ASCULAR ATHEROSCLEROTIC LESIONS are among the chief factors reducing survival in kidney graft recipients. Graft survival is seriously affected in patients with coexisting dyslipidemia. Routine use of immunosuppressive agents, contraindications to antilipemic agents, and adherence to improper dietary habits expose kidney graft recipients to atherosclerotic risk. It is now increasingly believed that risk minimization is possible with the use of specific diets such as the Mediterranean diet (MD), recommended since 1997 by the International Consensus of European countries. However, transition to an MD may be difficult in non-Mediterranean countries because of a shortage of or the substantial cost of some foods and different culinary habits. We have searched for a diet possessing all of the cardioprotective benefits of an MD but largely based on locally available, inexpensive food products. The modified diet now developed (mMD) contains more carbohydrates with a low glycemic index (amylose-poor/cellulose-rich), 30 mL of cold-pressed olive oil, and less cholesterol. It also features an improved daily calorie distribution through frequent meals.

The goal of the present study was to verify the effect of the new diet on some risk factors of atherosclerosis in a cohort of kidney graft recipients.

Materials and Methods

Kidney graft recipients with stable graft function were randomly allocated to two groups (see Table 1 for patient data). The study group consisted of 21 patients who were placed on the new diet. Weekly menus for 4-week periods were provided. Patients in the control group (n = 16) continued with their standard low-lipid diets (typical for Central European pattern) and iso-caloric with the study diet. Isocaloric intake was determined by estimation of kilocalories per kilogram as goal and then verified by diet records and food analysis computer program (Jumar Software, 2001, Poznan, Poland). Immunosuppressive (cyclosporin, prednisone, azathioprine) and hypotensive (diuretics, calcium channel blockers, angiotensin converting enzyme [ACE] inhibitors) regimens remained unchanged. No antilipemic agents were administered before or during the experiment. No patients presented clinical evidence of cardiovascular disease (previous myocardial infarction, angina). Dietary compliance was ascertained every 4 weeks using questionnaires (24-hour food diaries) and monitoring oleic acid content in plasma triacylglycerols. Data from questionnaires were analyzed using the Dietetic Food Analysis Program (Jumar, Poland). Patient measurements were done at 0, 1, 2, and 6 months from enrollment.
Study Diet

This diet featured carbohydrates with a low glycemic index (poor in glucose, simple carbohydrates, and amylose, but rich in cellulose). Approved diet constituents included cereals, pulse, whole-rye bread, vegetables (cooked or fresh), oat flakes (cooked), and noodles prepared al dente. Amylose-rich foods, sweets, and sweet drinks were prohibited. Breakfast was the main meal, providing 39% of daily calorie intake, whereas supper provided the least (16% of total). In the study group, daily energy intake was attributed as follows: 47% carbohydrates, 38% fatty acids (including 10% saturated, 22% monounsaturated, and 6% polyunsaturated species), and 15% protein. Cholesterol and fiber supply was 165 mg/day and 47 g/day, respectively. The significant content of fiber in the diet can be attributed to the use of fresh, unprocessed food, elimination of semiprocessed products, and daily intake of pulse/cereal (e.g., buckwheat, barley)/vegetables/whole-meal rye bread.

The dominating fatty acid was oleic acid from olive oil and erucic-acid-poor rapeseed oil. Patients consumed 30 mL cold-pressed olive oil per day (fresh salads) and prepared their cooked meals exclusively with rapeseed oil. All other oils were totally eliminated from the diet. Patients consumed approximately 30 g daily of products rich in α-tocopherol and α-linolenic acid C 18:3 n-3 (grains, flaxseed, nuts). The patients were advised to consume fresh vegetables with every meal. The daily animal protein consumption was 25 to 50 g for men and 23 to 46 g for women, representing one third of the total protein. No additional vitamin supplementation was offered.

Control Diets

Questionnaires were the basis for analyzing diets in the control group. In comparison with the study diet, the glycemic index of control diets was markedly higher because of a greater content of amylose (white bread, potatoes, rice). An inverse calorie distribution was noted, breakfast providing 25% of daily calorie intake and supper providing the least (38% of total). Details of both diets are presented in Table 1. In the control group, daily energy intake was attributed as follows: 57% carbohydrates, 26% fatty acids, 17% protein. Cholesterol and fiber supply was 257 mg/day and 24 g/day, respectively. The carbohydrate component was poor in cellulose and rich in starch (white bread, potatoes, rice). The fat content was lower than in the study group, with polyunsaturated (mainly C18:2 n-6) fatty acids dominating. Questionnaires revealed that butter and sunflower oil were the main sources of fat in this group. Daily animal protein consumption was higher than in the study group (approxi-
<table>
<thead>
<tr>
<th>Study Group (n = 21)</th>
<th>Control Group (n = 16)</th>
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<tbody>
<tr>
<td><strong>Follow-up (mo)</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>230 ± 58</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>51 ± 15†</td>
</tr>
<tr>
<td><strong>Total cholesterol/HDL</strong></td>
<td>4.83 ± 1.7</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>123 ± 38</td>
</tr>
<tr>
<td><strong>LDL cholesterol/HDL</strong></td>
<td>2.58 ± 0.98</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dL)</td>
<td>194 ± 76</td>
</tr>
<tr>
<td>Oleic acid in TG (μg/mL plasma)</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Insulin (mU/mL)</td>
<td>10.3 ± 6.2</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.0 ± 4.1</td>
</tr>
<tr>
<td>WHR</td>
<td>0.89 ± 0.1</td>
</tr>
<tr>
<td>BF (kg)</td>
<td>14.8 ± 7.8</td>
</tr>
<tr>
<td>LM (kg)</td>
<td>27.6 ± 4.1</td>
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Abbreviations: TG, triacylglycerols; BMI, body mass index; WHR, waist-hip ratio; BF, body fat; LM, lean body mass.

Significant difference in relation to starting value marked by # (Wilcoxon test): *p < 0.05; **p < 0.01; ***p < 0.001.

Significant difference between groups marked by † (Mann-Whitney U test): *p < 0.05; ††p < 0.01; †††p < 0.001.

Oleic acid was isolated from triacylglycerols in 1 mL of plasma.
mately 70 g for men and 50 g for women), whereas consumption of fruits and fresh vegetables was lower. The diet was not supplemented with vitamins.

**Anthropometry**

Body weight, height, and waist and hip circumference were measured. Body fat (BF) and lean body mass (LM) were determined using Futrex 6000 XL (Futrex, Inc, Gaithersburg, MD) infra-red instrument. The body mass index (BMI) and waist-hip ratio (WHR) were calculated.

**Plasma Lipids**

Total cholesterol (TCh), low-density lipoprotein (LDLCh), and high-density lipoprotein (HDLCh) cholesterol fractions and triacylglycerols (TG) were measured in fasting plasma with commercial test kits (reference numbers 61219, 61534, 61530, and 61236, respectively; Bio-Merieux, Marcy L’Etoile, France).

**Plasma Insulin**

Fasting blood was ethylenediaminetetraacetic acid (EDTA) anticoagulated and used to measure insulin concentration in plasma with the Abbott IMX Insulin assay—Microparticle Enzyme Immunoassay—MEIA (IMX System Insulin; Abbott Laboratories, Tokyo, Japan).

**Chromatography of Triacylglycerols and Fatty Acids**

One milliliter of plasma was extracted according to Folch.6 Aliquots were placed on thin-layer chromatography plates (Merck, Germany). The triacylglycerol band was scraped off, saponified with methanolic potassium hydroxide, and methylated in the presence of boron trifluoride in methanol (15 minutes at 65°C). Fatty acid methyl esters were extracted with hexane, concentrated under nitrogen, and injected into a gas chromatograph (Perkin–Elmer 8500) equipped with a 30-meter capillary column (RTX 5 Restek). Chromatographic conditions were: injector temperature, 220°C; detector temperature, 260°C; oven temperature, 50°C to 245°C; ramp rate, 4°C/min, isothermal time, 5 minutes. Fatty acids were identified by comparing their retention times with those of high-purity standards (>99%, Sigma-Aldrich; Cayman). Peak areas were integrated using the Chromed Chromatography Software, 2000 (Poznan, Poland).

**Statistics**

Because the distribution in most cases deviated from normal (Shapiro–Wilk test), nonparametric tests were used. Significance was first checked with Friedmann ANOVA, and significant results were next subjected to the Wilcoxon matched-pairs test. Groups were compared with the Mann–Whitney U test. Correlations were ascertained with Spearman rank correlation coefficient.

**Results**

TCh levels in the study group continued to decline during the study (from 230 mg/dL at the start to 210 mg/dL after 6 months; \( P < .02 \)) (Table 2). In the control group, cholesterol levels remained essentially unchanged. The difference between both groups was already significant after 2 months of the study diet, and persisted until the end of the experiment. The LDL cholesterol levels were significantly lower in the study group after 2 and 6 months of study diet (Table 2). No significant differences in HDL cholesterol levels were noted over time or between groups.

A continuous decline in triacylglycerol levels was observed in the study group (from 194 mg/dL at the start to 152 mg/dL after 6 months; \( P < .02 \)) (Table 2). The change was significant between the starting value versus 6 months of the diet (\( P < .0007 \)), 1 versus 2 months (\( P < .04 \)), and 1 versus 6 months (\( P < .0086 \)). Significance was not shown between starting and after 1 month of the diet. In the control group, triacylglycerol levels changed insignificantly (Table 2).

No significant changes were noted in either group regarding body weight, BF, or BMI. An increase in LM was noted in controls only (Table 2). Insulin levels decreased in the study group from 10.29 μU/mL at the start to 7.7 μU/mL after 6 months of the study diet. The decrease was significant after 2 months of the diet (\( P < .046 \); \( n = 10 \)). The study and control groups differed significantly after 1 month of the experiment (6.74 versus 13.8 μU/mL; \( P < .041 \)). Statistical significance disappeared thereafter.

The content of oleic acid in triacylglycerols continued to increase in the study group and remained unchanged in controls (Table 2). The difference between groups was significant after 1 month and remained such until the end of the experiment. From the second month on, oleic
acid content correlated positively (approaching significance) with levels of TCh and LDL cholesterol (Table 3). The correlation coefficient at the end of the study reached $R = 0.59$ ($P < .009$) for TCh and $R = 0.55$ ($P < .017$) for LDL cholesterol.

**Discussion**

The fate of organ graft recipients largely depends on successful management of short- and long-term complications. Accelerated atherosclerosis observed in these patients has been attributed to hyperlipidemia resulting from dietary restrictions and immunosuppressive therapy (steroids, cyclosporin, etc.).7–9 Low-cholesterol diets rich in polyunsaturated fatty acids recommended for prevention of atherosclerosis have not lived up to their expectations.10,11 Attention has now focused on the MD, with olive oil as the main fat rich in monounsaturated oleic acid and some polyphenols.5 There are reports that olive oil prevents the formation of atherosclerotic lesions by reducing the susceptibility of lipoproteins to oxidation. Moreover, the prevalence of ischemic heart disease is lowest in European populations with a high consumption of olive oil.12–14 Promising effects of the MD in kidney graft recipients relate to significant improvements in plasma lipid profile, such as reduced triacylglycerol, TCh, and LDL cholesterol levels. The study group baseline TCh:HDL ratio was 4.83 and at 6 months decreased to 4.19; the control group baseline TCh:HDL ratio was 4.23 and at 6 months increased to 4.30. In this study, differences between groups in the concentrations of HDL at the start of the experiment were on a random basis. Considering that we observed beneficial changes in TCh for the study group (unlike in controls), we believe that the risk of cardiovascular disease was reduced in the study group, although changes in HDL in both groups were not significant. Because HDL cholesterol levels remain essentially unchanged, the LDL/HDL cholesterol ratio is also reduced.1,13–15 For LDL:HDL ratio, the study group baseline was 2.58 and at 6 months decreased to 2.28; the control group baseline was 2.32 and at 6 months was essentially unchanged at 2.28 (Table 2).

We have now developed a diet based on the MD and shown that it improved the lipid profile in kidney graft recipients. The effect cannot be attributed to lower calorie intake because our diet was isocaloric with diets in the control group. Moreover, no significant differences in body weight or BMI were found between both groups. We are inclined to attribute reduced lipid levels in plasma to lower glucose and perhaps cholesterol intake rather than to the use of olive oil per se. Increasing concentrations of oleic acid observed from the second month of the experiment correlated positively with TCh and LDL cholesterol levels, although TCh and LDL cholesterol levels continued to decrease. It has been shown that a reduced dietary load of glucose is associated with suppressed synthesis of VLDL in the liver.16 Contrary to that, dietary fatty acids (including oleic) are a substrate for and thus stimulate VLDL synthesis. Oleic acid in olive oil has been shown to suppress peroxidation processes in plasma,11 improve endothelial function,17,18 and inhibit platelet activity.19–21 No direct effects of oleic acid on triacylglycerol, TCh, and LDL cholesterol levels in plasma have been observed. In our opinion, the unequivocally hypolipidemic action found in

<table>
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<th>Correlated Parameters</th>
<th>Number of Subjects Measured</th>
<th>$R$</th>
<th>Significance ($P$)</th>
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</thead>
<tbody>
<tr>
<td>Total cholesterol concentration in plasma after 2 mo with oleic acid content in triacylglycerols after 2 mo</td>
<td>17</td>
<td>+0.45</td>
<td>.067</td>
</tr>
<tr>
<td>Total cholesterol concentration in plasma after 6 mo with oleic acid content in triacylglycerols after 6 mo</td>
<td>18</td>
<td>+0.59</td>
<td>.009</td>
</tr>
<tr>
<td>LDL cholesterol concentration in plasma after 2 mo with oleic acid content in triacylglycerols after 2 mo</td>
<td>17</td>
<td>+0.45</td>
<td>.068</td>
</tr>
<tr>
<td>LDL cholesterol concentration in plasma after 6 mo with oleic acid content in triacylglycerols after 6 mo</td>
<td>18</td>
<td>+0.55</td>
<td>.017</td>
</tr>
</tbody>
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**Table 3.** Spearman Rank Correlation Coefficients ($R$) for Lipoprotein Levels and Oleic Acid Content in Plasma Triacylglycerols After 2 and 6 Months of Study.
these studies can be attributed to a radical reduction in dietary glucose supply.\textsuperscript{22,23} A reduced dietary glucose load limits the availability of acetyl-CoA for lipid synthesis (cholesterol, fatty acids, triacylglycerols) and suppresses fasting insulin levels. Carbohydrates with a low glycemic index, more frequent but lighter meals) reduce the concentrations of lipid (cholesterol, triacylglycerols) and nonlipid (insulin) risk factors of cardiovascular disease and protect recipients against progression of atherosclerosis.

References


