Effect of a Diet Low in Saturated Fatty Acids on Plasma Lipids, Lipoproteins, and HDL Subfractions

Christian Ehnholm, Jussi K. Huttunen, Pirjo Pietinen, Ulla Leino, Marja Mutanen, Ella Kostiainen, James M. Iacono, Rita Dougherty, and Pekka Puska

The effect on serum high density lipoprotein subfractions of a low fat diet with a high ratio of polyunsaturated-to-saturated fatty acids was studied in 38 middle-aged volunteers (19 men and 19 women) in North Karelia, Finland. The mean serum HDL₂ cholesterol decreased from 32 ± 2 mg/dl (mean ± se) to 28 ± 2 mg/dl (p < 0.001) during the experimental diet and returned to 33 ± 2 mg/dl (p < 0.001) after a return to the original diet. No changes were observed in the concentration of HDL₃ cholesterol. A highly significant decrease was observed in serum apoprotein A-I concentration, but not in apoprotein A-II concentration during the experimental diet. It is concluded that a low-fat, high-P/S ratio diet lowers LDL and HDL₂ cholesterol in healthy volunteers, but does not influence the level of HDL₃ subfraction.

(Arteriosclerosis 4:265-269, May/June 1984)

Elevated low density lipoprotein (LDL) cholesterol and decreased high density lipoprotein (HDL) cholesterol plasma levels have been independently associated with an increased risk of premature coronary heart disease in studies conducted within populations.¹ ² A major factor affecting plasma cholesterol and its distribution among lipoproteins is the composition of the diet. Thus, a reduction of saturated fatty acids and cholesterol in the diet has been shown³ ⁴ to cause a significant decrease in total cholesterol and LDL cholesterol in man, whereas a decrease in the ratio of polyunsaturated to saturated fatty acids (P/S ratio) has an opposite effect. Several studies⁵⁻⁷ have suggested that a diet with a low content of saturated fats also lowers the plasma concentration of HDL cholesterol. As a result, little change is observed in the ratio of LDL cholesterol to HDL cholesterol, a fact highly predictive of future coronary heart disease within populations.²

Within each lipoprotein class there exist several subspecies that vary in their density, their chemical composition, and possibly their site of synthetic origin. The two major subfractions of high density lipoproteins, HDL₂ and HDL₃, also differ in their response to environmental stimuli. Thus, factors like physical activity⁸ and alcohol consumption⁹ influence the plasma level of the lighter HDL₂ fraction, while the changes in HDL₃ are small or nonexisting. Only preliminary and partly conflicting information exists about the role of these subfractions in the development of coronary heart disease.¹⁰ ¹¹

We have recently shown that a low fat diet with a high ratio of polyunsaturated-to-saturated fatty acids effectively lowers the levels of serum cholesterol, LDL, and HDL cholesterol in free-living inhabitants of North Karelia, a county in Eastern Finland with an exceptionally high rate of coronary heart disease.⁷ In the present study, we investigated the effects of a similar diet on the HDL subfractions in another group of free-living volunteers in North Karelia.

Methods

Subjects

This study was carried out in connection with a dietary intervention study that assessed the effect of a low fat, high P/S ratio diet on blood pressure in subjects with normal or mildly elevated blood pressure.¹² The subjects and the design of the study have been described in detail elsewhere.¹² Briefly, 57 families were selected for the study in two semirural
communities in North Karelia. They were initially identified from a community-based hypertension register. Registered persons from 30 to 50 years of age and their spouses were invited to participate. Most of the eligible couples volunteered and gave their informed consent after a full explanation of the goals and design of the study. Based on the information from a questionnaire and medical examination, subjects with major diseases or a history of specific treatment for dyslipoproteinemia as well as those currently being treated with antihypertensive drugs were excluded. The study protocol was approved according to the guidelines of the National Public Health Institute, Finland.

Serum lipid analyses carried out before the beginning of the experiment revealed a high mean serum cholesterol concentration (± SE) in both men and women (239 ± 5 mg/dl and 221 ± 5 mg/dl, respectively). These values are in accordance with earlier reports on serum lipid concentrations in the North Karelian population (see reference 7).

Experimental Design

The study consisted of three periods: a 2-week baseline period, a 6-week intervention period, and a 4-week return-to-baseline period. During the baseline and return-to-baseline periods, all families were instructed to eat their usual diet. For the intervention period, the 57 families chosen for the study were randomly allocated into three groups: Group 1 (19 couples, n = 38), Group 2 (19 couples, n = 38), Group 3 (18 couples, n = 36). During the intervention period, Group 1 subjects consumed a low fat high P/S ratio diet and those in Group 3 remained on their usual diet. Careful assessment of diets, body weight, and serum lipids and lipoproteins were carried out during and at the end of each period in Groups 1 and 3. No lipid studies were carried out in Group 2.

Diet

The change in the diet for Group 1 during the experimental period was accomplished by substituting low fat items for high fat foods typically consumed in North Karelia and by partially substituting polyunsaturated fats for saturated fats. Strategic food items in the experimental diet were margarine with a high content of polyunsaturated fatty acids, skim milk, lean meat, low fat sausage, and low fat cheese. The use of vegetables, including beets, and berries and fruit was strongly encouraged. Factors in the diet such as salt, alcohol, and coffee were kept unchanged.

The dietitians visited the families at least twice a week to supervise their adherence to the diet regimen and to other study requirements. During these visits, the dietitians brought the food items of strategic importance, advised the families in the practical management of their diet, and checked the food consumption records. Each dietitian had the same number of families from each of the study groups (eight or nine families per dietitian).

The subjects kept a careful record of food consumption for 7 predetermined days during each of the three periods. In these periods, food weights and volumes were measured and the type of food and drink was described in detail. Afterward, the food records were coded by the dietitians, and the food consumption data were processed using Finnish food composition tables and a computer program developed for this purpose.

Measurements

A fasting venous blood sample was drawn for lipid and lipoprotein analysis at the end of each period. All measurements except the determination of apoprotein A-I and A-II were carried out immediately using fresh samples. Apoprotein A-I and A-II were analyzed from samples kept at −80°C for 3 to 6 months. Cholesterol and triglyceride concentrations in serum and in various lipoprotein fractions were analyzed using Boehringer kits No 236 691 and 297 771 (Boehringer GmBH, Mannheim, FRG) in a Kone OlliC Discrete Analyzer. The total concentration of HDL cholesterol in the serum was determined after precipitation of VLDL and LDL with dextran sulfate-MgCl₂.

Apoprotein A-I and A-II were determined using an immunodiffusion method. Ultracentrifuge fractionation of serum lipoproteins was carried out for samples from Group 1 according to the method described in the Lipid Research Clinic’s study protocol.

The results are expressed as means ± SEM. Differences in the mean values were tested with Student’s t test or paired t test.

Results

The detailed composition of the diet in each group and during each period have been published elsewhere. Briefly, the proportion of calories consumed as fat decreased in Group 1 from 39% during the baseline period to 23% during the intervention period and increased again to 37% during the return-to-baseline period (Table 1).

The reduction of fat intake was mainly due to a decrease in the consumption of saturated and monounsaturated fatty acids. As a result, the P/S ratio of the diet increased from 0.27 to 0.98 during the intervention period and returned to 0.29 during the return-to-baseline period. The cholesterol content of the diet increased from 475 ± 28 mg/day during the baseline period to 282 ± 17 mg/day during the intervention period and returned to the original level during the return-to-baseline period. The proportion of carbohydrates increased by approximately 10%, and that of proteins by 6% during the intervention period. The baseline diet of Group 3 closely resem-
Table 1. Composition of Diet in Group 1 during the Three Experimental Periods, Calculated on the Basis of Food-Consumption Records of 35 Subjects

<table>
<thead>
<tr>
<th>Period</th>
<th>Protein (% of energy)</th>
<th>Carbohydrate (% of energy)</th>
<th>Fat (% of energy)</th>
<th>Total saturated fats</th>
<th>Monounsaturated fats</th>
<th>Polyunsaturated fats</th>
<th>Ethanol (% of energy)</th>
<th>Cholesterol (mg)</th>
<th>P/S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>15.3±0.3</td>
<td>44.4±0.9</td>
<td>38.6±0.7</td>
<td>19.5±0.6</td>
<td>12.8±0.4</td>
<td>4.7±0.4</td>
<td>1.7±0.4</td>
<td>475±28</td>
<td>0.27±0.02</td>
</tr>
<tr>
<td>II</td>
<td>21.4±0.4</td>
<td>53.9±0.9</td>
<td>23.3±0.6</td>
<td>6.9±0.2</td>
<td>5.9±0.2</td>
<td>6.4±0.3</td>
<td>1.5±0.3</td>
<td>282±17</td>
<td>0.98±0.04</td>
</tr>
<tr>
<td>III</td>
<td>15.5±0.4</td>
<td>46.7±0.7</td>
<td>36.8±0.6</td>
<td>18.4±0.5</td>
<td>12.1±0.3</td>
<td>4.7±0.3</td>
<td>1.0±0.3</td>
<td>470±27</td>
<td>0.29±0.02</td>
</tr>
</tbody>
</table>

During Periods I and III, the subjects consumed their normal diet, and during Period II, a low fat-high P/S-ratio diet (experimental diet). Values are means ± SEM.

Table 2. Total Serum Cholesterol, Triglycerides, and HDL Cholesterol in Group 1 and Group 3 at the Ends of the Three Study Periods

<table>
<thead>
<tr>
<th>Period</th>
<th>Total cholesterol (mg/dl)</th>
<th>VLDL cholesterol (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>HDL2 cholesterol (mg/dl)</th>
<th>HDL3 cholesterol (mg/dl)</th>
<th>Apolipoprotein A-I (mg/dl)</th>
<th>Apolipoprotein A-II (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>223 ± 7*</td>
<td>24 ± 6</td>
<td>139 ± 6*</td>
<td>32 ± 2*</td>
<td>19 ± 1</td>
<td>142 ± 6*</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>II</td>
<td>193 ± 6</td>
<td>21 ± 4</td>
<td>113 ± 5*</td>
<td>28 ± 2</td>
<td>19 ± 1</td>
<td>118 ± 3</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>III</td>
<td>226 ± 6*</td>
<td>23 ± 3</td>
<td>135 ± 5*</td>
<td>33 ± 2*</td>
<td>19 ± 1</td>
<td>139 ± 3*</td>
<td>39 ± 1</td>
</tr>
</tbody>
</table>

During Period II, subjects in Group 1 consumed a low fat high-P/S diet (experimental diet), and subjects in Group 3, their normal diet (i.e., the same diet as during the baseline and return-to-baseline periods). Values are means ± SEM.

*Significantly different from the value for the intervention period (p < 0.001 paired comparison test).
†Difference between Groups 1 and 3 is statistically significant (p < 0.01).

The mean body weight of Group 1 decreased 0.7 kg during the intervention period and increased 0.2 kg during the return-to-baseline period. The concentrations of serum cholesterol, triglycerides, and HDL cholesterol in Groups 1 and 3 are shown in Table 2. A significant decrease in total cholesterol and HDL cholesterol was observed in Group 1 during the low fat high P/S-ratio diet, with a return to the original level during the return-to-baseline period. In keeping with our earlier experience, no change was observed in serum triglycerides despite the 10% increase in the dietary intake of carbohydrate. No significant changes were seen in serum lipids in Group 3 during the study.

The concentrations of various lipoprotein fractions (as lipoprotein cholesterol) in Group 1 at the end of each study are given in Table 3. The decrease in total cholesterol during the experimental diet was due to reductions in LDL cholesterol and HDL cholesterol. In contrast, the concentrations of VLDL cholesterol and HDL₃ cholesterol remained constant throughout the study. Since the percentage changes in LDL cholesterol and HDL₃ cholesterol were similar, the ratio of HDL₃ cholesterol to LDL cholesterol was not significantly changed (Table 4). The correlation between the individual changes in LDL choles-
Table 4. Ratios of HDL Cholesterol to LDL Cholesterol and HDL₂ Cholesterol to LDL Cholesterol in Group 1 at the Ends of Study Periods

<table>
<thead>
<tr>
<th>Period</th>
<th>HDL cholesterol/ LDL cholesterol</th>
<th>HDL₂ cholesterol/ LDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.38 ± 0.02</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>II</td>
<td>0.43 ± 0.03</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>III</td>
<td>0.40 ± 0.02</td>
<td>0.25 ± 0.02</td>
</tr>
</tbody>
</table>

During Periods I and III, the subjects consumed their normal diet, and during Period II, a low fat-high P/S diet (experimental diet). Values are means ± SEM.

The concentration of apolipoprotein A-I changed in parallel with LDL cholesterol and HDL₂ cholesterol during the experimental diet (Table 3). On the other hand, no changes were seen in the concentration of apolipoprotein A-II during the study period.

Discussion

The present results confirm and extend our previous observations on the effect of a fat-modified diet on plasma lipoproteins in free-living inhabitants of North Karelia.12 Thus, a highly significant decrease in LDL and HDL cholesterol concentrations was seen in the subjects of Group 1 during the low fat high P/S ratio diet, with a return to the initial levels after the return to the usual North Karelian diet. In contrast, no change in plasma lipids and lipoproteins was detected in the control subjects who remained on their usual diet throughout the study. This result confirms our previous conclusion that the high serum cholesterol level in this population is predominantly due to the local dietary patterns.

Only few studies have thus far been published on the effects of dietary modifications on HDL subfractions. The present results demonstrate that the reduction in HDL cholesterol during the low fat high P/S ratio diet is entirely due to a change in the HDL₂ fraction. Thus, a highly significant decrease took place in the concentrations of HDL₂ cholesterol and total serum apoprotein A-I, the major protein component of HDL₂. On the other hand, the levels of HDL₃ cholesterol and apoprotein A-II, the peptide more characteristic of HDL₃, remained constant during dietary changes. These results are in keeping with the recent reports by Kashyap et al.18 and Brussaard et al.19 who observed a decrease in HDL₂ cholesterol during a diet with drastically reduced fat content.

Since the dietary intervention in Group 1 included a decrease in total fat, saturated fat, and cholesterol content and an increase in the P/S ratio and in the intake of polyunsaturated fat and total carbohydrate, it is not possible to point out the exact reason for the reduction in serum HDL₂. The data from several recent reports suggest that all these factors may influence the level of HDL cholesterol, and hence probably also the level of HDL₂ subfraction. Thus, reduction in the dietary saturated fat has been shown to lower HDL cholesterol.5 6 18 20 A similar change has been observed after an increase in the P/S ratio in some20-23 but not in all studies.24 25 Dietary cholesterol may also raise HDL cholesterol although the effect may be limited to diets with a low content of polyunsaturated fatty acids.21 It should be noted that despite the substantial increase in the P/S ratio in the intervention diet of Group 1 in our study, the absolute increase in the amount of polyunsaturated fatty acids was rather small (from 4.7% to 6.4% of the total energy intake, see reference 12).

Parallel changes in the mean serum concentrations of LDL cholesterol and HDL cholesterol in our previous study, and between LDL cholesterol and HDL₃ cholesterol in this study, might be taken to indicate that the effects of various dietary components on the two lipoprotein fractions are identical. If this were true, it would be impossible to lower the level of the atherogenic LDL without a decrease in the presumably antiatherogenic HDL₂ fraction. Indirect evidence against this argumentation is, however, available. First, no correlation was observed in...
this study between the individual changes in LDL and HDL subfractions during the intervention period. Second, the results from a 6-year intervention study carried out in Oslo suggest that it is possible to lower serum cholesterol by dietary means without a change or even with an increase in HDL cholesterol. Thus, the long-term effects of the diet on HDL and its subfractions may not be similar to those observed during a 6-week intervention. Finally, it should be remembered that the evidence for the antiatherogenic properties of total HDL and HDL subfractions is still indirect. Thus, it is not known whether the changes in HDL fractions due to alterations in alcohol consumption, physical activity, or diet will in any way influence the course of atherosclerosis in man. In fact, low serum HDL cholesterol levels seems to be characteristic of several populations with a relatively low rate of coronary heart disease. 27, 28

In conclusion, our results demonstrate that a low fat high P/S ratio diet lowers LDL cholesterol, HDL cholesterol, and HDL subfractions in healthy volunteers, whereas under these conditions no significant changes are seen in the serum level of HDL cholesterol or in the ratio of HDL cholesterol and HDL subfractions to LDL cholesterol. The individual changes in LDL and HDL subfractions are not related to each other, suggesting that the dietary factors responsible for the alterations in the two fractions may not be identical.

Acknowledgments

We are indebted to Jouko Sundvall, Kirsi Ollila, and Sega Poonmilais for technical assistance, and A.-L. Luukkainen, the seven field dietitians, and the local health centers in Juuka and Lieksa.

References

27. Knulman JT, Herrus RJ, Hautvast JGA. Serum total and high density lipoprotein (HDL) cholesterol concentrations in rural and urban boys from 16 countries. Atherosclerosis 1980:36:529–537

Index terms: diet • fat • P/S ratio • cholesterol • triglyceride • lipoproteins • apoproteins • HDL • LDL • VLDL
Effect of a diet low in saturated fatty acids on plasma lipids, lipoproteins, and HDL subfractions.
C Ehnholm, J K Huttunen, P Pietinen, U Leino, M Mutanen, E Kostiainen, J M Iacono, R Dougherty and P Puska

*Arterioscler Thromb Vasc Biol.* 1984;4:265-269
doi: 10.1161/01.ATV.4.3.265

*Arteriosclerosis, Thrombosis, and Vascular Biology* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/4/3/265

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Arteriosclerosis, Thrombosis, and Vascular Biology* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Arteriosclerosis, Thrombosis, and Vascular Biology* is online at:
http://atvb.ahajournals.org//subscriptions/