A Nutritional Intervention Promoting the Mediterranean Food Pattern Is Associated with a Decrease in Circulating Oxidized LDL Particles in Healthy Women from the Québec City Metropolitan Area

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ABSTRACT The aim of the present study was to evaluate the effect of a nutritional intervention promoting the Mediterranean food pattern under free-living conditions on circulating oxidized LDL (ox-LDL) in a group of 71 healthy women from the Québec City metropolitan area. The 12-wk nutritional intervention consisted of 2 courses on nutrition and 7 individual sessions with a dietitian. A score based on the 11 components of the Mediterranean pyramid was established to determine the women’s adherence to the Mediterranean food pattern. Plasma ox-LDL concentrations were measured by a monoclonal antibody mAb-4E6–based competition ELISA. Among all women, plasma ox-LDL decreased by 11.3% after 12 wk of nutritional intervention (P < 0.0001) despite a lack of change in plasma LDL cholesterol (LDL-C). Also, an increase in the Mediterranean dietary score was significantly correlated with a decrease in ox-LDL concentrations (r = −0.30; P = 0.01). More specifically, increases in servings of fruits (r = −0.25; P < 0.05) and vegetables (r = −0.24; P < 0.05) were associated with decreases in ox-LDL concentrations. Changes in the food pattern in response to a nutritional intervention promoting the Mediterranean food pattern were accompanied by beneficial effects in circulating ox-LDL concentrations in healthy women. J. Nutr. 135: 410–415, 2005.

KEY WORDS: ● Mediterranean diet ● oxidized LDL ● nutritional intervention ● women

Cardiovascular disease and, more specifically, heart disease and stroke are the major causes of illness, disability, and death in Canada. A high oxidized LDL (ox-LDL) concentration has been related to an increase in coronary heart disease (CHD) risk (1). Indeed, oxidative modifications of LDL particles through the action of resident vascular cells such as endothelial cells, macrophages, and smooth muscle cells and their subsequent accumulation in the arterial wall play a substantial role in the development of atherosclerosis (2). These modified LDL particles induce a response of endothelial cells that increases the adherence and migration of monocytes from the blood to the intima and their differentiation into macrophages (3), a process that further stimulates peroxidation of LDL (4). Ox-LDL trapped in the artery wall are recognizable by scavenger receptors present on the surface of macrophages (5), which are the predominant cell type in the earliest atherosclerotic lesions known as fatty streaks (6).

The Mediterranean diet is characterized by the consumption of olive oil and a high intake of fruits and vegetables (7). Some studies have already demonstrated that consuming fruits and vegetables would have a protective effect against CHD (8,9) and ischemic stroke (10). Indeed, fruits and vegetables may reduce CHD risk through the beneficial combinations of micronutrients and antioxidants, such as flavonoids. Consumption of flavonols, a subclass of flavonoids, was shown previously to be inversely associated with CHD risk in free-living populations (11). Furthermore, flavonoids were shown to protect lipoproteins from oxidation, which could partly explain their cardioprotective potential (12).

The results of the Seven Countries Study demonstrated that the mortality rate from CHD was 30–50% lower in Southern Europe than in Northern Europe or in the United States (13). This supports the notion that the Mediterranean food pattern may help reduce CHD risk. However, the mechanisms by which the Mediterranean diet exerts its protective effects against CHD remain unclear. Indeed, the lower recurrence rate of coronary events in the Lyon Diet Heart Study was not associated with significant modifications in the basic plasma lipid profile such as total cholesterol, LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C) concentrations (14). However, increased consumption of antioxidant-rich foods may provide an explanation for the apparent benefits of the Mediterranean diet (7). We therefore investigated the effect of a nutritional intervention promoting the Mediterranean food pattern on circulating ox-LDL concentrations in 71 free-living, healthy women.
SUBJECTS AND METHODS

Healthy women from the Quebec City metropolitan area who were free of metabolic disorders requiring treatment (coronary heart disease, diabetes or dyslipidemias) were recruited through the laval University newspaper during the summer of 2001. To be part of the study, women had to be 35–65 y old, had to have a stable body weight for at least 3 mo before the study, and had to be in charge of food purchases and meal preparation most of the time. Only women with a diet concordant with the usual Canadian food pattern were included in the study. One hundred and twenty-six women were screened for an evaluation of their food habits. Among this initial group of women, 94 were selected for the study according to the above criteria. Seventy-seven women signed the informed consent form, which had been approved by Ethics Committees of laval University. Three women dropped out for personal reasons. Three other participants did not complete all 3-d food records or the FFQ. Therefore, 71 women completed the study.

Intervention. The methodology of the nutritional intervention was described previously (15). Briefly, the study was conducted in 2 phases involving 35 and 36 women, respectively. Each phase was conducted using a similar 12-wk intervention design. The first started in August 2001 and the second began in January 2002. Individual sessions took place during wk 1, 6, and 12 of the intervention to evaluate the dietary changes and to examine ways to improve adherence to the Mediterranean food pattern. Unannounced qualitative 24-h recalls were performed by telephone during week 2, 4, 8, and 10. The objective of these recalls was to provide support to the subjects and to reinforce the key principles of the Mediterranean diet. Three dietitians were trained to provide a standardized intervention, which promoted a high consumption of fruits, vegetables, legumes, nuts, and seeds; olive oil; dairy products; fish; and to reinforce the key principles of the Mediterranean diet. Three dietitians were trained to provide a standardized intervention, which promoted a high consumption of fruits, vegetables, legumes, nuts, and seeds; olive oil; dairy products; fish; and low consumption of sweets, meat, and meat products.

The 3-d food record. Each participant completed a 3-d food record at wk 0 and 12. At the screening of the nutritional intervention (wk 0), women were given oral and written instructions by the dietitian on how to keep a 3-d food record. In addition, participants were encouraged to consume their usual amount of food and drinks. Participants had to record all food and beverages they consumed on 3 d, 2 week days and 1 weekend day. Participants were not required to weigh foods but were asked to measure the volume of foods consumed with household measurement tools (e.g., cups, tablespoons) or to indicate the weight of commercial products when it was possible to assess portion sizes. Participants recorded information from recipes when necessary. Less than 1 wk after a participant completed the 3-d food record, the dietitian checked the recorded data and helped the participant to review all of the information for accuracy and completeness. An example of portion size was used for clarification if needed. The food record also included a section for recording dietary supplement intakes.

Mediterranean dietary score. A validated quantitative FFQ was administered by a dietitian at wk 0 and 12 of the nutritional intervention. The FFQ was described previously by Goulet and colleagues (16). Briefly, the FFQ contains 91 items and 33 subquestions and is based on typical foods, which are available in Quebec. Participants were questioned about frequency of intake for different foods during the last month and were asked to report the frequency of daily, weekly, and monthly consumption. The number of servings consumed for foods listed in the FFQ was used to calculate the global Mediterranean dietary score. The Mediterranean dietary score, indicating the adherence of the women to the Mediterranean food pattern, was constructed on the basis of Mediterranean pyramid components and was described in a previous publication from our group (15). Our Mediterranean dietary score was based on the following 11 components of the Mediterranean pyramid: grains; fruits; vegetables; legumes, nuts, and seeds; olive oil; dairy products; fish; poultry; eggs; sweets and red meat/processed meat. A maximum of 4 points/component of the pyramid was allowed. The total dietary score could therefore vary between 0 and 44 points (15). Other studies also used a score to evaluate adherence to the Mediterranean food pattern (17,18). An evaluation of nutrient intakes derived from the food record was performed using the Nutrition Data System for Research (NDS-R) software version 4.03, developed by the Nutrition Coordination Center (University of Minnesota, Minneapolis, MN), and the Food and Nutrient Database 31, released in November 2000. This database includes >16,000 food items for which the complete nutritional value of 112 nutrients is included.

Anthropometry. At wk 0 and 12, body weight, waist circumference, and height were measured according to the procedures recommended at the Airlie Conference on the standardization of anthropometric measurements (19); BMI was calculated by dividing weight (kg) by height (m) squared [(kg/(m)²].

Plasma lipid- and lipoprotein profile. Blood samples were collected at wk 0 and 12 from an antecubital vein into vacutainer tubes containing EDTA after a 12-h overnight fast for the measurements of plasma lipid and lipoprotein concentrations. Plasma was collected, divided into aliquots, and stored at −80°C. Plasma total cholesterol and triglyceride (TG) levels were determined by enzymatic methods using a Technicon RA-500 analyzer (Bayer), as previously described (20). HDL-C levels were also obtained using the autoanalyzer after precipitation of VLDL and LDL in the infranatant with heparin and MnCl₂ (21). Apolipoprotein (apo) B was measured by nephelometry (BN ProSpec, Dade Behring) with reagents provided by the company (N antisera to Human Apolipoprotein B). LDL-C was estimated with the standard equation of Friedewald and colleagues (22). Ox-LDL concentrations were measured at wk 0 and 12 using a commercial sandwich ELISA according to the manufacturer’s instructions (Alpco Diagnostics).

Statistical analysis. The descriptive values are presented as means ± SD. Data collected at the beginning (wk 0) and after the 12-wk intervention were compared using repeated-measures ANOVA to identify time effects. Changes in many dietary variables were not normally distributed; therefore, we used Spearman correlations to systematically quantify associations among variables. Partial Spearman correlations were performed between changes in ox-LDL and changes in Mediterranean dietary score to partial out the effect of changes in body weight, waist circumference, and plasma apo B concentrations.

Student t tests were also used to compare changes in ox-LDL in response to the intervention between users and nonusers of dietary supplements. Similarly, the effect of the intervention phase (Fall 2001 vs. Winter 2002) on changes in ox-LDL was also tested. Moreover, participants were divided on the basis of baseline circulating ox-LDL using tertiles of the distribution to establish whether the circulating ox-LDL response to the intervention might be modulated by baseline circulating ox-LDL. ANOVA was performed and in the presence of significant effects, Duncan’s test was used to determine the location of significant differences.

Once one subject displayed extreme changes in daily energy (~1793 kcal (~7502 kJ)), total fat (~169 g) on the SEA (~76 g) consumption in response to the nutritional intervention. This subject was clearly an outlier for many dietary variables and was therefore excluded from the analyses. All analyses were performed with the SAS statistical package version 8.02 (SAS Institute) and statistical significance was defined as P < 0.05.

RESULTS

Anthropometric and plasma lipid- and lipoprotein profiles of the study participants are presented in Table 1. Twelve weeks after the beginning of the nutritional intervention, weight decreased slightly but significantly by 0.6 kg (P < 0.01). Furthermore, waist circumference after the nutritional intervention was 1.2 cm lower than at baseline (P < 0.0001). Plasma ox-LDL concentrations decreased by 11.3% after the nutritional intervention (P < 0.0001). In addition, ox-LDL concentrations decreased more (~11.0 vs. −1.1 U/L; P < 0.05) in the women who had the most elevated ox-LDL concentrations at baseline (58.2 ± 7.8 U/L) based on tertiles of the distribution compared with women with the lowest ox-LDL concentrations (31.4 ± 4.5 U/L). Plasma apo B concentrations also decreased after the 12-wk intervention (P < 0.05). However, plasma concentrations of total, LDL, and HDL cholesterol did not change significantly.
HDL cholesterol as well as TG were not affected by response to the nutritional intervention.

Reductions in ox-LDL concentrations that occurred during the intervention were positively associated with decreases in LDL-C concentrations \((r = 0.30; P = 0.01)\) (Fig. 1). The small but significant weight loss (0.6 kg) by the group was not correlated with the change in ox-LDL concentrations. Moreover, the change in ox-LDL was not associated with changes in plasma apo B or waist circumference. Furthermore, changes in ox-LDL concentrations did not differ between the 2 intervention phases (Fall 2001 vs. Winter 2002: \(-7.9 \pm 11.0\) vs. \(-4.4 \pm 9.3\) U/L).

The Mediterranean dietary score increased from 21.1 ± 3.6 at baseline to 29.1 ± 4.4 after the 12-wk intervention \((P < 0.0001)\) (Table 2). The increase in Mediterranean dietary score was significantly correlated with a decrease in ox-LDL concentrations \((r = -0.30; P = 0.01)\) (Fig. 2). This association remained significant after controlling for changes in weight, waist circumference and plasma apo B \((r = -0.26; P < 0.05)\).

Further analyses were performed to examine which components of the Mediterranean food pattern would be associated with the diminution in ox-LDL concentrations. Servings determined by the FFQ for whole grain, vegetables, fruits, olive oil, fish and seafood, poultry and legumes, nuts, and seeds increased significantly in response to the nutritional intervention (Table 2). In contrast, servings from refined grain, sweets, and red meat/processed meat decreased significantly. Among the total sample of women, only increases in servings of fruits \((r = -0.25; P < 0.05)\) and vegetables \((r = -0.24; P < 0.05)\) were significantly correlated with decreases in ox-LDL concentrations (Fig. 3). Changes in the number of servings of...
other food groups were not associated with changes in ox-LDL (all $P > 0.10$).

Table 3 shows the effects of the nutritional intervention on dietary variables as measured by the 3-d food record. At 12 wk energy intake was reduced by 651 kJ/d ($P < 0.01$). The percentage of energy derived from lipids did not change after 12 wk. However, the percentage of energy derived from SFA decreased significantly from 11.1 to 9.8%. In addition, eicosapentaenoic acid [20:5(n-3)] and docosahexaenoic acid [22:6(n-3)] increased significantly ($P < 0.01$ and $P < 0.05$, respectively), whereas the percentage of energy derived from trans fatty acids (trans) decreased significantly ($P < 0.0001$). The percentage of energy derived from monounsaturated fatty acids (MUFA) and PUFA did not differ in response to the nutritional intervention. The percentage of energy from protein increased after the nutritional intervention ($P < 0.01$). An increase in β-carotene intake was also observed in response to the nutritional intervention ($P < 0.05$), but vitamin A, α-tocopherol, and ascorbic acid intakes were not affected.

Changes in dietary fat (SFA, MUFA, PUFA, and trans) after the nutritional intervention were not associated with changes in ox-LDL concentrations. An increase in ascorbic acid tended to be associated with a decrease in ox-LDL concentrations ($r = -0.22$; $P = 0.07$). Moreover, increases in niacin, panthenolic acid, and vitamin B-6 were all associated with a decrease in ox-LDL concentrations ($r = -0.26, -0.34, -0.30$; $P < 0.05$, respectively). However, no associations were found between changes in ox-LDL concentrations and changes in vitamin A, α-tocopherol, and β-carotene. In addition, a decrease in caffeine intake was associated with a decrease in ox-LDL concentrations ($r = 0.26$; $P = 0.03$). No significant difference was found in ox-LDL changes in response to the intervention between users and nonusers of dietary supplements (results not shown).

**DISCUSSION**

Our nutritional intervention promoting the Mediterranean food pattern resulted in significant changes in dietary intakes in a group of 70 free-living, healthy women. A significant decrease in ox-LDL concentrations occurred in response to this nutritional intervention. More specifically, the increase in Mediterranean dietary score, an indicator of the adherence to our nutritional intervention, was significantly correlated with the decrease in ox-LDL concentrations. To the best of our knowledge, this is the first study to demonstrate that an intervention promoting the Mediterranean food pattern under free-living conditions can lead to significant modifications of food habits, which in turn reduce plasma ox-LDL concentrations.

The Mediterranean diet is associated with healthy living and long life expectancy; people living in the Mediterranean region have one of the lowest rates of mortality from CHD (13). The Final Report of the Lyon Diet Heart Study (14), which promoted the Mediterranean diet in secondary prevention, reported a reduction in the rate of CHD recurrence after a first myocardial infarction. Interestingly, changes in major traditional risk factors such as total cholesterol and LDL-C could not explain the reduction in myocardial infarction risk in response to a Mediterranean diet (14). Thus, it is tempting to hypothesize that a large proportion of the reduction in risk of CHD observed with the Mediterranean diet could be attrib-

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**TABLE 3**

Daily intake of energy and selected nutrients as measured by the 3-d food record in healthy women at wk 0 and 12 of the nutritional intervention$^{1,2}$

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ</td>
<td>8505 ± 1919</td>
<td>7854 ± 1789$^{**}$</td>
</tr>
<tr>
<td>Carbohydrates, % of energy</td>
<td>48.9 ± 6.8</td>
<td>48.9 ± 7.0</td>
</tr>
<tr>
<td>Proteins, % of energy</td>
<td>16.4 ± 2.5</td>
<td>17.7 ± 2.9$^{**}$</td>
</tr>
<tr>
<td>Lipids, % of energy</td>
<td>33.4 ± 6.1</td>
<td>32.4 ± 6.2</td>
</tr>
<tr>
<td>SFA, % of energy</td>
<td>11.1 ± 2.7</td>
<td>9.8 ± 2.7$^{**}$</td>
</tr>
<tr>
<td>MUFA, % of energy</td>
<td>14.2 ± 3.2</td>
<td>14.2 ± 3.7</td>
</tr>
<tr>
<td>PUFA, % of energy</td>
<td>5.6 ± 1.6</td>
<td>5.8 ± 1.4</td>
</tr>
<tr>
<td>EPA, g</td>
<td>0.06 ± 0.07</td>
<td>0.13 ± 0.13$^{**}$</td>
</tr>
<tr>
<td>DHA, g</td>
<td>0.18 ± 0.23</td>
<td>0.28 ± 0.36$^{**}$</td>
</tr>
<tr>
<td>Trans, % of energy</td>
<td>1.43 ± 0.67</td>
<td>0.97 ± 0.51$^{**}$</td>
</tr>
<tr>
<td>Vitamin A, RE</td>
<td>3113 ± 1891</td>
<td>3512 ± 2259</td>
</tr>
<tr>
<td>α-Tocopherol, mg</td>
<td>10.11 ± 4.68</td>
<td>9.41 ± 3.39</td>
</tr>
<tr>
<td>Ascorbic acid, mg</td>
<td>138.7 ± 57.7</td>
<td>144.2 ± 62.6</td>
</tr>
<tr>
<td>β-Carotene, μg</td>
<td>4748 ± 3088</td>
<td>6107 ± 4498$^{**}$</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SD; $n = 70$. Asterisks indicate different from wk 0: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$.

$^2$ Abbreviations used: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RE, retinol equivalents.
uted to changes in ox-LDL concentrations. This hypothesis is consistent with data from Panagiotakos and collaborators, who demonstrated that the adoption of a Mediterranean diet did not influence the lipid profile, with the exception of a reduction in plasma ox-LDL, which was lower in people with a Mediterranean diet than in those who adopted a Westernized diet (23). Our results also agree with these previous studies because the adoption of Mediterranean food pattern was not associated with a diminution of LDL-C and total cholesterol, the traditional risk factors for CHD. Moreover, Esposito and collaborators demonstrated recently in a population with metabolic syndrome that a Mediterranean style diet may improve endothelial function and inflammatory markers, which are related to oxidative modification in the pathogenesis of atherosclerosis (24). Thus, the cardioprotective effects of the Mediterranean food pattern could be explained, at least to some extent, by reduced LDL particle oxidation.

Our data further suggest that the Mediterranean food pattern may be preventing early atherogenesis even in healthy women. In fact, we observed beneficial effects of the intervention despite the fact that women in our study were healthy. Our results also showed that ox-LDL decreased the most after 12 wk in the subset of women characterized by the most elevated ox-LDL concentrations at baseline. This suggests that more severely affected subjects could benefit more substantially from our nutritional intervention.

An increase in the consumption of fruits and vegetables appears to be the dietary change that is most likely to explain the beneficial effect of the Mediterranean diet on ox-LDL concentrations. In fact, increasing daily fruit and vegetable consumption by 1 serving each (for a mean of 4 servings of fruits and 4 servings of vegetables at the end of the intervention) was associated with a significant decrease in ox-LDL concentrations. In a prospective study of 75,596 women and 38,683 men, Joshipura and colleagues found that an increase of 1 serving of fruit or vegetables/d was associated with a 7% lower risk of ischemic stroke in women. Our study may help provide some information on the physiologic mechanisms by which this reduction in stroke events was obtained (10). Furthermore, our results could also bring further support to the relevance of public health programs that recommend the consumption of 5–10 fruits and vegetables/d (Heart and Stroke Foundation of Canada and the Canadian Cancer Society).

Further analyses were performed to investigate the contribution of nutrients on the determination of ox-LDL concentrations. An increase in vitamin C tended to be associated with a decrease in ox-LDL concentrations. Vitamin C is an antioxidant that may have a direct effect on preventing the oxidation of LDL particles (25). Because fruits and vegetables are the major sources of vitamin C, the association between increases in fruit and vegetable consumption and decrease in ox-LDL concentrations could therefore be explained to some extent by the antioxidant properties of vitamin C present in fruits and vegetables. However, other components of fruits and vegetables such as flavonoids could explain this association. In fact, dietary flavonoid intake was shown to be inversely associated with the risk of CHD (8,9) and ischemic stroke (10). It was also demonstrated that flavonoid consumption can lead to a significant decrease in the susceptibility of LDL to oxidative modification (26). Unfortunately, the database used for the nutritional analysis did not contain adequate and reliable information on levels of flavonoids in fruits and vegetables. In fact, no database for dietary assessment including complete food composition for all of the flavonoids classes is currently available. This is largely attributable to the fact that there are between 11 and 26 classes of flavonoids and >4000 compounds (27). Moreover, many factors that affect flavonoid content such as seasonal variation, light, climate, degree of ripeness, food preparation and processing (28) are very difficult to control for. It is therefore complicated to obtain information of adequate quality regarding the flavonoid content of foods. For that reason, we cannot confirm an association between changes in flavonoid content of the diet and changes in ox-LDL concentrations in our study.

Increases in niacin, pantothenic acid, and vitamin B-6 were also all significantly correlated with a decrease in ox-LDL concentrations. To the best of our knowledge, no intervention study has reported such associations. However, Hu and colleagues found that pantothenate inhibited microsomal lipid peroxidation induced by FeCl3/ascorbate, whereas pyridoxine stimulated this process in vitro (29). The fact that intakes of these micronutrients are intercorrelated may also complicate the interpretation of results. Further studies are clearly warranted to investigate the specific role of vitamins on ox-LDL concentrations.

Our results showed that a decrease in caffeine intake was also associated with a decrease in ox-LDL concentration. These results are not in line with those of Lee and colleagues who showed that in vitro, some caffeine compounds significantly reduced the level of TBARS and conjugated dienes produced from the LDL peroxidation (30). Differences in the study design and in the method used to assess LDL oxidative susceptibility may explain the discordance in results obtained.

We recognize that the present study has some limitations such as the lack of a control group. However, women with higher ox-LDL concentrations at baseline experienced notable changes in ox-LDL concentrations in response to dietary changes, thus supporting our thesis that the changes observed were related to the intervention rather than being explained by a study effect.

In conclusion, a nutritional intervention promoting the Mediterranean food pattern under free-living conditions was associated with a decrease in ox-LDL concentrations in healthy French-Canadian women. These changes in ox-LDL concentrations could be explained by an increase in the consumption of fruits and vegetables. Our study suggests that the decrease in ox-LDL concentrations might be one of the mechanisms that may explain the cardioprotective effects of the Mediterranean food pattern, which is recognized for its abundance of fruits and vegetables.

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LITERATURE CITED


