Apolipoprotein E phenotype and diet-induced alteration in blood pressure¹⁻⁴

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ABSTRACT The purpose of the study was to answer the following two questions. First, are the diet-induced changes in the plasma cholesterol concentration associated with a change in blood pressure? Second, is the possible diet-induced change in blood pressure related to the apolipoprotein E (apo E) phenotype? Two hundred employees of our hospital volunteered and among those, 23 subjects with the apo E3 (E3,3) and 21 with the apo E4 phenotype (E4,3 or 4,4) were selected. The apo E groups were age-and sex-matched. Study subjects were healthy, had normal body weights, and their mean (± SD) age was 37.9 ± 7.7 y. The total energy derived from dietary fat was 37%, 26%, and 38% during the baseline, low-fat, and high-fat diet periods, respectively. The two intervention diets were consumed by the study subjects for 4 wk at a time. During the trial blood pressure was measured once a week with an automatic device under standardized conditions. Systolic, diastolic, and mean arterial pressures were significantly reduced during the low-fat diet period compared with baseline, but not compared with the high-fat diet period among the apo E4 subjects only (−6%, −4.5%, and −6%, respectively). The high-fat diet was associated with elevation of blood pressure among 70% of study subjects. A slight but significant positive correlation was noted between the plasma total cholesterol concentration and blood pressure, more so among the apo E4 subjects. Furthermore, age was correlated with blood pressure response in apo E4 subjects. In conclusion, both the systolic and diastolic blood pressures were significantly altered during the different diet periods. The dietary response of blood pressure seemed to differ between subjects with the apo E4 and those with the apo E3 phenotype. Am J Clin Nutr 1997;65:543–50.

KEY WORDS Cholesterol, diet, fat, apolipoprotein, blood pressure

INTRODUCTION

Elevated blood pressure and elevated plasma cholesterol concentrations are independent risk factors for coronary artery disease (CAD), and the simultaneous presence of these two risk factors confers an additional risk for CAD (¹–³). On the other hand, there is evidence of an interaction between blood pressure and plasma cholesterol concentration. As shown in several population-based studies (⁴, ⁵), recently reviewed by Goode et al (⁴), plasma cholesterol concentration and blood pressure are positively correlated. Also, the reduction in plasma cholesterol concentration achieved either by dietary intervention or by hypolipidemic medication is associated with lowered blood pressure and even a reduction in the incidence of hypertension (⁴, ⁶).

The diet-induced alteration of blood pressure has been attributed to several dietary components. During dietary trials it is difficult to change one single factor at a time, and, thus, it has been difficult to separate the effects on blood pressure of the various dietary components. Dietary changes designed to lower the plasma cholesterol concentration (ie, by reducing the total and saturated dietary fat and cholesterol and simultaneously enriching the diet with polyunsaturated fat) generally reduce blood pressure in humans (⁷–¹²), although some controversial results have also been published (¹³–¹⁶).

Extensive changes in plasma cholesterol concentrations can be achieved by dietary changes (¹⁷–²³) but there are some interindividual differences in responsiveness (²⁴–²⁹). The apolipoprotein E (apo E) phenotype has a powerful role in the regulation of the plasma cholesterol concentration and it has also been suggested to be one of the genetic factors that partly explain CAD risk (³⁰). The apo E phenotypes 4,4 and 4,3 (E4) are associated with a higher cholesterol concentration than is the apo E3,3 phenotype (E3) (³¹, ³²). There have also been some studies on the effect of apo E phenotype on the dietary response of plasma cholesterol concentration (³³–³⁹), whereas other studies (²³, ⁴⁰–⁴⁸) have revealed equal alterations in plasma cholesterol concentrations among apo E3 and E4 phenotypic subjects. An association of the apo E4 phenotype with high systolic blood pressure has been observed (⁴⁹). On the other hand, there is some evidence suggesting that the distribution of the apo E phenotypes among hypertensive patients is identical to that found in the general population (⁵⁰). These findings prompted us to investigate the possible role of apo E as a link between blood pressure and plasma cholesterol concentration.

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SUBJECTS AND METHODS

Subjects

The study subjects were employees of the Oulu University Central Hospital. Of the 200 subjects who volunteered for the study, we selected 23 subjects with the apo E3 phenotype (E3,3) and 21 with the apo E4 phenotype (17 subjects with E4,3 and 4 subjects with E4,4). The subjects were eligible if they did not have a history of hypertension, CAD, diabetes, or any other disease of major importance. The ages of the subjects ranged from 22 to 54 y, the mean age being 37.9 y for men and 39.4 y for women. The body mass index (BMI, in kg/m²) ranged from 19 to 28, the mean being 23. A physical examination and routine laboratory tests ruled out any abnormalities of clinical importance. None of the subjects were taking any medication affecting blood pressure. They were encouraged to maintain their normal living habits and amount of exercise during the trial. Eleven subjects were cigarette smokers, and they were distributed equally into both of the apo E subgroups. The apo E3 and E4 groups were age- and sex-matched (Table 1). Plasma cholesterol concentrations at baseline were similar in both apo E groups (Figure 1). All subjects were working either in the technical department, the administrative office, or the kitchen of the hospital. The study was approved by the Ethical Committee of the University of Oulu.

Design of the trial

A more detailed description of the design of the trial and the lipid data were published earlier (23). During the baseline diet period, the study subjects consumed their habitual diets. After the baseline period of 3 mo, all subjects received a low-fat, low-cholesterol diet for 4 wk and immediately after that a high-fat, high-cholesterol diet for another 4 wk. All food was delivered from the hospital kitchen to be consumed in the hospital dining room during the working days (~75% of the daily energy intake) or at home (~25% of the daily energy intake).

Diets

Seven-day food consumption records were kept during the baseline diet period. The food records were collected after the first visit to our laboratory. All study subjects were advised about how to prepare food diaries, and all food diaries that were returned were also checked by a dietitian at the second visit to our laboratory. At baseline, blood pressure was measured once before and once after the food records were obtained. This food record information was used to calculate the nutrient content of the baseline diets. The baseline diet of the study subjects represented a typical Western diet and consisted partly of meals from the hospital kitchen and partly of the subjects’ habitual food eaten at home (Table 2). According to the food diaries collected during the baseline period and the clinical interview (data not shown), the amount of alcohol used by the study subjects was negligible and therefore alcohol was not included in the calculations of diets.

The two intervention diets were designed on the basis of the regular hospital meals. The hospital meals were analyzed for 7 d. The original goal for the composition of the diet was as follows: 1) the low-fat diet provided 25% of energy from total fat, with 34% of total fat from saturated fat, 33% from monounsaturated fat, and 33% from polyunsaturated fats, and 2) the high-fat diet provided 38% of energy from total fat, with 57% from saturated fat, 31% from monounsaturated fat, and 12% from polyunsaturated fat. After the trial, the nutrient contents of the intervention diets were recalculated according to the actual daily records, and according to these calculations the dietary composition was as follows: 1) 26% of energy from fat, with 32% from saturated fat, 35% from monounsaturated fat, and 33% from polyunsaturated fat, and 2) 38% of energy from fat, with 60% from saturated fat, 31% from monounsaturated fat, and 9% from polyunsaturated fat. The most suitable energy intake was chosen according to the subjects’ food consumption records and daily physical activity; mean daily energy intakes were 12.6 MJ for men and 8.4 MJ for women. The 7-d food consumption records were repeated during the second week of the low-fat diet period to control compliance with the trial. To calculate the individual dietary compositions, the NUTRICIA (Social Insurance Institution, Turku, Finland) and AIVO (AIVO Finland Oy, Turku, Finland) nutrition software programs were used. The calculations of the compositions of the diets were based on the Food and Nutrient Database of the Social Insurance Institution (51).

Blood pressure measurements

All blood pressure measurements were made by using an automatic, microprocessor-controlled device (Critikon Dinamap 1846 SX/P; Critikon Inc, Tampa, FL), in which an oscillometric technique is used and which determines the systolic blood pressure, the mean arterial pressure (MAP), the diastolic blood pressure, and the pulse rate. The pulse pressures (difference between the systolic and diastolic blood pressures) were calculated by using the Dinamap values. With this device, the CV (SD by the mean) calculated from 13 sequential measurements made on the same person at 3-min intervals was 6% for systolic blood pressure, 3% for diastolic blood pressure, and 5% for both the pulse and MAP.

Blood pressure was measured on at least two occasions during the baseline period and once a week during both of the intervention periods. Blood pressure was registered by the same experienced nurse on all occasions. Before each measurement, the subjects rested for ≥10 min and blood pressure was measured from the right arm with the subjects sitting. Two measurements were taken at a time and the second value was recorded. For the final results, the mean of the two values from the baseline period and the mean of the last two values from each intervention period were used.
Body weight

The study subjects were asked to weigh themselves daily during the trial and they were further weighed once a week in our laboratory with a digital weighing scale (Lindetronic 4000; Lindells, Uppsala, Sweden).

Lipoprotein analysis

Blood samples were collected for analysis of the plasma total cholesterol concentration after an overnight fast. The cholesterol concentrations (enzymatic colorimetric method, catalogue no. 236691; Boehringer Diagnostica, Mannheim, Germany) were analyzed by using a Gilford IMPACT 400E Clinical Chemistry Analyzer (Gilford Instruments Laboratories Inc, Oberlin, OH). The apo E phenotype was determined from the plasma after delipidation by using the isoelectric focusing and immunoblotting techniques (52, 53).

Statistical analysis

The normality of variables was tested by using the Shapiro-Wilk W test. The intervention-induced changes in systolic blood pressure and diastolic blood pressure, MAP, pulse pressure, and pulse, and especially the effects of apo E phenotype and sex were tested with respect to the intrasubject variation during the dietary intervention periods. This was done by using a layered design in the form of repeated measures across time. The top layer in the model was the between-subject layer, in which the effect of having an apo E3 or E4 phenotype (or being male or female) was tested with respect to the variation from subject to subject. The bottom layer was the within-subject layer, in which the repeated-measures factor for the diet periods (baseline, low-fat, and high-fat) was tested with respect to the variation from one dietary period to another. For the significant effects revealed by analysis of repeated measures, a further paired Student’s t test (or signed rank test when appropriate) was performed to evaluate the respective P values. The results are expressed as means ± SEMs.

Correlations were tested between the changes induced by dietary interventions and the baseline values of systolic blood pressure, diastolic blood pressure, BMI, plasma cholesterol concentration, and different dietary constituents. The corre-
Nutrient composition of the diets per megajoule

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Baseline period(^1) (n = 43)</th>
<th>Low fat(^2) (n = 44)</th>
<th>High fat(^2) (n = 44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (g)</td>
<td>10.3 ± 1.6</td>
<td>11.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>26.8 ± 3.6</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>9.6 ± 1.6</td>
<td>6.7</td>
<td>10.1</td>
</tr>
<tr>
<td>Saturated fats (g)</td>
<td>4.3 ± 1.0</td>
<td>1.9</td>
<td>5.6</td>
</tr>
<tr>
<td>Monounsaturated fats (g)</td>
<td>3.2 ± 0.6</td>
<td>2.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Polyunsaturated fats (g)</td>
<td>1.3 ± 0.4</td>
<td>2.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>38.8 ± 9.3</td>
<td>27</td>
<td>40</td>
</tr>
<tr>
<td>Palmitic acid (g)</td>
<td>2.1 ± 0.4</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Stearic acid (g)</td>
<td>0.9 ± 0.2</td>
<td>0.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Linoleic acid (g)</td>
<td>1.1 ± 0.4</td>
<td>1.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Linolenic acid (g)</td>
<td>0.2 ± 0.02</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>2.7 ± 0.7</td>
<td>4.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>461 ± 85</td>
<td>462</td>
<td>466</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>476 ± 90</td>
<td>670</td>
<td>516</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>156 ± 41</td>
<td>205</td>
<td>173</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>45.9 ± 8.1</td>
<td>52</td>
<td>40</td>
</tr>
</tbody>
</table>

\(^1\) \(\bar{x} \pm SD\). Calculated from 7-d food diaries.  
\(^2\) Calculated from 7-d food records.

RESULTS

The initial values and percentage change of systolic blood pressure, diastolic blood pressure, and MAP during the trial are shown in Table 3. Subjects with the apo E4 phenotype had higher MAPs than did apo E3 subjects (P < 0.01). After the MAP of the baseline period was adjusted for BMI, age, and plasma total cholesterol concentration, values were adjusted separately for women and men. All calculations were carried out by using JMP statistical software (SAS Institute Inc, Cary, NC).

mm Hg, P < 0.01) and diastolic blood pressure (−2.6%, −2.2 mm Hg, P < 0.01) in all study subjects during the low-fat diet period. The low-fat diet altered the systolic blood pressure values more in the apo E4 (−5.8%, −8.6 mm Hg) than the apo E3 subjects (−1.0%, −1.3 mm Hg, P < 0.05; Table 3 and Figure 1). A similar trend was observed in diastolic blood pressure (−4.5%, −3.9 mm Hg in apo E4 subjects and −0.8%, −0.7 mm Hg in apo E3 subjects, P = 0.07). The MAP declined during the low-fat diet, the reduction being larger in the apo E4 subjects (−6.1%, −6.5 mm Hg) than the apo E3 individuals (−1.5%, −1.5 mm Hg, P < 0.01).

When the subjects were switched from the low-fat to the high-fat diet, blood pressure increased (Table 3 and Figure 1). Systolic blood pressure was elevated by an average of 2.9 mm Hg (P < 0.01) and diastolic blood pressure by 1.6 mm Hg (P < 0.05). Also, during the high-fat diet period the apo E4 subjects tended to show a greater increase in systolic blood pressure
(2.7%, 3.5 mmHg) than did the apo E3 subjects (2.1%, 2.4 mmHg), but this difference was not significant. By contrast, diastolic blood pressure during the high-fat period responded similarly in both the apo E groups (2.2%, 1.4 mmHg and 2.2%, 1.7 mmHg). On average, MAP was significantly elevated in both the apo E groups. Only 30% of the subjects (n = 13) did not respond with an elevation of MAP; their MAP was reduced. Those who responded with an elevation of MAP (n = 31) during the high-fat period were on average younger (aged 37 ± 1 y) and their mean plasma cholesterol concentration was higher at the beginning of the study (5.42 ± 0.16 mmol/L) compared with the rest of the subjects (40 ± 2 y and 4.73 ± 0.24 mmol/L). The number of responders was equal among apo E3 and E4 subjects (n = 15 and n = 16, respectively).

The plasma total cholesterol concentration decreased by 17% (P < 0.05) from the baseline period to the end of the low-fat period and increased by 28% (P < 0.001) from the low-fat to the end of the high-fat period (Figure 1). Lipid changes were equal in the apo E3 and E4 groups, their mean cholesterol concentrations being 5.28 ± 0.19 and 5.13 ± 0.20 mmol/L at baseline, 4.51 ± 0.18 and 4.46 ± 0.18 mmol/L in the low-fat period, and 5.72 ± 0.21 and 5.61 ± 0.22 mmol/L in the high-fat period, respectively. A slight but significant positive correlation was noted between the plasma total cholesterol concentration and the systolic (r = 0.30, P < 0.05) and diastolic (r = 0.34, P < 0.05) blood pressures at baseline. These correlations were stronger for apo E4 subjects than for apo E3 subjects (Table 4).

No significant correlation was observed between the change in plasma cholesterol concentration and the change in blood pressure when the subjects were switched from the baseline period to the low-fat period. The change in plasma cholesterol concentration of the apo E4 subjects when switched from baseline to the low-fat diet correlated significantly and positively with the change in MAP (r = 0.52, P < 0.05). Furthermore, the age of the apo E4 subjects correlated negatively with the diet-induced change in blood pressure (Figure 2).

Body weights of the study subjects during the trial are shown in Figure 1. Only a slight reduction of body weight was noted in women during the whole trial (~0.5 ± 0.2 kg) but weight changes were the same in the two apo E groups. Overall, a positive correlation was noted between the BMI and blood pressure values at baseline (r = 0.47, P < 0.01), and this correlation was stronger in the subjects with the apo E4 phenotype for both systolic blood pressure and diastolic blood pressure (Table 4).

According to the 7-d food records collected during the baseline period, dietary intakes of total fat, carbohydrates, protein, fiber, cholesterol, saturated fats, polyunsaturated fats, monounsaturated fats, linoleic acid, and linolenic acid did not differ between the apo E groups at baseline. Also, dietary intakes of sodium, calcium, potassium, and magnesium were the same in the apo E3 and E4 individuals. There was a negative correlation between the dietary intake of fiber and systolic blood pressure in the apo E4 subjects (r = −0.46, P < 0.05), whereas apo E3 subjects showed no significant correlation. Furthermore, the amount of dietary linoleic acid correlated negatively with the systolic blood pressure at baseline, and this correlation was significant only in apo E4 subjects (r = −0.69, P < 0.01). Most of the dietary components listed in Table 2 were significantly altered between the intervention diets. Among the various dietary constituents, changes in dietary intake of linoleic acid correlated with the reduction of diastolic blood pressure (r = −0.35, P < 0.05), whereas changes in dietary intakes of fiber, calcium, cholesterol, and linolenic acid showed uniform but insignificant correlations with the decline in systolic blood pressure (r = −0.26, r = 0.25, r = 0.26, and r = −0.20, respectively).

### DISCUSSION

To our knowledge, this is the first prospective study of dietary effects on blood pressure in subjects with different apo E phenotypes. In this study, the low-fat diet significantly reduced both systolic and diastolic blood pressures and MAP in all study subjects during the 4-wk intervention period, confirming the results from previous studies (7–12). Moreover, the low-fat diet especially reduced the blood pressure of the subjects with the apo E4 phenotype compared with those with the apo E3 phenotype. This difference was found in both systolic blood pressure and MAP, and a trend was noticed in diastolic blood pressure.

The blood pressure measurements were standardized and every participant visited the same nurse at least twice during the 3-mo baseline period for blood pressure measurement. To allow the blood pressure to stabilize during both intervention periods, only the values recorded at the end of the last 2 intervention weeks were used for analysis. However, some
apo E groups at baseline, and this result is in accord with previous results (49). On the other hand, after adjusting systolic blood pressure, diastolic blood pressure, and MAP for age, BMI, and plasma total cholesterol concentration, the blood pressure values of only the apo E4 subjects tended to be higher than those of the apo E3 individuals in our trial. Thus, more information on the association between blood pressure and apo E phenotype is needed to explore this question more extensively.

The apo E4 phenotype is associated with higher plasma cholesterol values than the apo E3 phenotype (31, 32) and this is thought to be the major reason for the enhanced CAD risk among apo E4 individuals (30). On the other hand, the plasma cholesterol concentration is known to be associated with blood pressure, and cholesterol lowering by either a dietary intervention or medication has been associated with a reduction in the number of subjects developing hypertension (4–6). In this trial, the responses to diet of the plasma cholesterol concentrations in the apo E3 and E4 groups did not differ from each other, a result that has been confirmed by some (40–48), but not all other studies (33–39). In agreement with previous studies (4), baseline blood pressure was related to the plasma cholesterol concentration, the correlation being approximately the same for both systolic and diastolic blood pressures. Interestingly, the correlation between blood pressure and plasma cholesterol concentration was stronger in subjects with the apo E4 phenotype than in those with the apo E3 phenotype, which supports the hypothesis that apo E may act as a link between blood pressure and plasma cholesterol concentration.

Even though the alterations in blood pressure and especially in plasma cholesterol concentrations were significant, these changes among all the study subjects were not positively correlated with each other. The lack of any such correlation may be due to the small sample size. Also, the relatively short intervention period in this study might be a factor. At any rate, the reactivity of vascular smooth muscle cells has been related to the plasma cholesterol concentration, and one explanation for changes in blood pressure after the reduction of plasma cholesterol concentration could be improvement in the regulation of endothelial function (55–58). It is possible that the changes in plasma cholesterol concentration have long-term effects on the arterial wall, especially on the function of the endothelium and, thus, on the reactivity of blood vessels. A longer intervention could have resulted in an even greater reduction in blood pressure and a better correlation between the alterations in the blood pressure and the alterations in plasma cholesterol concentration. Furthermore, other mechanisms, still related to apo E, that regulate blood pressure might outweigh the effects of a change in plasma cholesterol. One of these might be aging. In this study, a subject’s age explained the blood pressure response: apo E4 subjects’ ages correlated negatively with their blood pressure response during the high-fat diet (Figure 2). This may reflect reduced endothelial function in the apo E4 subjects.

BMI is known to be related to blood pressure (59), and this correlation was also observed in our study. This positive correlation was stronger in the apo E4 than the E3 subjects. Nevertheless, because body weights did not change significantly in this study, neither the alterations in blood pressure nor the differences in dietary responsiveness of blood pressure are likely to be explained by alterations in BMI.
The numbers of smokers and nonsmokers did not differ between the study groups, and all of the smokers continued smoking during the trial, which excludes changes in smoking habits as a confounding factor. Of the nutrients reported at baseline, only the consumption of dietary linoleic acid was slightly different between subgroups, the men consuming less than the women. Compliance with the intervention diet was good, as shown by the marked alterations in plasma cholesterol concentrations. Furthermore, all the study subjects continued their physical exercise habits and full-time work during the trial, thus minimizing the potential effects of changes in lifestyle.

Dietary modifications, including sodium restriction, limitation of the amount of saturated fatty acids, and increased dietary intake of n-6 polyunsaturated fatty acids as well as dietary fiber are known to be related to blood pressure reduction. One of the main purposes of the dietary changes during the trial was to lower the plasma cholesterol concentration with the low-fat diet and, on the other hand, to raise the plasma cholesterol concentration with the high-fat diet. The amount of dietary sodium was planned to remain unchanged, and it was indeed shown to be the same during the different periods according to the nutrient content calculations based on the food diaries. However, because the urinary excretion of sodium was not measured, some alteration in sodium intake cannot be ruled out. Several changes occurred simultaneously in the composition of the diet when subjects were switched from one diet phase to another (Table 2). Hence, many of the dietary changes may have contributed to the alteration in blood pressure, the final effect being the sum of them.

Both CAD and hypertension are common diseases in many Western populations, the prevalence of the latter being almost 30% for subjects aged 30–59 y in Finland (60). The Finns also have high plasma total cholesterol concentrations (61), high prevalence of the apo E4 phenotype (53), and a relatively high intake of fat (62). High blood pressure has a significant role in CAD risk, especially in populations with high cholesterol concentrations (63). According to previous results, endothelial dysfunction and elevated blood pressure can be induced by elevated plasma cholesterol concentration, and according to our results, altered responsiveness during a dietary treatment may be a consequence of altered endothelial function. In conclusion, significant blood pressure alteration can be achieved with a diet designed to change plasma cholesterol concentration, the dietary response in blood pressure of subjects with the apo E4 phenotype being more marked than that of subjects with the apo E3 phenotype.

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