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Magnitude of dietary effects on plasma cholesterol concentration: role of sex and apolipoprotein E phenotype

Markku J. Savolainen, Maire Rantala, Kari Kervinen, Leea Järvi, Kaisu Suvanto, Tapio Rantala and Y. Antero Kesäniemi

Department of Internal Medicine, University of Oulu, Kajaanintie 50, SF-90220 Oulu (Finland)

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Summary

The effects of fat-controlled, low-cholesterol and high-fat, high-cholesterol diets pursued for 4 weeks on plasma lipids and lipoproteins were studied in 44 healthy middle-aged subjects (22 women and 22 men). All the calories were supplied from the hospital kitchen. When the subjects were switched from the fat-controlled, low-cholesterol diet to the high-fat, high-cholesterol diet the average increase in total cholesterol was 1.2 mmol/l (28%), ranging from 0.2 to 2.7 mmol/l (4–56%). At the same time the average increase in LDL cholesterol was 1.0 mmol/l (39%), ranging from 0.1 to 2.4 mmol/l (3–90%). Interestingly, the men responded to the dietary changes more sensitively than the women. The increase in total cholesterol from the low-fat to the high-fat diet was 31% for the men and 25% for the women ($P < 0.05$), the corresponding increases in LDL cholesterol being 42% and 37%, respectively ($P < 0.05$). A marked increase in HDL cholesterol was observed when the subjects were switched from the low-fat to the high-fat diet, the increase being 30% for the men and 20% for the women. The absolute and percentage lipid changes on the two diets were equal in the subjects with the common apolipoprotein E phenotype 3/3 and in those homozygous and heterozygous for the epsilon 4 allele (E4/4 and E4/3). Overall, the data show that middle-aged men, the main risk group for coronary heart disease, seem to be particularly responsive to this diet modification, and the effects of a prudent diet can also be obtained in subjects with a genetic predisposition to a moderate elevation in plasma cholesterol level, i.e., those who fall into the phenotypic categories E4/4 and E4/3.

Key words: Hypercholesterolemia; Cholesterol; Apolipoprotein E; Sex; Atherosclerosis; Triglycerides; Diet; Coronary heart disease

Introduction

A high plasma cholesterol concentration is a well-established risk factor for coronary heart dis-

Correspondence to: Y. Antero Kesäniemi, Professor of Medicine, Department of Internal Medicine, University of Oulu, Kajaanintie 50, SF-90220 Oulu, Finland.

case, and plasma cholesterol levels can be significantly reduced by dietary modification [1–4]. More importantly, the incidence of coronary heart disease can also be reduced by long-term dietary treatment [5], particularly in conjunction with smoking intervention [6].

The magnitude of dietary effects on plasma cholesterol level seems to vary between individuals [3,4,7]. While the average lowering of plasma cholesterol by diet is often only 10%, some subjects may respond to a fat-controlled, low-cholesterol diet with a remarkable 30% reduction in their cholesterol level [3,4]. These differences could obviously be due to true inter-individual variation in the response to dietary changes, but poor adherence to the diet might also be responsible. These data and recent findings on both plasma cholesterol levels [8–11] and the intestinal absorption of cholesterol [12] being associated with the genetically determined phenotypic pattern of apolipoprotein E prompted us to explore the possibility that the response to a lipid-lowering diet might be related to inherited factors such as the apolipoprotein E phenotype.

The present trial was designed to investigate whether the magnitude of the dietary effects on plasma total cholesterol and lipoprotein cholesterol concentrations depend on the apolipoprotein E phenotype in middle-aged individuals living normal lives when their diets are well controlled. In addition, we wanted to study how frequently the recently launched target value for plasma cholesterol, 5.2 mmol/l [13,14], can be achieved by dietary modification and whether the response to diet is equal in both sexes.

Materials and methods

Subjects

Employees of the technical department, administrative office and kitchen of Oulu University Central Hospital were invited to participate in the trial. Two hundred out of 320 (62.5%) volunteered. Their initial plasma cholesterol concentration and apoprotein E (apoE) phenotype were analysed. The mean cholesterol concentration was $5.41 \text{ mmol/l} \pm 0.09 \text{ (SEM)}$ (range 3.06–11.7 mmol/l). The distribution of apoE phenotypes was the same as previously found in the Finnish population

[10,12], the gene frequency being 20%, 75% and 5% for the epsilon 4, 3 and 2 alleles, respectively.

Of the population screened for their apoE phenotype, 21 subjects with the phenotype E4/4 or E4/3 volunteered for the diet intervention trial. These subjects were matched for age, sex and body weight with 23 individuals having the phenotype E3/3. On the evidence of a medical history, a physical examination and routine clinical laboratory tests all the subjects were free of any cardiovascular, thyroid or hepatic disease, diabetes mellitus or renal dysfunction. None of them was receiving any regular medication. Twenty-two of the subjects were men and 22 women, the mean ages being 36 years and 37 years, respectively (Table 1). Two men and 2 women had phenotype E4/4, and 9 men and 8 women the E4/3 pattern. The subjects who were homozygous and heterozygous for the epsilon 4 allele were analysed together and will be later designated E4 individuals. Eleven men and 12 women had the phenotype E3/3 (designated below E3). The body mass index among all the subjects (weight/height^2) varied from 19 to 29 kg/m^2 . The men with the E4 phenotype were slightly more obese than those with the E3 phenotype. All subjects volunteered for the study, which was approved by the Ethical Committee of the University of Oulu.

TABLE 1
CHARACTERISTICS OF THE SUBJECTS

The results are means $\pm$ SEM. Body mass index (BMI) is the weight (in kg) divided by the square of the height (in metres). E3 indicates individuals with the apoprotein E phenotype E3/3 and E4 indicates those with the apoprotein E phenotypes E4/4 and E4/3.

Group	No. of subjects	Age (years)	BMI
All subjects	44	37.9 ± 1.2	23.4 ± 0.4
Women			
E3	12	38.9 ± 2.4	22.4 ± 0.8
E4	10	39.1 ± 2.8	22.1 ± 0.8
Men			
E3	11	39.1 ± 1.9	23.4 ± 0.6
E4	11	34.7 ± 2.2	25.6 ± 0.6^a

^a Significantly different from the E3 men ($P < 0.05$).

Design of the trial

The trial consisted of a 3-month baseline period when the subjects consumed their habitual diets, an intervention period A involving a low-fat diet lasting for 4 weeks, an intervention period B with a high-fat diet for 4 weeks and a 1-month switch-back period when the subjects returned to their usual diets. All the subjects continued their regular daytime work at the hospital. All their food during the intervention periods was supplied from the hospital kitchen. Two meals comprising about 75% of the daily energy intake were served at the hospital, and evening snacks and breakfasts, both to be consumed at home, were also prepared by the hospital. The meals for the weekends were prepared and distributed on Fridays or Saturdays to be consumed at home. All the food was served free of charge.

Plasma lipids were analysed twice during the baseline period, once a week during the intervention periods and twice during the switch-back period. The mean values for the last two weeks of periods A and B were used for analysis. Mean values were determined for the baseline and switch-back periods using both measurements for each. Body weight was monitored every day during the intervention periods and twice each during the baseline and switch-back periods.

Diets

The baseline and switch-back diets consisted partly of meals consumed in the hospital during working hours, and partly of the subjects' habitual home food. The hospital meals were analysed for one week (courtesy of the Agricultural Research Centre, Jokioinen, Finland). The diet contained 18.5% protein, 50.0% carbohydrate and 31.5% fat (Fig. 1). The daily cholesterol intake was 390 mg/12.6 MJ, the P/S ratio was 0.3 and 51% of the fatty acids were saturated, 32% monounsaturated and 16% polyunsaturated.

The intervention diet A involved reduction of the amount of fat to 25% of total energy and adjustment of the composition of the fatty acids to contain equal amounts of saturated, monounsaturated and polyunsaturated fatty acids (P/S ratio = 1.0). The cholesterol content was 240 mg/12.6 MJ per day. The diet was prepared by limiting the amount of dairy products and adding

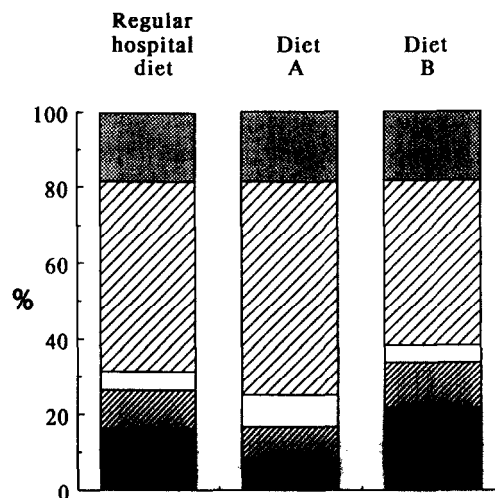


Fig. 1. Percentage distribution of calories derived from protein (▨), carbohydrates (□) and polyunsaturated (▤), monounsaturated (▥) and saturated (■) fats.

margarine, polyunsaturated salad dressings and vegetables. In diet B the amount of fat was increased to 38% of total energy, and the fatty acid composition was adjusted to be equal to the average Finnish diet (57% saturated, 31% monounsaturated and 12% polyunsaturated fatty acids) with a P/S ratio of 0.2. The cholesterol content was 420 mg/12.6 MJ per day. The protein content remained the same throughout (18–19%). The diets were adjusted to be isocaloric, the mean daily individual energy intakes being 12.6 MJ for men and 8.4 MJ for women.

Lipid analyses

All blood samples were taken after a 12-h fast. Cholesterol and triglyceride concentrations were analysed enzymatically [15,16] using a Gilford IM-PACT 400E Clinical Chemistry Analyser. HDL cholesterol was determined after precipitation of the plasma sample with heparin-manganese [17]. Since the subjects were normotriglyceridemic, LDL cholesterol was calculated according to the Friedewald formula [18]. The apoE phenotype was determined from the plasma after delipidation using isoelectric focusing and immunoblotting techniques [10,19].

Statistical analysis

The results were expressed as means $\pm$ SEM.

The significances of the differences were calculated using the two-sample *t*-test, and the paired-difference *t*-test or a one-way analysis of variance where appropriate. Correlations were calculated using the least-squares method. The Statistical Analysis System (SAS) [20] was used.

Results

Plasma total cholesterol and triglyceride values during the various dietary periods are shown in Table 2. Plasma cholesterol in general decreased significantly during period A (low-fat, low-cholesterol diet) and increased in period B (high-fat, high-cholesterol diet). When the subjects were switched from period A to period B their mean plasma cholesterol level increased by 1.24 mmol/l (27.8%) the range being from 0.2 to 2.7 mmol/l. This increase was larger for the men ($31 \pm 2\%$) than for the women ($25 \pm 3\%$) ($P < 0.05$), the percentage increases for the males and females with the apoprotein E3 phenotype being 31.0 and 25.0, respectively, and those for the males and females with the E4 phenotype 30.6 and 24.7%. Plasma triglyceride levels were slightly increased during

period A compared to the baseline period, but returned to the baseline values in period B. Overall plasma cholesterol and triglyceride values during the switch-back period were similar to those at the baseline period.

The plasma LDL and HDL cholesterol concentrations during the various diet periods are shown in Table 3. LDL cholesterol decreased significantly during period A and increased during period B in all the groups. The mean LDL cholesterol level among all the subjects was 1.02 mmol/l (39%) higher in period B than in period A, the increase varying between 0.08 and 2.42 mmol/l and being higher ($42 \pm 4\%$) for the men than for the women ($37 \pm 5\%$) ($P < 0.05$). The corresponding changes were 41.9 and 33.7%, respectively, for the men and women with the E3 phenotype, and 41.2 and 39.8% with E4. HDL cholesterol was reduced in each group in period A from the values in the baseline period (from 11.5 to 20.3%), but returned to the baseline level in period B. No significant sex differences were observed in the changes in HDL cholesterol concentrations on the various diets, with the exception of the E4 female subjects, in whom HDL

TABLE 2

PLASMA TOTAL CHOLESTEROL AND TRIGLYCERIDE VALUES ON VARIOUS DIETS

The results are means $\pm$ SEM. E3 and E4 indicate the same groups as in Table 1. Diet A = low-fat, low-cholesterol diet. Diet B = high-fat, high-cholesterol diet. Baseline and switch-back periods involve the subjects' habitual diet.

Group	Number of subjects	Baseline period	Diet A	Diet B	Switch-back period
Total cholesterol (mmol/l)					
All subjects	44	5.21 ± 0.14	4.49 ± 0.13^b	5.73 ± 0.15^d	5.38 ± 0.14
Women E3	12	5.16 ± 0.23	4.46 ± 0.23^a	$5.56 \pm 0.31^{a,c}$	5.17 ± 0.25
E4	10	4.85 ± 0.22	4.23 ± 0.16^a	$5.28 \pm 0.26^{a,d}$	4.99 ± 0.21
Men E3	11	5.42 ± 0.32	4.57 ± 0.35^b	$6.00 \pm 0.37^{a,d}$	5.58 ± 0.31
E4	11	5.39 ± 0.33	4.66 ± 0.25^b	$6.06 \pm 0.26^{a,c}$	5.78 ± 0.30
Total triglycerides (mmol/l)					
All subjects	44	0.96 ± 0.09	1.17 ± 0.09	0.95 ± 0.08^d	1.01 ± 0.09
Women E3	12	0.84 ± 0.11	0.94 ± 0.08^a	$0.70 \pm 0.06^{a,d}$	0.79 ± 0.09
E4	10	0.74 ± 0.05	0.95 ± 0.05^a	0.68 ± 0.05	0.77 ± 0.03
Men E3	11	1.04 ± 0.10	1.31 ± 0.11	1.20 ± 0.19	1.10 ± 0.11
E4	11	1.19 ± 0.30	1.48 ± 0.30^a	1.24 ± 0.22	1.38 ± 0.29^a

^a and ^b Significantly different ($P < 0.05$ and $P < 0.001$, respectively) from the baseline period.

^c and ^d Significantly different ($P < 0.05$ and $P < 0.001$, respectively) from period A.

TABLE 3

PLASMA LDL AND HDL CHOLESTEROL VALUES ON VARIOUS DIETS

The results are means $\pm$ SEM. E3 and E4 and the various periods are the same as in Table 2.

Group	Number of subjects	Baseline period	Diet A	Diet B	Switch-back period
LDL cholesterol					
All subjects	44	3.21 $\pm$ 0.10	2.65 $\pm$ 0.10 ^b	3.67 $\pm$ 0.10 ^{b,d}	3.41 $\pm$ 0.11 ^a
Women 3/3	12	3.03 $\pm$ 0.22	2.57 $\pm$ 0.19 ^a	3.44 $\pm$ 0.27 ^{a,c}	3.21 $\pm$ 0.18
4/3 or 4/4	10	2.68 $\pm$ 0.16	2.29 $\pm$ 0.11 ^a	3.22 $\pm$ 0.21 ^{a,c}	2.92 $\pm$ 0.17
Men 3/3	11	3.56 $\pm$ 0.31	2.81 $\pm$ 0.30 ^a	3.95 $\pm$ 0.30 ^{a,d}	3.68 $\pm$ 0.28
4/3 or 4/4	11	3.56 $\pm$ 0.22	2.88 $\pm$ 0.16 ^b	4.03 $\pm$ 0.17 ^{a,c}	3.78 $\pm$ 0.21
HDL-cholesterol					
All subjects	44	1.58 $\pm$ 0.06	1.32 $\pm$ 0.04 ^b	1.64 $\pm$ 0.04 ^{a,d}	1.53 $\pm$ 0.05
Women 3/3	12	1.71 $\pm$ 0.07	1.49 $\pm$ 0.08 ^a	1.83 $\pm$ 0.09 ^c	1.63 $\pm$ 0.11
4/3 or 4/4	10	1.84 $\pm$ 0.16	1.51 $\pm$ 0.08 ^a	1.76 $\pm$ 0.08 ^c	1.73 $\pm$ 0.10
Men 3/3	11	1.39 $\pm$ 0.09	1.17 $\pm$ 0.06 ^a	1.51 $\pm$ 0.08 ^{a,c}	1.40 $\pm$ 0.08
4/3 or 4/4	11	1.40 $\pm$ 0.06	1.12 $\pm$ 0.04 ^b	1.47 $\pm$ 0.05 ^c	1.37 $\pm$ 0.06

^a and ^b Significantly different ($P < 0.05$ and $P < 0.001$, respectively) from the baseline period.

^c and ^d Significantly different ($P < 0.05$ and $P < 0.001$, respectively) from period A.

cholesterol rose by only 17.6% compared with 32.2% in the E4 men ($P < 0.05$) on switching from diet A to diet B. The LDL/HDL cholesterol ratio did not differ significantly between the baseline, diet A, diet B, and switch-back period for the total series of subjects (2.18, 2.13, 2.32, 2.34, respectively). Only in the female individuals with apoE4 did the LDL/HDL cholesterol ratio increase significantly from period A (1.57) to period B (1.87) ($P < 0.006$).

Body weight generally remained constant throughout the various diet periods (Table 4), although a minor reduction was observed among

the women with E3 in period A, the level remaining unchanged after that. A small reduction was also found in period B for the women with E4.

Discussion

The results show that the difference in plasma cholesterol concentration between the low-fat, low-cholesterol and the high-fat, high-cholesterol diets, when adequately controlled, is quite large in middle-aged people living normal lives. Providing that the subjects keep carefully to their diets, the magnitude of the effects on plasma total and LDL

TABLE 4

BODY WEIGHTS DURING THE DIETARY TRIAL

The results are expressed in kilogrammes and indicate mean values $\pm$ SEM. E3 and E4 and the various periods are the same as in Tables 1, 2 and 3.

Group	Number of subjects	Baseline period	Diet A	Diet B	Switch-back period
All subjects	44	67.1 $\pm$ 1.9	66.9 $\pm$ 1.9	66.8 $\pm$ 1.9	67.4 $\pm$ 2.0
Women E3	12	57.5 $\pm$ 2.3	57.0 $\pm$ 2.3 ^a	56.6 $\pm$ 2.2 ^a	56.7 $\pm$ 2.3
E4	10	59.8 $\pm$ 2.9	59.4 $\pm$ 3.0	58.9 $\pm$ 3.0 ^a	59.4 $\pm$ 3.0
Men E3	11	70.1 $\pm$ 2.3	70.3 $\pm$ 2.1	70.8 $\pm$ 2.1	70.9 $\pm$ 2.1
E4	11	81.1 $\pm$ 2.4	81.2 $\pm$ 2.5	81.2 $\pm$ 2.4	81.7 $\pm$ 2.4

^a Significantly different from the baseline period ($P < 0.05$).

cholesterol concentrations can be as high as 28 and 39%, respectively, when they are switched from the prudent to the high-fat diet. These results match well with many previous reports, as extensively reviewed by Goodnight et al. [2]. The lower percentage changes sometimes obtained [2] may be due to the inadequate following of the dietary recommendations. The present findings are of importance particularly at the moment, when potent new cholesterol-lowering agents are widely available and Western societies are becoming aware of the need for action to lower plasma cholesterol levels in the population. The current trial certainly emphasizes that the treatment of hypercholesterolaemic patients should include effective dietary instructions, monitoring and check-ups before hypocholesterolaemic drugs are used. It is also significant that no special or expensive ingredients are necessary for a cholesterol-lowering diet. Simply an increase in the amount of ordinary vegetables, whole-grain products and some vegetable oils and a decrease in fatty dairy products is sufficient to achieve substantial results.

Individuals with the apoprotein E3 and E4 phenotypes both responded well to the dietary changes. This is very important, as it shows that, in addition to the people with the most common phenotype, E3/3, subjects who are heterozygous or homozygous for the epsilon 4 allele (E4/3 and E4/4), who have an enhanced likelihood of elevated plasma cholesterol concentrations [8–12], are also responsive to dietary changes. Interestingly, subjects with the quite rare epsilon 2 allele (phenotype E2/2, E2/3) were recently reported to be poor responders to dietary changes compared with those with phenotypes E4/3 or E4/4 [21]. Thus, some of the inter-individual variation in responsiveness to dietary changes may be related to the genetically determined phenotypic pattern of apolipoprotein E, the mechanism presumably being at least partly related to the recently discovered differences in the efficiency of cholesterol absorption among the various apoprotein E phenotypes [12]. Other factors that may well also be of genetic origin, such as apoprotein B and AI polymorphism, may apparently be involved and should be explored to uncover the causes of inter-individual variation in dietary response.

Most of the present subjects were not initially

markedly hypercholesterolaemic, although the majority had baseline cholesterol values above 5.0 mmol/l, the area typical of many Western populations. The fact that the values were somewhat low for Finns was presumably due to the dietary habits of these subjects on their baseline diets before the intervention periods. In fact, the hospital diet that the subjects had been consuming in the past and received during the baseline period was already moderately fat-controlled, at least by Finnish standards, even though saturated fats still made up 16% of the total energy consumption. It is nevertheless interesting that when on the fat-control, low-cholesterol diet as many as 38 out of the 44 subjects (86%) had plasma cholesterol levels below 5.2 mmol/l, the target value recently launched by both the American Heart Association and the European Atherosclerosis Society [13,14].

Low-density lipoprotein is the particle that carries most of the cholesterol in plasma and is also the main atherogenic element in the bloodstream. The present dietary interventions particularly affected the concentrations of these particles, even though HDL cholesterol was also reduced by the fat-controlled diet. The latter phenomenon is known to be associated with a reduction in dietary fat [1] and the replacement of saturated fats with polyunsaturated ones [2], but not with monounsaturated ones [22,23]. Even so, the LDL/HDL cholesterol ratio was not enhanced by the present low-fat diet, indicating that the overall effect can be regarded as beneficial. In fact, when on the fat-controlled diet, only 3 of the subjects had HDL cholesterol levels under 0.9 mmol/l, a value regarded as another potential risk factor for coronary heart disease [24,25], and these three males also had quite low total (< 5 mmol/l) and LDL cholesterol (< 3.5 mmol/l) levels during the same period.

Interestingly, the men showed a more pronounced response to the dietary changes than the women. This was mainly true of plasma total and LDL cholesterol concentrations. It is somewhat difficult to envisage the mechanisms which might generate a sex difference of this kind in dietary responsiveness, for there is no reason to believe that the female subjects adhered to the dietary regulations any less strictly than the men, and there was in any case no indication of any of the

participants diluting the dietary effect by poor adherence. It is significant that 75% of the total daily calories were consumed in a controlled situation in the hospital dining room. Thus, hormonal differences between males and females are more likely to be responsible for the different dietary effects. All but two of the present females were of the fertile age, and the well-known oestrogen-related effects [26–29], presumably responsible for the low-cholesterol values observed in the baseline period, may also have influenced their dietary responses in some unknown manner. Greater age of the female subjects and subsequent hormonal differences in some previous studies [3,4] may also be the reason that the sex difference in the response to dietary changes has not been noted.

Similarly to the data from trials carried out using hypolipidaemic agents [30,31], the extensive changes in plasma cholesterol concentration following dietary modification could be converted into calculations of the risk of developing coronary heart disease. In subjects with moderate hypercholesterolemia a 1% rise in plasma cholesterol increases the risk of developing coronary heart disease by 2% [30]. Although the figures may not be directly applicable to subjects with mild hypercholesterolemia the present data suggest that the risk for coronary heart disease can be reduced considerably when the high-fat diet is replaced by a fat-controlled diet. This is obviously of great importance in all Western societies, particularly in countries such as Finland where the population still has high-cholesterol values and where the incidence of coronary heart disease is still extremely high [32]. No doubt effective dietary guidance and control are crucial factors if we wish to reduce the numbers of premature deaths, early incapacitations, and enormous demands for hospitalization, medication and coronary artery bypass operations. It is notable that all of these unfortunate events and expensive manoeuvres affect in particular men who are still of working age [33]. Fortunately, these people also seem to be especially responsive to dietary modification.

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