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Response of serum cholesterol to dietary cholesterol in relation to apolipoprotein E phenotype

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Abstract. The responsiveness of serum cholesterol concentration to dietary cholesterol in humans shows modest, but reproducible, interindividual differences. We studied the relation of this between-subject variation with the apolipoprotein E (apo E) polymorphism in 20 healthy men and 12 women. Each participated in three controlled dietary trials, each consisting of a low-cholesterol diet period (106–129 mg/day) and a high-cholesterol period (625–989 mg/day). Responsiveness was defined as a subject's average increase of serum cholesterol in the three experiments. Mean responsiveness (±SD) for 32 subjects amounted to 0.46±0.33 mmol/l (18±13 mg/dl); range −0.3 to 1.1 mmol/l (−12 to 43 mg/dl). Among the volunteers we identified the following phenotypes (with number of subjects): E2/2 (1), E2/3 (4), E3/3 (17), E3/4 (6) and E4/4 (4). No relation was found between apo E phenotype and responsiveness ($r = -0.11$, $n = 32$). Exclusion of the E2/2 homozygote - a clear-cut hyperresponder - yielded a correlation of $r = -0.07$, with a one-tailed upper 95% confidence limit for r of +0.24. Thus, at the very most, $0.24^2 = 6\%$ of the variance in response could be due to variance in apo E. The difference in response between E4/4 and E3/3 subjects was −0.11 mmol/l (−4 mg/dl), and its one-tailed simultaneous upper 95% confidence limit +0.15 mmol/l. Addition of data from two more experiments on the same subjects, one involving dietary cholesterol ($n = 13$), and one involving exchange of saturated for polyunsaturated fatty acids ($n = 22$) again produced no difference in response between apo E phenotype classes. Thus, differences in apo E phenotype were not the major determinant of interindividual differences in susceptibility of serum cholesterol concentrations to diet.

Key words: Apolipoprotein E - Serum cholesterol - Diet

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Introduction

Dietary cholesterol affects serum cholesterol concentrations in some individuals more than in others. In several animal species this variation in responsiveness to diet has a genetic basis, and hypo- and hyperresponding strains of monkeys, rabbits and rats have been bred (reviewed in [1]). In repeated dietary experiments with 32 volunteers, we have recently established that modest, but consistent interindividual differences in responsiveness of serum cholesterol to dietary cholesterol also exist in humans [2]. We also established a congruence between the responsiveness of serum cholesterol to dietary cholesterol and to dietary fatty acids in this group [3].

The metabolic basis of variation in responsiveness to dietary cholesterol has not been elucidated [1]. The extent of the increase in serum cholesterol concentrations after cholesterol consumption may depend on the capacity for depressing endogenous cholesterol synthesis [4] or stimulating receptor-mediated clearance of low-density lipoprotein (LDL) [5], but the primary determinant might be the degree of intestinal absorption of cholesterol [6].

Recently, Utermann [7] suggested that some of the variation in the responsiveness of serum cholesterol to dietary cholesterol arises from the apolipoprotein E (apo E) polymorphism. Apo E plays a key role in the clearance of cholesterol-rich lipoproteins from plasma

[8]. Three major isoforms of apo E are known, E2, E3 and E4, with typical population frequencies of 8%, 77% and 15% [6, 7, 9]. In population studies [6, 10, 11], the apo E phenotype has been associated with a gradient in serum LDL and total cholesterol levels, subjects with the E2/2 phenotype having the lowest and those with the E4/4 phenotype, the highest serum levels. Apo E2-containing lipoproteins (chylomicron remnants, VLDL remnants, and HDL with E) show a markedly deficient ability to bind to liver lipoprotein receptors compared with apo E3 and apo E4. As a result, in subjects with the E2/2 or E2/3 phenotype such lipoproteins might accumulate in plasma, so that less cholesterol would enter the liver from the intestine (chylomicrons) and from the periphery (HDL with E). In compensation, LDL-receptors might be upregulated, resulting in an enhanced uptake of LDL and a lowering of LDL in plasma. In addition, the production of LDL from VLDL remnants might be decreased because it depends on the presence of functional apo E on the remnants [12].

If apo E-mediated uptake of lipoproteins influences LDL-receptor activity on liver cells, and thereby LDL concentrations in plasma, then it is conceivable that the responsiveness of plasma LDL to dietary cholesterol also increases from apo E2 to apo E3 to apo E4. Fisher et al. [13] attempted to delineate the relation between apo E phenotype and response to dietary cholesterol by comparing five subjects with phenotype E3/3 and three with phenotype E2/3, but found no correlation. However, the within-subject variability of the response to dietary cholesterol is such that firm conclusions are only possible when there are a fairly large number of subjects and measurements and a wide spread in phenotypes [2].

We have investigated the relation between responsiveness and apo E phenotype in 32 subjects who had each participated in at least three controlled trials on dietary cholesterol; 22 also took part in an experiment in which the saturated and polyunsaturated fat intake was varied whilst cholesterol intake remained constant.

Methods

Subjects and diets. All subjects, 12 women and 20 men, were healthy normolipemic volunteers. Their recruitment and selection have been described elsewhere [2]. Their baseline characteristics are presented in Table 1.

Table 1. Baseline characteristics (mean ± SD) for the 32 subjects while consuming their habitual diets

Age at entry (years)	33 ±13
Height (cm)	177 ± 8
Weight (kg)	71 ±10
Body mass index (kg/m^2)	22.2 ± 2.5
Serum total cholesterol (mmol/l)	4.96± 0.78
Serum HDL cholesterol (mmol/l)	1.40± 0.30
Serum triglycerides (mmol/l)	0.91± 0.34

Cholesterol, 1 mmol/l=39 mg/dl; triglycerides, 1 mmol/l=89 mg/dl

The response of serum cholesterol to increased dietary cholesterol intake was measured in 1982 in three trials of similar design [2]. Thirty of the subjects had participated in all three trials, and two more, each of phenotype E3/E3, in the first two trials only. Each trial consisted of a low-cholesterol period followed by a high-cholesterol one. The low-cholesterol periods lasted 14, 11, and 25 days, and the high-cholesterol diets 13, 13, and 28 days for experiments 1, 2, and 3, respectively. Subjects returned to their habitual diet and lifestyle for 1 month between studies 1 and 2, and for 5 months between studies 2 and 3. Subjects took their meals daily in the Department and received the rest of their daily rations ready packed. The diets consisted of natural foodstuffs and were formulated so that cholesterol was the only variable. Cholesterol was fed in a constant proportion to calories. Cholesterol intakes in the low- and high-cholesterol period were 12 and 56 mg/MJ, respectively in experiment 1, 10 and 57 mg/MJ in experiment 2, and 11 and 84 mg/MJ in experiment 3. Mean energy intake was 11.2 MJ (2685 kcal) per day; it was continuously adjusted individually so as to keep changes in body weight during the trials to a minimum. Intake of other nutrients was constant throughout all three trials. Two to four separate fasting blood samples were obtained at the end of each low- and high-cholesterol period and the response calculated as the difference of the mean levels of serum cholesterol with the two diets. Responsiveness to dietary cholesterol was defined as the mean of the responses in the three experiments. In addition, 23 subjects participated in a fourth experiment, in which cholesterol intake was kept constant and the fatty acid composition of the diet was varied [3]; apo E was known for 22 of them. The saturated, monounsaturated and polyunsaturated fatty acid content of the diet was 11%, 12%, and 21% of energy, respectively, in the first 3 weeks, and 23%, 14%, and 5% in the second period, which also lasted 3 weeks. Finally, 13 subjects took part in a fifth experiment (J. F. C. Glatz et al., in preparation), which comprised a high-cholesterol (900 mg/day), a low-cholesterol (140 mg/day) and another high-cholesterol (900 mg/day) period, each of which lasted 18–30 days each.

Informed consent was obtained from all participants, and the studies were approved by the Human Ethics Committee of the Department.

Analytical methods. Total serum cholesterol was measured in experiments 1–3 with the Liebermann-Burchard reagent under rigidly standardized conditions [14] and in experiments 4 and 5 enzymatically [3]. LDL was isolated by density gradient centrifugation (density range 1019–1055 g/ml) of fresh serum. LDL apolipoprotein B concentration was measured by electroimmuno assay. Further experimental details are described elsewhere [2]. The apo E phenotype was determined by isoelectric focusing [15, 16].

Statistical analyses. The data from multiple experiments were jointly analyzed by three-way analysis of variance with three classifications: person, apo E phenotype, and experiment, individual persons being nested into apo E classes. Total serum cholesterol responsiveness was the dependent variable. The data recorded in the E2/2 homozygote were excluded from this analysis. Tukey's studentized range test was used to test differences between the mean responses of the various apo E phenotype classes and to calculate simultaneous confidence limits for these differences. The SAS Generalized Linear Models procedure [17] was used.

For the analysis of trends across apo E classes, the following dummy values were assigned to the apo E phenotype variable: E2/2=20, E2/3=25, E3/3=30, E3/4=35, and E4/4=40. Correlation coefficients were calculated between these apo E phenotype dummy values and the cholesterol response per subject for each experiment separately, and for the mean response per subject over multiple experiments (Fig. 1). Both product-moment (Pearson) and rank order (Spearman) correlation coefficients were tested. As these produced essentially the same results, only the product-moment correlations are given. Confidence intervals for correlation coefficients were calculated using Fischer's z-transformation. One-sided tests and one-tailed 95% confidence in-

tervals were calculated in all cases, as the only plausible alternative for the zero hypothesis was the existence of a positive relation between apo E phenotype value and response.

Results

Among the 32 subjects we identified the following apo E phenotypes (with numbers of subjects): E2/2 (1), E2/3 (4), E3/3 (17), E3/4 (6) and E4/4 (4). The frequencies of phenotypes in this group appear similar to those reported for 2000 Dutchmen by Klasen et al. [9], except for the absence of E2/4 and a greater preponderance of E4/4.

Apolipoprotein E phenotype and serum cholesterol levels

When plotted by increasing apo E phenotype value from E2/2 to E4/4, the apo E phenotype showed a weak positive correlation with the serum level of total cholesterol, both when it was measured while the subjects were consuming their habitual diet, and when they were keeping to a strictly controlled low- or high-cholesterol diet (Table 2). The relationship of apo E phenotype rank order with the concentration of LDL cholesterol or of LDL apo B was closer than that with total cholesterol (Table 2).

Table 2. Linear correlation coefficients of the serum levels of total cholesterol, LDL cholesterol, and LDL apolipoprotein B (apo B), as measured during various diets, with the apolipoprotein E phenotype value (E2/2=20, E2/3=25, E3/3=30, E3/4=35, and E4/4=40) in 32 normolipemic volunteers participating in a series of experiments on variability of response of serum cholesterol to diet

Diet	Coefficient of correlation with apo E phenotype value		
	Total cholesterol[b]	LDL-cholesterol[c]	LDL apo B[d]
Habitual[a]	0.12		
Low-cholesterol[a]	0.26	0.37*	0.46*
High-cholesterol[a]	0.20	0.31*	0.57**

[a] Mean cholesterol intakes were 305 mg/day on the habitual diet, 121, 106, 129 and 140 mg/day on the low-cholesterol diets (experiments 1, 2, 3, and 5, respectively) and 625, 673, 989 and 900 mg/day on the high-cholesterol diets (experiments 1, 2, 3, and 5, respectively)

[b] Mean of six to ten blood samples for the habitual diet or of eight values obtained during the appropriate periods of experiments 1, 2, and 3 (low- and high-cholesterol diet)

[c] Determined in experiment 2 by ultracentrifugation of pools of two serums per subject per diet

[d] LDL apo B was measured in plasma in 23 subjects who participated in the first two periods of experiment 5

* $P<0.05$; ** $P<0.01$ (one-tailed tests)

Variation in responsiveness of serum cholesterol to dietary cholesterol

The effect of increased cholesterol intake on the serum concentration of total cholesterol was measured in three separate, but similar, experiments. The mean (±SD) increase in serum cholesterol for all 32 subjects was 0.56±0.53 mmol/l (22±20 mg/dl) in experiment 1, 0.18±0.38 mmol/l (7±15 mg/dl) in experiment 2, and 0.66±0.36 mmol/l (26±14 mg/dl) in experiment 3. Changes in HDL cholesterol averaged only 0.08 mmol/l [2], and in our experience VLDL does not rise in response to dietary egg yolk cholesterol [18]; therefore, the increase in total cholesterol reflected essentially a rise in LDL. Individual response values were significantly correlated between trials [2]. For each subject the response values from the three trials were averaged to obtain the best estimate of true responsiveness to cholesterol consumption.

In the subjects participating in the fatty acid experiment (expt 4), serum cholesterol rose by 1.04 mmol/l when polyunsaturated fatty acids in the diet were replaced by saturated fatty acids. Individual responses to saturated fat in this experiment and to dietary cholesterol in the other trials showed significant congruence ($r=0.62$) [3].

Finally, the serum cholesterol response measured in the fifth controlled study was again significantly correlated with the mean response to dietary cholesterol in the first three experiments ($r=0.86$, $n=13$, $P<0.05$) (J. F. C. Glatz et al., manuscript in preparation).

Apolipoprotein E phenotype and responsiveness to dietary cholesterol

The apo E phenotype rank value, plotted from E2/2 to E4/4 (Fig. 1), showed no significant correlation with the mean responsiveness of serum cholesterol in the

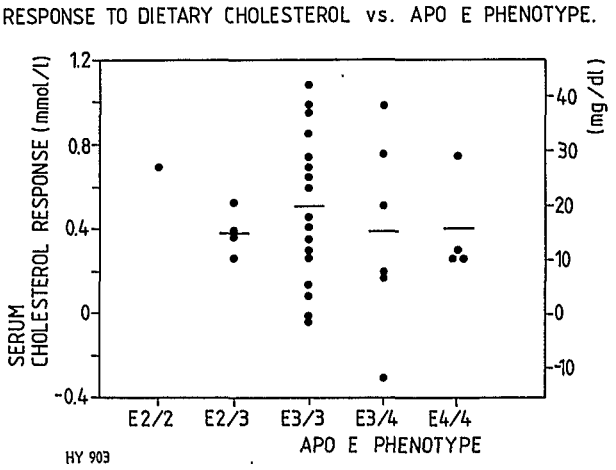

Fig. 1. Relation between apolipoprotein E phenotype and the individual responsiveness of serum cholesterol to increased dietary cholesterol, averaged over three experiments. For each apo E phenotype class, mean responsiveness is indicated by a *horizontal bar*

Table 3. Differences in the response of serum cholesterol to dietary cholesterol between subjects with different apo E phenotypes. Data are given for the first three experiments combined (experiments 1+2+3; 91 individual response observations used for data analysis); for all four experiments where dietary cholesterol was the experimental variable (experiments 1+2+3+5; 103 observations used); and for the first three experiments plus experiment 4, where saturated fat was the dietary variable (experiments 1+2+3+4; 112 observations used). Simultaneous upper 95% confidence limits are given in parentheses. None of the values differed significantly from zero

Phenotypes compared	Mean difference in response between phenotypes (95% upper limit)		
	Experiments 1+2+3	Experiments 1+2+3+5	Experiments 1+2+3+4
	(mmol/l)		
E3/3-E3/2	0.12 (0.38)	0.13 (0.36)	0.08 (0.32)
E4/3-E3/3	−0.11 (0.11)	−0.10 (0.09)	−0.12 (0.08)
E4/4-E4/3	0.01 (0.31)	−0.07 (0.20)	0.04 (0.32)
E4/4-E3/3	−0.11 (0.15)	−0.17 (0.07)	−0.08 (0.16)

first three experiments ($r = -0.11$, $n = 32$, $P > 0.05$; one-tailed 95% upper confidence limit for r, 0.18) (Fig. 1). Exclusion of the E2/E2 homozygote did not materially change this value ($r = -0.07$, $n = 31$, $P > 0.05$; one-tailed 95% upper confidence limit for r, 0.24). Analysis of variance, with exclusion of the data of the E2/2 homozygote, showed that there were significant differences in response between individuals ($P = 0.0002$), but not between the responses of the various apo E phenotype groups ($P = 0.40$). Differences in response between phenotype classes and the simultaneous one-tailed 95% upper limits of these differences for various combinations of experiments are given in Table 3. All differences were close to 0; upper confidence limits ranged from 0.07 to 0.38 mmol/l.

Analysis of variance of all four dietary cholesterol experiments combined, excluding the E2/2 phenotype, yielded a P-value of 0.30 for an effect of phenotype on response. The differences in response between phenotype classes were again close to zero (Table 3).

The apo E phenotype value was also not correlated with the response to dietary saturated fatty acids in the fourth experiment ($r = -0.15$, $n = 22$). Combined analysis of these data with the data from experiments 1–3 again produced no difference in response between apo E phenotypes (Table 3). Multivariate analyses with age, sex and body mass index included as explanatory variables produced essentially the same results (not shown).

Discussion

Through its effect on plasma apo B concentration, polymorphism of human apo E contributes substantially to the normal variance of plasma lipoprotein concentrations found within populations [19]. The data presented in this study agree with the findings recorded in previous studies [6, 10] in that the C_2 allele was associated with lower levels of total or LDL cholesterol than in the presence of the more common homozygous E3/3 phenotype. LDL and total cholesterol levels did not differ between the groups with the E3/3, E3/4 and E4/4 phenotypes; this may limit the applicability of our findings somewhat.

The responsiveness of a subject's serum cholesterol to dietary cholesterol, averaged over three separate strictly controlled experiments, was not related to the apo E phenotype. The consideration of data from two more trials in which subsets of our subjects participated – one involving dietary fatty acids and the other also dietary cholesterol – strengthened this conclusion. The various sets of data in Table 3 are not, of course, independent of each other. They are presented here merely to emphasize that there was no relation between apo E phenotype and response in our subjects, no matter which set of data was considered. Analysis of the data of the first three experiments alone put an upper 95% confidence limit of 0.24 on the correlation between apo E and response when the E2/2 subject was excluded; therefore, no more than $0.24^2 = 6\%$ of the variance in response could be due to variance in apo E phenotype. Analysis of variance showed that there were marked, sustained differences in susceptibility of serum cholesterol to dietary cholesterol between individuals, but produced no evidence that mutations in the amino acid sequence of apo E explained any of this variability. Specifically, our data put an upper confidence limit of about 0.1 mmol/l on the difference between E4/4 and E3/3 homozygotes in serum cholesterol response to a fairly large dose of dietary cholesterol – a dose that caused total cholesterol in our subjects to rise by almost 0.5 mmol/l. This upper limit of 0.1 mmol/l is appreciably less than the differences in serum cholesterol levels between E4/4 and E3/3 subjects in various European populations consuming high-cholesterol, high-saturated fat diets; such differences typically range from 0.2 to 0.6 mmol/l [7, 19, 21]. Our data are at variance with the findings of Miettinen et al. [20]. They measured serum cholesterol in 16 men, one with the E2/2, five with the E2/3, nine with the E3/4 and one with the E4/4 phenotype, on low-fat diets providing either 150–200 mg or 900 mg of cholesterol per day. Total cholesterol was significantly increased in the E4 men but not in the E2 men; the respective rises in LDL cholesterol were 16% and 3%. On the other hand, Goldberg et al. [22] and Brenninkmeijer et al. [23] failed to observe a relation between apo E phenotype and the response of serum total or LDL cholesterol concentration to dietary lipid changes. One difference between the experiment of Miettinen et al. [20] and ourselves was the background diet, which was low in fat in the Finnish study and high in saturated fat in ours.

Although the apo E response hypothesis was formulated to explain the effect of the C_4 allele on serum cholesterol compared with the C_3 allele [7], it still seems pertinent to stress that our sole E2/2 homozygote subject was a highly consistent hyperresponder. This lean, healthy, symptom-free man showed no signs of dyslipo-

proteinemia, but he did have the low LDL-cholesterol level and high VLDL-cholesterol/triglyceride ratio typical for E2/2 subjects. When challenged with dietary cholesterol or saturated fat, his serum cholesterol consistently hyperresponded on every occasion (expt 1, +0.70 mmol/l; expt 2, +0.67; expt 3, +0.77; expt 4 (saturated fat), +1.36; and expt 5, +1.23 mmol/l). Still, his free-living serum cholesterol at 4.3 mmol/l was low for his sex and age (42 years), which again is typical for E2/2 homozygotes. Miettinen et al. [20] did not present separate data for the response of their E2/2 homozygote.

In conclusion, our findings did not suggest that differences in sensitivity to the cholesterol and saturated fat present in the usual diet are a major cause of the differences in serum cholesterol concentration that are associated with differences in apo E phenotype.

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