Carotenoid Round-Robin conducted by the National Institute of Standards Technology were used for calibration curves.

2.5 Statistical Analyses

Changes in blood levels over time were modeled using repeated measures analysis of variance (ANOVA). The main grouping variables of diet arm, time and diet x time interaction were used as factors in all models with each plasma measure as an outcome. Model diagnostics was carried out through residual plots to check the distributional and homo-scedasticity assumption. For variables that showed departure from model assumptions, the Box-Cox family of transformations [34] was employed to identify the 'best' transformed model. Log transformation was used to normalize IGF1, cholesterol, triacylglycerol, LDL, total PUFA, phospholipid measures of MUFA, 18:1 and 18:3, both tocopherols, lycopene, zeaxanthin and β -cryptoxanthin. Square root transformation was used for total MUFA and 18:1, while fourth root was used to normalize α -carotene, β -carotene, total carotenoids and lutein. An inverse square root transformation was used for insulin.

Co-variates for all blood measures included age and body mass index (BMI) at baseline. For fat-soluble micronutrients, total fat intake at baseline (grams/day) was an additional covariate in the models. The correlation between the measurements at baseline, three months, and six months within each subject was modeled using a completely unstructured variance-covariance matrix, thereby providing a robust option. The mixed model approach used all available data

from each subject, yielding an intention-to-treat analysis which minimizes bias in the interpretation of clinical trials. Since the time X diet arm interaction term was insignificant for all measures, we presented the analyses without it for ease of interpretation. All post-hoc tests used a Bonferroni correction protecting overall Type-I error at 5%. In order to identify clusters of measurements that change in synchrony in the Mediterranean diet arm, we (a) calculated Spearman's pairwise correlations and (b) conducted a principal component analysis of the six-month change scores.

3. RESULTS

3.1 Subjects

A total of 69 women were enrolled on study and 60 completed six months of participation. The mean body mass index of enrolled women was 24 kg/m² (range 19–30 kg/m²) and mean age was 44 years (range 25–59 years). As reported previously, most (59 of 69) of the women were Caucasian, most (40 of 69) were married and most (63 of 69) were college graduates [23]. There were no statistically significant differences in any of these demographic characteristics, or in body weight, between the two diet groups. Mean body weight did not change significantly but it did increase at 6 months in the control arm by 0.24 kg and it decreased by 1.21 kg in the Mediterranean arm [23].

3.2 Measures of Insulin Sensitivity and Blood Lipids

For glucose and IGF-bp3, age at baseline was a significant covariate, with higher values of glucose, and lower values of IGF-1 and IGF-bp3 being associated with increasing age. Glucose handling is known to be compromised with age, perhaps as related to decreases in IGF-1 [35]. For insulin, insulin/glucose ratio, and C-reactive protein, BMI at baseline was a significant covariate, with higher values of the measures associated with heavier subjects, consistent with the important influence of body weight on insulin sensitivity. Both age and BMI at baseline were significant covariates that were positively associated with total cholesterol, LDL and triacylglycerol, and negatively associated with IGF-1 in the regression models. In the ANOVA model, plasma levels of insulin, glucose, C-reactive protein, IGF-1 and IGF-bp3 did not change significantly in either arm with time (Table 1). There were also no statistically significant differences in any of these measures between the two diet arms averaged over time. There was some suggestion for decreases in total cholesterol and triacylglycerol at 3 months in the Mediterranean arm, which may be related to the slight weight loss that was noted at 3 months [23].

3.3 Fatty Acid Blood Levels

Age and BMI were not significant covariates for any of the fatty acids, except BMI at baseline for 18:2 in total plasma fatty acids, but both were included in the regression models as they were for all other plasma measures. Both age and BMI have been shown to modulate fatty acid desaturase activities and thus could possibly influence phospholipids fatty acid levels [36]. There were significant differences in the two diet arms with respect to levels of plasma fatty acids (Table 2). There was no difference between the diet arms at baseline (verified by means of independent-samples t-tests), so the difference could be attributed to the intervention.

The patterns with time were similar when examining either total fatty acids or phospholipid fatty acids; however, statistically significant diet group differences were observed only in the total fatty acids. This may be because phospholipid fatty acids are more highly regulated, and it has been shown that total plasma fatty acids are better correlated with dietary intakes [30]. No significant changes over time were observed within the control arm for any of the measures. The Mediterranean diet group had significantly higher average MUFA and 18:1 levels than the control group, while the average PUFA and 18:2 measures were significantly lower in the

Mediterranean arm. The increases in MUFA and 18:1 within the Mediterranean arm were also significant for phospholipid fatty acids. Levels of 18:2 in phospholipids reached borderline significance ($p=.07$) for differences by diet group. In the Mediterranean arm, the increase in total MUFA was about 25% while SFA and PUFA each decreased by about 5%. Interestingly 18:3 (n-3) decreased, likely because canola oil was classified as an “occasional” use fat in the intervention. Plasma levels of 20:4, n-6, increased about 10% and 18:2 decreased 8% indicating that with a high MUFA diet, delta-6-desaturase activity may be increased (Table 2).

3.4 Micronutrients

For fat-soluble micronutrients, total fat intake was included along with age and BMI in the ANOVA as covariates. Total fat intake was not a significant covariate for any of the fat-soluble micronutrients in this study, but it was included nonetheless since there is some indication that increased fat intake might facilitate absorption of carotenoids, especially the more lipophilic ones [37]. BMI at baseline was a significant co-variate for γ -tocopherol, α -carotene, β -carotene, β -cryptoxanthin, and total carotenoids. The association was positive with γ -tocopherol, but inverse with the remaining micronutrients. This is in agreement with previous studies showing that increased BMI was a negative predictor of both carotenoid levels and increases in carotenoids (specifically α -carotene, β -carotene and β -cryptoxanthin) after a high fruit-vegetable intervention [38,39]. Age also was a significant predictor for β -carotene, β -cryptoxanthin, and 2,6-cyclolycopene-1,5-diol in this present study, with older subjects more likely to have higher levels of these micronutrients. In this regard, it is interesting to note that 2,6-cyclolycopene-1,5-diol is an oxidation product of lycopene [40].

Several micronutrients exhibited a significant diet arm difference in time-averaged values (Table 3). The Mediterranean arm had significantly higher average intakes of α -carotene, β -carotene, total carotenoids, and β -cryptoxanthin compared to the control arm. On the other hand, the average intake of γ -tocopherol in the control arm was significantly higher than in the intervention arm. Lack of any significant diet arm difference at baseline with respect to any of these measures confirmed that the observed difference over the duration of the study was primarily due to the intervention and it was sustained over the six-month period.

In the Mediterranean arm, statistically significant increases at 6 months, relative to baseline, were evident in zeaxanthin by 31%, β -cryptoxanthin by 115%, α -carotene by 151%, and β -carotene by 75%. The three-month values were also significantly higher compared to the baseline for α -carotene, and for β -carotene there was a trend ($p=.07$). Total plasma carotenoids were increased by 37% at 3 months and 55% at 6 months.

3.5 Clustering of Changes in Micronutrients and Fatty Acids

Finally, we examined possible sources of inter-individual variability within the Mediterranean arm. Principal component analysis was conducted to address this (Table 4). The results indicated that changes in lutein, α -carotene and β -carotene were significantly clustered with each other and accounted for 26% of the variance in changes in plasma measures. These carotenoids are all high in vegetables, and we termed this a “vegetable pattern” in Table 4. A second cluster was a decrease in PUFA and an increase in MUFA, which would be indicative of substituting oils such as corn and sunflower for olive oil, and we termed this an “oils” pattern. Lycopene and saturated fat were linked positively, indicating that this carotenoid was likely consumed in mixed dishes such as pizza that has both cheese and tomato sauce. Interestingly, increased plasma lycopene levels have been linked with Western acculturation [41]. Finally, zeaxanthin and β -cryptoxanthin clustered together, and these carotenoids are high in fruit [42,43]. The four patterns accounted for 78% of the variance in the plasma measures. These findings were confirmed by the correlations between changes in plasma levels of these nutrients and dietary micronutrients (not shown). These clusters of foods could be useful in

understanding the types of dietary changes subjects make when asked to follow a Mediterranean type of diet.

4. DISCUSSION

The changes in blood levels of fatty acids and carotenoids indicated that subjects were making the appropriate dietary changes, but changes in blood levels were of smaller magnitude than the changes in dietary intakes. In the Mediterranean arm, the increase in total MUFA was about 25% while SFA and PUFA each decreased by about 5%. These changes in plasma fatty acids reflected, but were smaller than the large changes in dietary intakes of fats, which included a 48% increase in dietary MUFA from 7-day food records [23]. The increases in dietary and plasma MUFA were larger than that obtained in the Lyon Heart Study, which had several positive health endpoints and utilized counseling for a Mediterranean diet together with providing a canola-oil based margarine [44].

Similar to the changes in plasma fatty acids, the changes in plasma carotenoids in the Mediterranean arm reflected the increase in fruits and vegetables, but were smaller. While reported fruit and vegetable intake doubled [23], total carotenoids only increased by 55%. This increase in total carotenoids is similar to that in our previous 9-a-day high fruit-vegetable intervention of women at increased risk for breast cancer and that achieved in a large intervention study of breast cancer survivors [45,46]. This indicates that blood carotenoids likely changed as much as could be expected with the Mediterranean intervention that requires consumption of 7–9 fruits and vegetables/day. The changes in the control arm were generally smaller and not statistically significant.

Most of the changes in blood fatty acids and carotenoids with the Mediterranean diet were larger at 6 than at 3 months, indicating that it took some time to comply with the requested dietary changes. In our previous study with a 9-a-day fruit and vegetable intervention, the increases were significant at 3 months and reached a plateau after that [45]. This could be due to the less complex nature of the intervention when focusing only on fruits and vegetables versus the more complex changes required with a Mediterranean diet.

The Mediterranean diet did appear to result in a decrease in blood levels of γ -tocopherol. This was not significant over 6 months of study, but is of interest and likely results from decreased intakes of vegetable oils rich in vitamin E as subjects started using more olive oil. Vitamin E intakes were not addressed with the exchange list diet. This may be an area for concern; however, vitamin E needs may be lower when PUFA intake decreases [47]. A low-fat diet can also decrease γ -tocopherol levels [45].

Despite the seemingly good compliance to the Mediterranean diet, changes in blood measures indicative of diabetes and cardiovascular risks were not evident. A review of other Mediterranean interventions has indicated that many did find favorable effects on LDL, but most of these studies were conducted in persons with increased cardiovascular risks or metabolic syndrome [26]. In a Canadian study of healthy women, LDL did not decrease overall but it did decrease in person in the highest tertile of LDL at baseline [48]. Favorable effects on insulin resistance were also seen in many but not all studies, and in two large studies with favorable effects on insulin, there was a weight loss associated with the Mediterranean intervention [26,49]. Changes in blood lipids with Mediterranean diets likely would be evident if there was a concomitant weight loss [49]. In this study, however, weight maintenance was a goal to isolate the effects of diet quality, as much as possible.

Important limitations of the present study are that the women who volunteered for the study were highly educated, the study was small and it only lasted 6 months. In addition, the exchange list diet targeted only fat, fruit and vegetable intakes. The exchange list could be further

modified to include omega-3 fats and whole grains, both of which appear important for cancer prevention.

In summary, the changes in blood levels of fatty acids and carotenoids in the Mediterranean diet arm were consistent with the previously reported dietary changes [23]. In particular, plasma levels of MUFA increased by about 25% and total carotenoids increased by 55%. The increases in plasma carotenoids were likely related to increases in fruits and vegetables, except for increases in lycopene which clustered with an increase in saturated fat. This may help explain the poor correlations observed previously for dietary and serum lycopene [50]. The increases in plasma MUFA were associated with decreases in plasma PUFA, likely representing a shift in the type of oils consumed. Despite these changes in diet, there were no measurable effects on plasma lipids, glucose, insulin and C-reactive protein in this study population of young, healthy women. Effects of the Mediterranean intervention on other measures of health and biomarkers of cancer risk, however, have yet to be explored.

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ABBREVIATIONS

MUFA	Monounsaturated fatty acid
PUFA	polyunsaturated fatty acids
BMI	body mass index
IGF-1	insulin-like growth factor 1
IGF-bp3	insulin-like growth factor binding protein 3

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Table 1
Plasma measurements in the study subjects over the course of the study.

Measurement ^a	Non-intervention Group			Mediterranean Group		
	Baseline n=34	Month 3 n=34	Month 6 n=33	Baseline n=35	Month 3 n=27	Month 6 n=27
Glucose, mg/dl	90 ± 6	89 ± 6	92 ± 8	91 ± 7	91 ± 7	92 ± 5
Insulin, ng/ml	9.9 ± 3.4	9.7 ± 3.6	10.7 ± 4.2	10.6 ± 3.4	11.4 ± 4.0	11.0 ± 2.4
Insulin/glucose	0.11 ± 0.04	0.11 ± 0.04	0.12 ± 0.06	0.12 ± 0.04	0.13 ± 0.07	0.12 ± 0.02
IBF-bp3, mg/ml	5.08 ± 1.05	5.09 ± 0.91	5.11 ± 0.72	5.18 ± 0.94	5.00 ± 0.93	5.20 ± 1.20
IGF-1, ng/ml	153 ± 43	154 ± 41	153 ± 40	149 ± 49	140 ± 43	143 ± 36
C-Reactive Protein, mg/l	1.61 ± 2.39	1.29 ± 1.71	1.43 ± 2.20	1.45 ± 1.39	1.48 ± 1.52	1.55 ± 1.89
Cholesterol, mg/dl	186 ± 35	185 ± 32	182 ± 31	198 ± 39	191 ± 34	197 ± 36
HDL, mg/dl	72 ± 14	70 ± 13	72 ± 15	70 ± 12	68 ± 13	71 ± 12
LDL, mg/dl	100 ± 28	101 ± 29	95 ± 25	111 ± 32	107 ± 30	109 ± 34
Triacylglycerol, mg/dl	69 ± 34	70 ± 26	72 ± 38	83 ± 37	78 ± 33	86 ± 45

^aData shown are means ± SD. No significant ($p < .05$) diet arm differences were found in the repeated measures ANOVA analysis, neither was there any significant time effect within either group.

Table 2

Plasma fatty acid levels in the study subjects.

Group Fatty Acid ^a	Non-intervention Group			Mediterranean		
	Baseline n=34	Month 3 n=34	Month 6 n=33	Baseline n=35	Month 3 n=27	Month 6 n=27
Total plasma fatty acids						
18:1, n-7 ^{b,c}	17.9 ± 5.2	18.4 ± 5.7	17.7 ± 5.0	19.3 ± 3.9	21.8 ± 6.4	24.1 ± 6.6 ^c
18:2, n-6 ^{b,c}	31.1 ± 5.1	31.2 ± 5.8	29.7 ± 5.8	29.2 ± 6.2	27.5 ± 4.3	27.0 ± 5.2 ^c
18:3, n-3	0.46 ± 0.52	0.31 ± 0.27	0.31 ± 0.23	0.53 ± 0.82	0.38 ± 0.42	0.37 ± 0.48
20:4, n-6	11.2 ± 4.1	11.1 ± 3.8	12.1 ± 4.5	9.8 ± 3.7	11.5 ± 4.5	11.0 ± 4.2
20:5, n-3	0.8 ± 0.8	0.7 ± 0.9	1.0 ± 1.3	0.6 ± 0.7	0.9 ± 1.1	0.6 ± 0.8
22:6, n-6	1.9 ± 1.3	1.8 ± 1.1	1.9 ± 1.2	2.4 ± 1.9	2.4 ± 1.4	1.8 ± 1.2
Phospholipid fatty acids						
SFA	44.0 ± 6.2	43.6 ± 5.8	43.9 ± 5.8	44.3 ± 5.7	44.0 ± 6.9	42.1 ± 3.9
MUFA ^c	11.2 ± 4.0	11.2 ± 4.1	11.3 ± 3.8	10.4 ± 2.5	12.2 ± 3.6	13.0 ± 3.8 ^c
PUFA	44.9 ± 5.3	45.6 ± 5.6	44.8 ± 5.6	45.3 ± 5.1	44.0 ± 7.3	44.7 ± 7.2
18:1, n-9 ^c	10.2 ± 4.2	10.3 ± 3.9	10.4 ± 3.7	9.5 ± 2.6	11.4 ± 3.7	12.1 ± 3.5 ^c
18:2, n-6	25.0 ± 4.1	24.8 ± 4.6	24.4 ± 3.8	24.3 ± 4.3	23.1 ± 3.9	22.3 ± 3.5
18:3, n-3	0.14 ± 0.14	0.16 ± 0.23	0.15 ± 0.19	0.19 ± 0.18	0.17 ± 0.20	0.14 ± 0.12
20:4, n-6	13.6 ± 4.8	14.7 ± 5.0	14.6 ± 5.4	14.6 ± 4.7	14.4 ± 4.7	15.9 ± 5.2
20:5, n-3	1.4 ± 1.3	1.1 ± 1.5	1.2 ± 1.4	1.0 ± 1.1	1.1 ± 1.3	0.9 ± 1.3
22:6, n-3	3.0 ± 1.8	2.9 ± 1.4	2.8 ± 1.9	3.1 ± 1.8	3.2 ± 2.0	2.8 ± 1.7
22:6, n-3	3.0 ± 1.8	2.9 ± 1.4	2.8 ± 1.9	3.1 ± 1.8	3.2 ± 2.0	2.8 ± 1.7

^a Values are the mean percentages for each fatty acid ± SD.

^b Significant (p < .05) diet arm difference from the ANOVA.

^c Significant differences between baseline and six-month values within Mediterranean Group were found.

Table 3

Plasma micronutrient levels in subjects over time.

Micronutrient ^d	Non-intervention Group			Mediterranean Group		
	Baseline n=34	Month 3 n=34	Month 6 n=33	Baseline n=35	Month 3 n=27	Month 6 n=27
Lutein, ng/ml	146 ± 120	195 ± 204	206 ± 229	135 ± 80	147 ± 118	191 ± 139
Zeaxanthin, ng/ml ^b	32 ± 17	34 ± 17	33 ± 19	29 ± 10	33 ± 16	38 ± 14
β-Cryptoxanthin, ng/ml ^{b,c}	87 ± 42	864 ± 45	96 ± 47	89 ± 35	140 ± 96	191 ± 55 ^c
α-Carotene, ng/ml ^{b,c,d}	84 ± 73	104 ± 93	113 ± 86	68 ± 40	158 ± 167 ^d	171 ± 115 ^c
β-Carotene, ng/ml ^{b,c}	261 ± 180	285 ± 204	276 ± 170	249 ± 181	398 ± 327	438 ± 295 ^c
Lycopene, ng/ml	383 ± 180	403 ± 167	442 ± 177	399 ± 168	455 ± 233	493 ± 223
Total carotenoids, ng/ml ^{b,c}	989 ± 508	1,107 ± 568	1,165 ± 536	968 ± 394	1,331 ± 805	1,501 ± 712 ^c
Cyclolycopene, ng/ml ^e	179 ± 54	181 ± 65	177 ± 48	169 ± 49	184 ± 58	191 ± 56
γ-tocopherol, μg/ml ^b	1.86 ± 0.59	1.81 ± 0.38	1.84 ± 0.52	1.84 ± 0.63	1.52 ± 0.51	1.65 ± 0.52
α-tocopherol, μg/ml	8.89 ± 0.33	9.15 ± 5.5	8.73 ± 4.38	8.57 ± 3.46	8.63 ± 3.89	10.07 ± 4.77

^a Values shown are mean and SD for each micronutrient.^b Significant (p < .05) diet arm difference^c Significant differences between baseline and six-month values within Mediterranean Group.^d Significant differences between baseline and three-month values within Mediterranean Group.^e This refers to 2,6-cyclolycopene-1,5-diol, an oxidation product of lycopene.

Table 4

Principal component factor analysis of changes in blood measures in the Mediterranean diet arm.

Change in Blood Measure	Component and Percentage of Variance ^a			
	1: vegetables	2: oils	3: pizza	4: fruit
	26%	20%	18%	14%
Lutein	0.703 ^b	−0.009	0.089	0.301
Zeaxanthin	0.086	0.180	−0.159	0.866
β-Cryptoxanthin	0.035	−0.271	−0.006	0.786
α-Carotene	0.824	−0.060	−0.005	−0.091
β-Carotene	0.937	−0.032	−0.004	0.026
Lycopene	0.291	−0.071	0.752	−0.094
MUFA	0.015	0.919	−0.215	−0.089
PUFA	0.122	−0.921	−0.326	−0.014
SFA	−0.183	0.111	0.879	−0.062

^aThe four components listed accounted for 78% of the variance for changes in blood measures in the Mediterranean diet arm. Data shown are the factor loading values for each blood measure in each component.

^bThe bolded values are the strongest contributors to each component.